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PHYLOGEOGRAPHY AND HABITAT ASSOCIATIONS OF FRANKLIN'S GULLS IN THEIR UNITED STATES BREEDING RANGE

by

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Bachelor of Science, Colorado State University, 2008

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This thesis, submitted by AnnMarie Krmpotich in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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ABSTRACT

The Franklin’s Gull (*Leucophaeus pipixcan*) is a long distance migrant which is listed as a species of concern by U.S. state wildlife management agencies and in the Northern Prairie and Parkland Waterbird Plan for the U.S. and Canada. The designation of this status is due to few, isolated breeding colonies and unknown population dynamics. Few attempts have been made to address the unknown population dynamics or to quantify habitat use at breeding sites of this species. Recognizing there is a need for this information; this thesis uses deoxyribonucleic acid (DNA) evidence to show the relationships of Franklin’s gull breeding colonies across the U.S. as well as quantifies habitat use through geographic information system (GIS) and remote sensing technologies. This thesis describes data collected on population structure and habitat use of Franklin’s gulls. I found Franklin's gulls in the breeding range of the United States are a panmictic population. It was found habitat use of Franklin's gull colonies at breeding sites depends on multiple variables suggesting landscape management to include wetland complexes is key to persistence of nesting colonies in the United States.

CHAPTER I

INTRODUCTION TO PHYLOGEOGRAPHY AND HABITAT ASSOCIATIONS OF FRANKLIN'S GULLS

Wetlands

General Description

The dynamic nature of wetlands complicates their definition and classification. In general, wetlands are defined by plants (hydrophytes), soils (hydric soils), and frequency of flooding (Cowardin et al., 1979). A wetland is a transitional area between true aquatic habitats (e.g. rivers, lakes, estuaries, or oceans) and a dry, terrestrial (upland) habitat (Mitsch & Gosselink 2007, Dahl, 2011). A classification system for wetlands is intended to provide a standard definition at a variety of hierarchy levels to be useful to natural resource managers for the purpose of science, education, and administration. Although many wetland classification systems have been developed, the one most commonly adopted for natural resource management in the U.S. today is the "Classification of Wetlands and Deep Water Habitats" (Cowardin et al., 1979). At a regional scale for the prairie pothole region (PPR), the classification system most widely adopted is the "Classification of Natural Ponds and Lakes in the Glaciated Prairie Region" (Stewart & Kantrud, 1971). Due to its practicality, this system is favored by many field biologists. Seven major classes of wetlands are distinguished by vegetational zones which include: Class I (ephemeral ponds), Class II (temporary ponds), Class III (seasonal ponds and lakes), Class IV (semi-permanent ponds and lakes), Class V (permanent ponds and lakes), Class VI (alkali ponds and lakes), to Class VII (fen ponds).

Wetland Functions and Trends

Wetlands serve many valuable functions on the landscape; including ecological, economic, and social benefits. Wetlands serve to mitigate floods, recharge ground water, protect shoreline, and cleanse polluted water coming from surrounding lands. These functions are lost when destruction, degradation, or any form of alteration occurs; additionally negatively impacting biotic factors (Mitsch & Gosselink, 2007). Public recognition of the value of wetlands has triggered a movement of protection over the past 30 years. Despite progress towards wetland protection, there still exists continuing pressure from both anthropogenic and environmental stressors that threaten these habitats. At the time of European settlement, there were an estimated 221 million acres of wetlands in the conterminous U.S. (Dahl, 1990, Dahl, 2000). From the 1950s to the 1970s the average annual loss of wetlands was 380,000 acres (Frayer et al., 1983). With the enactment of The Clean Water Act (1972) and the Food Security Act of 1985 ("Swampbuster"; P.L. 99-198), wetland drainage slowed considerably (Reynolds et al., 2006). However, wetlands are still being lost at alarming rates; the amount of wetland habitat loss annually in the U.S. is 58,500 acres (Dahl, 2000). As of 2009, it was reported the total wetland acreage was 110.1 million (Dahl, 2011), which is less than half of the pre-settlement wetlands found in the continental U.S. With the continued loss of freshwater wetlands come severe consequences in hydrologic and ecosystem connectivity (Dahl, 2011).

Wildlife Impacts

Wetland habitats have been lost or degraded due primarily to agricultural practices. There is a conflict of interest for wildlife managers between locations of land optimal for supporting wetland wildlife and land with high agricultural value being drained and converted to crops. Severely affected by these wetland alterations are the wildlife species in the PPR. One major concern in wildlife management is breeding bird populations, as the PPR is a geographical 'hot spot' for migratory bird use during the breeding season, and available habitat will ultimately determine their success. Loss of wetland habitats has negative consequences to wetland bird populations (Johnson, 2001). In particular, wetlands play a critical role in migratory bird breeding habitat and the loss of this habitat has severe impacts to these populations. Consequently, populations of many waterbird species have been designated as a species of concern (Beyersbergen et al., 2004).

Understanding the impacts of landscape change is important, especially for migratory species that rely on a mosaic of habitats to meet their annual needs during the breeding, non-breeding, and migration periods (Skagen et al., 1999; Drake et al,. 2001). Reductions in the availability of wetland stopover sites could negatively impact migratory bird populations by reducing the overall quality of the landscape and increasing the distance between suitable stopover sites (Skagen, 2006, Smith, 2008). There is much concern for migratory bird species that rely on wetlands in the PPR either as stopover sites or for their breeding habitat as the availability of quality wetlands within this landscape continues to decline.

Gene Flow

Connectivity

Habitat loss through anthropogenic landscape alteration (conversion of wild lands to logging, development, or agricultural practices) can result in isolated habitat patches which results in a significant impact at the population level for wildlife species (Funk et al., 2010). Habitat loss can segregate subpopulations even further, leaving them vulnerable to reduced genetic diversity (Howes et al., 2009). Segregated populations often have reduced numbers of individuals, increasing the chance of genetic drift (Evans et al., 2008), reduced genetic variation within a population, and increased genetic diversity between populations, possibly leading to speciation (Irwin et al., 2001). The consequence of this segregation and reduction in population size is a loss of local alleles (Lacy, 1987). Importantly, changes in gene frequency can occur over a relatively short period of time (Rolshausen et al., 2009), which has implications for population survival and conservation. Some concerns of gene frequency change occurring quickly is a bottleneck, inbreeding, and the population's ability to evolve in response to environmental change- all directly affects fitness. In light of these considerations, understanding how populations within a species are distributed across its geographic range is important in conservation biology and to species management.

Genetic monitoring at various spatial scales can assist in detecting changes in species abundance and diversity. Situations where this is important include species designated as a conservation priority and threatened and endangered (T & E) species where this information can help determine both management strategies and intensity. Further, determining the amount of connectivity among sub-populations can allow inferences about the status of population inter-mixing from panmixia to geographic isolation, which impacts management approaches. Managing disjunct populations of species is crucial, as survival at a regional scale often depends on population growth and dispersal characteristics at the local scale (Fahrig & Merriam, 1994). Successful management of disjunct populations includes fine-scale determination of what types of areas and protections are necessary; whereas, management of a panmictic species where there are no restrictions in the population for mating (i.e., all individuals are potential mates), will involve different strategies including conservation prioritization of a species' needs at various life stages, preserving intraspecific genetic diversity, and protecting the ecological and evolutionary processes necessary for the species' persistence.

Migratory Species

Understanding genetic diversity is key to determining metapopulation dynamics and for developing species conservation plans for migratory species (Esler, 2000; Taylor, & Norris, 2010). A migratory species' range depends on habitat availability, including necessary stopover areas (Skagen, 2006) and on a species' response to climate in both wintering and breeding grounds (Mustin et al., 2007). Migratory birds depend on stopover sites to refuel their energy stores depleted during travel between distant summering and wintering areas (Weber, 1999). If major stopover points are lost (not available), migratory behavior must adapt in order to maximize fitness (Smith & Deppe, 2008; Weber, 1999). New migration routes are known to occur with changing environmental conditions (Sutherland, 1998). However, the extent to which species are capable of shifting migration patterns to occupy new ranges is a major concern, especially given predicted impacts of global climate change (Mustin et al., 2007).

Most migratory species, although dispersed over a large geographic area, exhibit some degree of genetic population structure (Avise & Hamrick, 1996; Webster et al., 2002). Causes of population structure are the result of restricted gene flow, due to physical or behavioral barriers that act to isolate breeding among subpopulations (Slatkin, 1987). The movement of individuals and their genes influences a number of ecological processes including population persistence and adaptive response to environmental change (Frankham et al., 2002). The use of genetic approaches in wildlife management can help address complex species management issues, such as deciphering meta-population dynamics, provided a basic knowledge of gene flow is known.

Phylogeography

The study of a species' geographic distribution and the study of a species' evolutionary patterns are known as phylogeography (Avise, 2000). Phylogenetic approaches can provide a myriad of ecological applications, from hypothesis testing to conservation planning. Previous studies have used phylogenetic methods to decipher a species range (Zeisset & Beebee, 2001), identify biogeographic barriers (Sonsthagen et al., 2011), distinguish movement patterns of migratory species (Mehl et al., 2004), provide insight to landscape genetics (Oomen et al., 2011), and assist in endangered species management (Lei et al., 2003). The use of phylogeography can provide important information for conservation purposes through determining population structure and by providing evidence for demographic events, such as changes in population size or dispersal (Avise, 2000).

The Franklin's Gull

The Franklin’s gull (*Luecophaeus pipixcan*) is a species that utilizes wetlands in the PPR and exhibits segregated populations, an ideal candidate to investigate habitat loss in a migratory species for this region. The Franklin's gull relies on Class IV & V wetlands (Stewart & Kantrud, 1971) for nesting which are the predominant wetland types, in terms of total acreage, throughout the PPR. The wetland nesting locations in this region used by Franklin's gulls are geographically segregated. The Franklin’s gull is a migratory species, traveling up to 10,000 km between its breeding areas in North America to wintering areas along the coasts of central Peru and northern Chile (Burger & Gochfeld, 2009). It is only one of two species of North American gulls to migrate south of the equator (Burger & Gochfeld, 1994).

The Franklin's gull arrives on its breeding grounds in mid-to late April and remains until August (Soos, 2004, Burger & Gochfeld, 2009). Following the nesting period, in late July the species makes broad, multi-directional post-breeding movements throughout its breeding range. Between September and early October, larger flocks begin to assemble as they prepare for their departure to southern wintering areas (Burger & Gochfeld, 2009). The Franklin’s gull winters primarily along the western portion of coastal South America with a small isolated inland wintering population in Peru (Burger, 1996; Burger & Gochfeld, 2009). Due to the dynamic nature of prairie wetlands and their vulnerability to both periodic drought and drainage, breeding colonies of Franklin's gulls often shift colony locations from year to year, and in some years local populations are suspected to forego annual breeding opportunities altogether (Burger and Gochfeld, 2009). Franklin's gull breeding populations on wetlands have become reduced and segregated over time, likely because of habitat loss and increased human disturbance at the breeding sites (Burger & Gochfeld; 1994). During the Dust Bowl era (1930s) most of the historic breeding sites were lost as a result of large-scale drainage projects but now with the establishment of state and federally protected wetlands (national wildlife refuge system; NWR) Franklin's gulls nest almost exclusively on public lands (Burger & Gochfeld, 1994).

Population Dynamics

The restoration and creation of large wetland complexes, mainly on protected national wildlife refuges over the past century has aided in expansion of Franklin's gull populations (Burger & Gochfeld, 1994), however population size and dynamics remain uncertain and a conservation concern. Since the early 1900s, the largest Franklin's gull breeding colonies in the U. S. (colonies of more than 100,000 breeding pairs) have been located on marshes in the prairie pothole region (PPR), primarily in North Dakota and Minnesota (Burger & Gochfeld, 1994). Segregated small sub-populations are located in Oregon, Nevada, and Utah (Burger & Gochfeld, 1994). In any given year there are less than 50 colony site locations across the North American breeding grounds for the continental population (Burger & Gochfeld 1994) with approximately 15-20 sites of those located in the U.S.

A paucity of information exists on total population size at both the continental-level and within the Prairie & Parkland Region for some colonial nesting waterbird species, according to *The Northern Prairie and Parkland Waterbird Conservation Plan* (Beyersbergen, 2004). Of those colonial species, the Franklin's gull has been estimated as approximately 2.5 million birds in the Northern Prairie & Parkland Region during migration (Beyersburgen, 2004). Another estimate of the global population of Franklin's gull was 315,000-991,000 adults (Milko et al., 2003). However, exact numbers are unknown, due to this species' typically remote nesting locations and an inability to assess population status from standard surveying methods (e.g. North American Breeding Bird Survey).

Detection of these colonies relies heavily on visual identification of colonies or observation of concentrated numbers of gulls in foraging areas (e.g. tilled farm fields in spring), indicating a possible colony in the vicinity. The most common and widespread survey methods to monitor the status and trends of North American bird populations (e.g. North American Breeding Bird Survey), are typically conducted roadside. A roadside survey methodology is often of limited value for detection of colonial nesting waterbird species that utilize marsh habitats. Colonies of these species, including those of Franklin's gulls, are typically located on the interior of wetlands within dense vegetation and away from areas of potential disturbance (e.g. roadsides, uplands, Burger & Gochfeld, 2009). Franklin's gull behavior exacerbates these issues. Adults rarely wander away from a nesting colony when eggs and/or chicks are present (Burger, 1974), which decreases the chance of detecting colonies. Despite these sampling limitations, some literature suggests that continental populations of Franklin's gulls have declined. Breeding Bird Survey data from 1968-1991 suggests an overall 90% decline (Sauer et al., 2008) but causes of this decline are unknown and trends in recent surveys show conflicting results (Beyersbergen, 2004; Kushlan, 2004).

The Breeding Season

The Franklin's gull requires large semi-permanent and permanent freshwater marshes with emergent vegetation and open water (Beyersbergen, 2004) on its breeding range. They are a colonial nesting species that build nest structures in the form of over water platforms or utilize existing muskrat houses (Burger & Gochfeld, 1994). These nests require continual maintenance by adding vegetation to prevent sinking or flooding until young-of-the-year gulls reach fledging stage. Nest characteristics vary between colony sites: types of vegetation (*Typha* spp*., Schoenoplectus* spp.); density of vegetation (live and dead); and location of nests within each wetland (center versus edge; Burger, 1974). Quantified land cover habitat characteristics which serve a role in nest site selection at breeding colony localities of Franklin's are currently limited for this species.

Clutches range from two to four eggs (mean *n*=3) (Burger & Gochfeld, 2009); only one brood is reared each season (Burger & Gochfeld 2009). Duration of pair bonds is unknown (Burger & Gochfeld, 2009). Parents remain in close vicinity of nests (within 30km) when searching for food (Burger & Gochfeld, 2009; Beyersbergen, 2004). It is common to see Franklin’s gulls in flocks when searching for food, usually over water or in agriculture fields; dominating their diet composition is earthworms, grubs, grasshoppers and midges (Chironomidae; Beyersbergen, 2004).

Conservation Status

Nesting colonies throughout the breeding range of Franklin's gulls share a common threat: habitat loss and disjunct populations. Across North America, breeding populations of Franklin’s gulls are threatened by habitat loss and other human encroachments on wetlands (Hagen et al., 2005; Bakker, 2005; MNDNR, 2008; Idaho

Department of Fish and Game, 2005; Montana Fish, Wildlife, and Parks, 2009; Ivey & Herziger, 2006). The Franklin's gull was recently listed as a species of high concern in the Northern Prairie and Parkland Region (Beyersbergen, 2004). Within its breeding range in the U.S., the Franklin's gull is currently listed as a species of conservation concern in all states where nesting colonies occur. Table 1 summarizes the status of Franklin’s gull throughout its U. S. breeding range by state.

Management Needs for the Franklin's Gull

Management of Franklin’s gulls is hampered by a lack of information about habitat preferences and population structure. Colonial waterbird species are dependent on relatively few critical wetland sites within their breeding range. With naturally fluctuating water levels of prairie wetlands due to periods of drought and deluge, individual sites may not serve as suitable nesting sites in consecutive years. The ephemeral nature of these sites forces colonial nesting waterbird species to relocate to other nearby locations or to forgo reproduction during a given year. This complicates resource managers’ efforts to analyze habitat use at existing nesting sites and to determine criteria for wetland preservation at nesting areas. Quantification of habitat use at successful nesting colony locations may assist in these efforts. Further, there are no data on population structure for the Franklin's gull; understanding the relationships among colonies within their breeding range provides critical information about population dynamics. As Franklin's gull populations may be forced to relocate to different breeding areas annually, it is particularly important to understand the relationships of individuals to one another.

Study Objectives

Population genetics is a powerful tool for wildlife managers when coupled with spatial data (Hitt et al., 2003; Haig et al., 2004; Schwartz et al., 2007; Kendall et al., 2009). Understanding how a species is connected across its geographic range is important, especially given ongoing continental declines in optimal wildlife habitat. Further, spatial information can identify key habitat elements for successful breeding grounds for a species. The goal of this study is to investigate the relationship among landscape composition at breeding sites and population genetic structure of the Franklin's gull in the U.S. To achieve this overall goal, the objectives of this study are to: 1) estimate the genetic diversity among and between Franklin's gull colonies on U.S. breeding grounds to infer levels of philopatry and dispersal, and 2) quantify habitat land cover at nesting sites to determine use trends at successful nesting colony locations. A combination of findings related to both objectives will provide information for landscape management approaches for Franklin's gull breeding colonies within the U. S.

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Table 1. Conservation status of the Franklin's gull (*Leucophaeus pipixcan*) within its U.S. breeding range by natural resource agencies, including reasons for this species' designation.

State	Conservation Status	Implications	Reference
ND	Level 1: Species in greatest need of conservation	The state has one of the largest breeding colonies in the world	Hagen et al. 2005
SD	Level 1: Priority bird species	High maximum abundance of the species within its range in South Dakota; The species is showing population declines in the state/across its range	Bakker 2005
MN	Special concern	Extremely uncommon in the state and has unique or highly specific habitat requirements that deserve careful monitoring of its status	Minnesota Department of Natural Resources 2008
MT	Species of concern	Native taxa at risk to state extirpation due to declining population trends, threats to their habitats, and restricted distribution	Montana Natural Heritage and Montana Fish Wildlife and Parks 2009
ID	Protected nongame; Imperiled breeding	Habitat threats and disjunct populations; habitat stresses including fluctuating water levels, exotics.	Idaho Department of Fish and Game 2005
UT	Priority species of moderate concern	Breeding, migrant of the state. Fluctuating, uncertain breeding trend, need for population assessment. Management issues primarily concerned with providing ideal colony site conditions.	Ivey and Herziger 2006

CHAPTER II

FRANKLIN’S GULL POPULATIONS REVEAL PANMIXIA DESPITE GEOGRAPHICALLY SEGREGATED BREEDING SITES

Abstract

Managing species with segregated populations is a challenging task and requires an understanding of population structure. For segregated populations, the conservation of genetic diversity is especially important. As habitat fragmentation increases, so does the importance of genetic diversity. Within this chapter I assessed genetic diversity among and between segregated colonies of Franklin’s gull to understand population structure across their breeding range in the U.S. To determine genetic variation and gene flow among these sub-populations, approximately 330 base pairs of the mitochondrial DNA (mtDNA) D-loop were amplified and sequenced. Samples represented 208 individual Franklin's gulls from 14 separate breeding colonies. I used likelihood and Bayesian algorithms to examine phylogeography and population structure, that revealed high levels of genetic diversity among segregated populations. I found complete lack of population structure for this widely dispersed waterbird, indicating low levels of natal site fidelity. I concluded that Franklin’s gulls be managed as one panmictic population across the U.S. rather than distinct sub-populations.

Introduction

Many migratory species are dispersed over a large geographic area during their breeding and wintering periods, yet exhibit some degree of population structure (Avise &

Hamrick, 1996; Webster et al., 2002). The movement of individuals and their genes influences a number of ecological processes including population persistence and adaptive response to environmental change (Frankham et al., 2002). Causes of restricted gene flow that lead to population structuring can be due to physical or behavioral barriers that act to isolate breeding potential among subpopulations. Habitat loss can segregate subpopulations even further, leaving them vulnerable to reduced genetic diversity (Howes et al., 2009).

Understanding how a species is geographically distributed can help provide information at various scales for management purposes. Managing disjunct populations is important because survival at a larger, regional scale often depends on growth and dispersal characteristics at the local scale (Fahrig & Merriam, 1994). Management concern and decisions will be different for a species exhibiting separate populations (i.e., sub-populations) compared to population panmixia. Management concerns of species exhibiting panmixia include reduced genetic diversity over time, how a species responds to fluctuating environmental conditions, lower adaptive potential, and the opportunity for a significant portion of a population to be impacted by disease (Avise, 2004). Panmixia can offer a greater potential of mate choice and reproduction with the high level of interconnectedness among individuals within a population. These factors directly impact a species at the local level which ultimately impacts the global population.

For migratory species, a high degree of female philopatry to natal sites is necessary for mtDNA to be conserved (Mortiz et al., 1987; Miller-Butterworth et al., 2003; Avise, 2004). Thus, to maintain genetic diversity in the case of high natal philopatry, segregated subpopulations of a migratory species are recognized and sometimes managed as separate units, since intermixing among subpopulations is unlikely to occur.

Management concerns for maintaining genetic diversity for migratory colonial nesting waterbird species is especially important given they have large, dense nesting colonies but depend on a select few breeding localities. Semi-permanent and permanent wetland quality and availability should be a management focus, as these species are more likely to exhibit a higher degree of site fidelity (Prevot-Julliard et al., 1998; Kushlan et al., 2002). Colonial birds that concentrate in large numbers of select wetlands experience increased threats and vulnerability to more individuals compared to species that are non-colonial. For example, the occurrence of predation, encroachment of invasive species, destruction or degradation of a wetland (e.g. sedimentation decreases invertebrate diversity), pollutants, or disturbances (human use too close to nest sites: boats, vehicles, agriculture machinery) have potential negative impacts that result in an exponentially larger effect than would be predicted with solitary nesting sites (Kushlan et al., 2002). These variables emphasize the importance of understanding population structure for informing management practices of colonial nesting migrants.

The value of agricultural land within the PPR has resulted in conversion of more than half of the historic eight million hectares of wetlands (Dahl & Johnson, 1991). Between 2004 and 2009, the total wetland loss was estimated to be 25,210 hectares (a 140% increase of wetland loss over 1998-2004) bringing the nation's total wetland acreage to just over 44.5 million hectares (Dahl, 2011). Remaining wetlands in the PPR continue to face threats, including intensification and expansion of agricultural production, development, and climate change (Johnson et al., 2005).

As a result of wetland loss, most of the existing wetlands that are used by nesting colonies of Franklin's gulls are managed impoundments with associated water control structures, dikes, and ditches. Water levels are managed and monitored to maintain a variety of wetland habitats; primarily in the PPR they are to meet waterfowl production objectives (Fredrickson & Reid, 1988). Management of these impoundments also often include regularly scheduled draw-downs. During this management technique water from wetlands is drained periodically to improve seed production, increase invertebrate abundance, and perform habitat maintenance such as disking, grazing, or burning (Cross & Vohs, 1988). Habitat requirements and more specifically water level preferences, differ among waterbird species (Kaminski et al., 2006). Often times many species of different guilds utilize the same wetland basin. Optimal depths for foraging of shorebird species such as sandpipers (*Calidris* sp.) and yellowlegs (*Tringa* sp.) are less than 8 cm whereas most dabbling duck species range from 8-23 cm. Many species need deeper water for nesting purposes, as is the preference of redheads (*Aythya americana*) at 61 cm of water (Fredrickson, 1991). Thus, managed wetlands often have different water levels depending on the time of year trying to meet the needs of a variety of phenological events but not all species preferences can be met.

All of these factors are important when considering the status of the Franklin's gull. The Franklin’s gull is a migratory species, traveling up to 10,000 km between its breeding areas in North America to wintering areas along the coast of central Peru to northern Chile. It is only one of two species of North American gull to migrate south of the equator. The Franklin's gull arrives on the breeding grounds in mid to late April and remains until August (Soos, 2004; Burger & Gochfeld, 2009). Following the nesting period, in July the species scatters and wanders throughout the breeding range in all directions. By September to early October larger flocks begin to assemble as they prepare for their departure south (Burger & Gochfeld, 2009).

The species breeds colonially on large, deep water marshes (0.3-0.6 m) with an interspersion of emergent vegetation for construction and attachment of floating nests. Mis-timed management of water levels can lead to failure to establish or abandonment of Franklin's gull colonies (Burger & Gochfeld, 2009). In the Northern Prairie and Parkland Region the Franklin's gull is listed as a waterbird species of High Concern, due to inadequate population information and large portions of the continental population using this region (Beyersbergen et al., 2004). Population trends for Franklin’s gull are not well understood, partly because the remote breeding locations make population estimates logistically difficult. Understanding the genetic population structure of the Franklin's gull is important because it will aid in the overall understanding of the structure of colonial breeders and serve to inform management decisions, especially given population concerns, disjunct populations, and threats of further habitat loss.

In this study I used mitochondrial DNA (mtDNA) markers to gain insight into Franklin's gull population structure. MtDNA is a valuable tool for examining population structure. Successful uses include the elucidation of butterfly migration patterns (Salazar et al., 2008), range expansion in fish (Coscia & Mariani, 2011) and bird philopatry (Avise et al., 1992). The mitochondrial region D-loop was chosen based on its rapid rate of known mutation compared to other markers. MtDNA lacks recombination found in nuclear DNA and is uni-parentally inherited (Avise, 2004) making it useful to track recent population relationships.

My objectives were to document the genetic differentiation within and between active Franklin's gull breeding colonies throughout the U.S. by using the mitochondrial D-loop to estimate levels of gene flow. Based on other species, I predicted that Franklin's gull colonies would exhibit population structure across the range sampled. To examine this observation, I determined the level of dispersal and phylogeographic population structure of Franklin's gulls breeding in the U.S. In addition I determined the level of genetic diversity within and between each individual nesting colony to inform more effective management practices. I also predicted to find population structure among sub-populations to the east and west of the Rocky Mountains, due to philopatry and based on previous work with other avian populations (Burg et al., 2003).

Methods

Field Methods and Study Area

During the 2012 breeding season, Franklin's gull feather samples (molted feathers on floating nest platforms) were collected from fourteen nesting colonies across the species' U.S. breeding range (Fig. 1). To collect a representative sample of Franklin's gulls found at each colony, nests were chosen randomly throughout the colony. Once feathers (1-5) were collected from a nest bowl, they were placed in uniquely labeled paper coin envelopes for storage until processing. All feathers were stored at room temperature. Proper permits were obtained and a proper feather collection and possession protocol was followed (Appendix A).

The sampling sites for feather collection included: Thief Lake Wildlife Management Area (WMA), MN; J. Clark Salyer National Wildlife Refuge (NWR), ND; Lake Alice NWR, ND; Beaver Lake Waterfowl Production Area (WPA), ND; Rush Lake WPA, ND; Sand Lake NWR, SD; North Detroit Township, Brown County, SD; Benton Lake NWR, MT; Bowdoin NWR, MT; Knutson's Bay, MT; Red Rock Lakes NWR, MT; Grey's Lake NWR, ID; Oxford Slough WPA, ID; and Bear River Migratory Bird Refuge, UT (Fig. 1, Table 2). The number of nests feathers were collected from was approximately 50 at each colony location. This number was calculated based on the number of viable sequences needed and buffered against laboratory processing errors. The number of nests which were processed to represent individual DNA sequences was determined using similar studies investigating avian population structuring through DNA evidence (Oomen et al., 2011; Reudink et al., 2011; Eo et al., 2010; Barrowclough et al., 2004); in this literature a range of 4-35 individuals per sampling location was used. Sample size by site in this study ranged from 6-20 (Table 2).

Laboratory Methods

The calamus (quill) was clipped off from the rest of the feather shaft for collected feather samples. MtDNA was extracted from the blood in the calamus using a Qiagen DNeasy kit using standard protocols (QIAGEN Inc., Valencia CA, USA). Each extraction set had a blank control that did not contain feather material to test for contamination across samples. Following successful DNA extraction the DNA was suspended in EB buffer and stored in a -70°F freezer. DNA was quantified in the extraction samples using a Nanodrop Spectrophotometer (ND 1000).

A portion of the D-loop of mitochondrial DNA (approximately 330 base pairs) was selected for this study. The mitochondrial region D-loop was chosen based on its rapid rate of known mutation compared to other markers. Further, mtDNA lacks recombination found in nuclear DNA and is uni-parentally inherited (Avise, 2004). Because of these factors, mtDNA is able to track recent population relationships. Two primers were manually designed for this study using existing sequences deposited in NCBI Genbank, accession numbers: FM209692-FM209694 (Sternkopf et al., unpublished) using conserved regions, specifically HVR-1 region of the D-loop; Betty (forward: GGAGGTTTACATTAACCTAT) and Fred (reverse: CTAGCTTCAAGACCATAC).

Polymerase chain reaction (PCR) reactions were performed with the Ex Taq Kit (Takara biotechnology Co., Ltd) using standard procedures in an Eppendorf thermocycler (Eppendorf, Hamburg, Germany). The cycling conditions for the PCR reaction was 1 minute at 94˚C (DNA denaturation), 1 minute at 47˚C (DNA annealing), and 1 minute at 72˚C (DNA elongation). This process was repeated for 29 cycles. One final cycle of 1 minute at 94˚C, one minute at 47˚C, and 10 minutes at 72˚C (final elongation phase) completed this process (Simmons & Scheffer, 2004). Samples were maintained at 4˚C until further processing.

PCR products were visualized using gel electrophoresis on a 2% agarose gel containing 0.1 µg/ml ethidium bromide via UVP Bio-Imaging System (Cambridge, UK) under an AutoChemi ultraviolet transilluminator. Successful amplifications were cleaned using a Qiagen purification kit (Santa Clarita, CA) according to the manufacturer's standard protocols. Purified PCR products were sequenced using a Big Dye Xterminator kit and visualized with ABI 3100 capillary sequencer. Sequences have been deposited in NCBI Genbank (accession numbers: to be submitted).

Data Analysis

Sequence data were verified via NCBI GenBank using a BLAST search to ensure no contamination from other possible sources of DNA. Sequences for individuals were assembled with Sequencer 4.6 (GeneCodes Corp.) and consensus sequences for each sampled individual were manually aligned by eye. Sequences for individual Franklin's gulls were assembled to produce a consensus sequence for an individual bird assuming feathers found in a nest bowl were from the same individual. Consensus sequences for each individual were aligned and manually adjusted when appropriate. Mutations at each position were verified with original chromatograms for each individual. All individual Franklin's gull sequences were complied into a matrix for phylogenetic analyses. Haplotypes (individual DNA sequences) were identified using the statistical parsimony approach in program TCS version 1.21 (Clement et al., 2000).

Haplotype frequency data were analyzed in program ARLEQUIN v. 3.5.1.2 (Excoffier, 2010) to obtain F_{ST} (gene flow) and nucleotide diversity (π) to infer the level of gene flow among and between populations. Arlequin was also used to perform exact tests of population differentiation (Raymond & Rousset, 1995), as well as haplotype diversity to measure the uniqueness of a particular haplotype within each population and analysis of molecular variance (Excoffier et al., 1992).

Two related methods were used to select the appropriate model for phylogenetic analyses. For likelihood analyses, model selection was obtained using Akaike Information Criterion (AIC) as the criterion in Modeltest 3.7 (Posada & Crandall, 1998;

Posada & Buckley, 2004). Model selection was based on Bayesian Information Criterion (BIC) scores and performed using Mr.Modeltest v. 2.2 (Nylander, 2004) for Bayesian searches and executed in program PAUP (Posada & Crandall, 1998). The most appropriate model for both algorithms was GTR+I+G.

Phylogenetic trees were generated using maximum likelihood criteria in GARLI (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006) and MRBAYES (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Maximum likelihood was used to search all possible combinations of tree topology and branch length. Maximum likelihood trees were constructed in GARLI using 1,000,000 generations of trees after likelihood scores became stationary. Resulting nodes of the final tree were assessed by re-sampling the matrix for 1,000 parametric bootstrap replications. Bayesian methods were used to produce a phylogenetic tree assuming posterior probability distribution. Eleven million generations of random trees were generated in MRBAYES, using two searches with four Markov Chain Monte Carlo chains (one heated, three cold). Cold chains were sampled every 100 runs. Analyses were determined when less than 0.01 difference between split frequencies of the two searches were reached. Twenty-five percent of resulting trees were discarded as burnin, as recommended (Huelsenbeck & Ronquist 2001; Ronquist and Huelsenbeck 2003). Posterior probabilities were used to assess the strength of relationships.

Results

Haplotype Diversity and Genetic Structure

Of the 208 individual Franklin's gull mtDNA D-loop sequences sampled, 115 unique haplotypes were present. Thirty-four haplotypes had more than one individual assigned to it (Table 3), whereas the remaining haplotypes were only found in one sample. The amount of unique sequences indicates a high degree of genetic diversity. Each of the 14 sub-populations (colonies) contained a variety of haplotypes ranging from 6-17 (Table 4). Arlequin revealed high haplotype diversity (h = 93.4-100%) across all sub-populations and high nucleotide diversity (0.0-4.2%) measuring the mtDNA sequences of individual birds within each colony (Table 4). Pairwise distances between all individuals revealed high diversity, ranging from 0.0-4.2%. The AMOVA showed low variation (0.06%) among sub-populations, but high genetic variation (99.40% explained) within groups (Table 5). Pairwise F_{ST} values between colonies were very low (Table 5) as was overall F_{ST} (0.00597, $p < 0.05$). F_{ST} values range from 0 (population panmixia) to 1 (two separate populations). This indicates populations sampled are interbreeding freely.

Phylogenetic Analysis

The haplotype network from GARLI revealed few resolved clades (-*ln* likelihood = 1701.10512). Bootstrap support ranged from 54-60% (Fig. 2). Bayesian methods also were unable to resolve haplotype groupings (–*ln* likelihood = 1557.62088). Posterior probabilities ranged from 57-91% (Fig. 3) for supported relationships. Overall, populations did not show genetic structure based on sampling location or any spatial scale thus indicating individuals sampled represent a panmictic population.

Discussion

Contrary to our predictions, we found high levels of genetic variation (115 haplotypes in 208 individuals across 330bp) and no population structure across the U.S. breeding areas for this species (Figs. 2 and 3). These results suggest a lack of natal site fidelity for the population, as a whole. Although Franklin's gull breeding sites are geographically isolated in their breeding range, congregations of large numbers of Franklin’s gulls on the wintering area have been documented (Burger, 1996), likely providing opportunities for population mixing to occur. Given the ephemeral nature of prairie wetlands, the benefits of returning to the same wetland in subsequent years are likely diminished (Covich et al., 1997); therefore, spring migration may occur in flocks of mixed breeding origin, whereby a female of one breeding location may follow those of another to different area, depending on year and previous nest and chick success (Haas, 1998; Catlin et al., 2005; Lecomte et al., 2008; Robinson & Oring, 1997).

Other waterbird species with similar colonial nesting traits display a lack of genetic structure. American white pelicans (*Pelecanus erythrorhynchos*) are long distance migratory waterbirds, nest colonially on inland sites, and are geographically segregated across their breeding range. MtDNA evidence indicates that American white pelicans are a panmictic population; reasons suggested include having low natal site fidelity, high rates of mobility, and lack of reproductive isolating barriers (Oomen et al., 2011, Reudink et al., 2011). An examination of lesser snow goose (*Chen caerulescens*) genetic structure found that individuals of this species, despite having disjunct populations in the breeding range, come together on the wintering grounds and intermix during the mate selection period (Cooke et al., 1975), therefore, gene flow is maintained through males returning to a female's natal site. Despite a recent population decline, Peruvian booby (*Sula variegata*) populations have shown to maintain high levels if genetic diversity and are genetically panmictic (Taylor et al., 2011) through high levels of dispersal and interaction due to ample availability of breeding locations, and opportunities for genetic exchange with many individuals for this colonial seabird.

Movement of just a few individuals between populations can be enough to prevent genetic differentiation among populations (Lacy, 1987; Oyler-Mccance, 2005). A general rule of thumb in conservation biology for long-term persistence of a species is one migrant individual per generation (OMPG rule), for isolated populations. The OMPG rule states one migrant into a subpopulation per generation is sufficient to maintain healthy levels of gene flow between isolated populations (Mills & Allendorf, 1996). Dispersal among breeding populations reintroduces genetic variation to subpopulations, causing within-subpopulation heterozygosities to stabilize after an initial rapid decline in genetic diversity. Genetic variation is required for a population to adapt to changing environments, new predators, diseases, parasites, changing climatic conditions, competitors, and changing food supplies (Lacy, 1987). Other species such as spiny lobster (*Palinurus gilchristi*, Naro et al. 2011; Tolley et al. 2005), bees (Beveridge & Simmons, 2006), and fish (White et al., 2009) reveal panmictic populations despite widely dispersed populations and long migratory paths.

Though mtDNA is informative for examining population genetic structure, additional markers (microsatellites) would prove beneficial for examining mate fidelity and annual pair formation. Further, expansion of sampling Franklin's gulls in the northern extent of their breeding range in Canada would further augment these results in determining if the trend it throughout or unique to the southern extent of the Franklin's gull breeding range. Additional studies regarding adaptive potential to a changing climate, and particularly the effects it may have on reproductive success are needed. More research on both wintering and breeding areas would facilitate a more comprehensive approach to management of this species in the breeding areas. Expanding results to encompass the Canadian breeding range, as well as wintering sites would provide information over the entire range of the species. It would also help to identify timing of pair formation and detect dispersal patterns.

Results of this work will aid in informing future management decisions. Based on the aforementioned results, Franklin's gull management decisions can be made with an understanding that each colony of breeding adults is genetically similar. The populations sampled have a high genetic diversity revealing healthy levels of population interaction during some point in this species' annual cycle. Given the concentrated use of few select wetlands, a mosaic of wetland habitats will be important for maintaining breeding localities and offer the Franklin's gull more options to establish a successful nesting colony. Identifying preference of why sites are chosen for nesting among breeding areas will provide a better understanding for this long distance migrant's habitat needs and ultimately the species' long-term success.

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Table 2. Sampling locations for each colony included in genetic analyses from the breeding range of the Franklin's gull. The codes in this table correspond to locations mapped in Figure 1, N = sample size, coordinates are given in decimal degrees.

Sampling site	State	Lat	Lon	Datum	N	Code
Thief Lake Wildlife Management Area	MN	48.491	-95.888	WGS 84	14	TL
J. Clark Salyer National Wildlife Refuge	ND	48.612	-100.698	WGS 84	18	JCS
Lake Alice National Wildlife Refuge	ND	48.371	-99.102	WGS 84	13	LA
Beaver Lake Waterfowl Production Area	ND	48.868	-102.629	WGS 84	6	BV
Rush Lake Waterfowl Production Area	ND	48.373	-100.224	WGS 84	10	RU
Sand Lake National Wildlife Refuge	SD	45.793	-98.238	WGS 84	10	SL
North Detroit County	SD	45.783	-98.010	WGS 84	19	NDT
Knutson's Bay	MT	48.388	-104.571	WGS 84	11	KB
Bowdoin National Wildlife Refuge	MT	48.386	-107.665	WGS 84	16	BOW
Benton Lake National Wildlife Refuge	MT	47.674	-111.327	WGS 84	14	BL
Red Rock Lakes National Wildlife Refuge	MT	44.642	-111.841	WGS 84	19	RR
Grays Lake National Wildlife Refuge	ID	43.059	-111.446	WGS 84	19	GL
Oxford Slough Waterfowl Production Area	ID	42.253	-112.000	WGS 84	19	OS
Bear River Migratory Bird Refuge	UT	41.486	-112.278	WGS 84	20	BR
Total samples size					208	

Table 3. Frequencies for 34 shared mtDNA haplotypes (115 total haplotypes) for 14 populations of Franklin's gulls sampled throughout breeding colonies found in the United States. Dashes indicated the haplotype was not found in the population.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Thief Lake WMA	-	-	-	-	1	-	-	-	-	1	-	-	1	-
Sand Lake NWR	-	-	-	-	-	1	-	-	-	-	-	2	-	-
North Detroit County	-	-	1	-	2	1	-	-	-	-	-	-	1	1
Rush Lake WPA	-	-	-	-	1	-	-	-	-	-	-	1	-	1
J. Clark Salyer NWR	1	-	-	-	-	-	2	-	-	-	-	1	-	-
Lake Alice NWR	-	-	-	-	-	-	-	-	-	1	-	1	2	-
Beaver Lake WPA	1	1	1	-	-	-	-	-	-	-	-	-	-	1
Knutson's Bay	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Bowdoin NWR	-	3	1	1	-	-	-	-	1	1	1	1	1	-
Benton Lake NWR	-	-	-	1	5	1	1	1	-	-	-	-	-	-
Red Rock Lakes NWR	-	-	-	-	-	-	2	-	-	-	-	1	3	-
Oxford Slough WPA	-	-	-	-	2	-	-	1	1	-	1	-	1	-
Gray's Lake NWR	-	-	1	-	1	-	-	-	-	-	-	-	2	-
Bear River MBR	-	-	-	-	-	-	-	2	-	-	-	3	1	1
Total Haplotypes Freq.	2	4	4	2	12	3	5	4	2	2	2	10	13	4

	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
Thief Lake WMA	1	1	-	-	-	-	-	-	-	-	-	-	-	1
Sand Lake NWR	-	1	-	-	-	-	-	1	-	-	-	-	-	-
North Detroit County	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Rush Lake WPA	-	-	-	-	-	-	-	1	-	-	-	1	-	-
J. Clark Salyer NWR	-	1	-	1	1	1	1	2	1	-	-	-	-	-
Lake Alice NWR	-	-	-	-	1	-	-	-	-	1	1	1	1	-
Beaver Lake WPA	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Knutson's Bay	-	1	-	-	-	-	1	-	1	-	1	-	-	1
Bowdoin NWR	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benton Lake NWR	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red Rock Lakes NWR	-	3	-	-	-	-	-	-	-	1	-	-	1	-
Oxford Slough WPA	-	-	-	-	-	-	-	-	-	-	-	-	1	2
Gray's Lake NWR	1	1	2	-	-	-	-	-	-	-	-	-	-	-
Bear River MBR	-	2	-	1	1	1	-	-	-	-	-	-	1	-
Total Haplotypes Freq.	2	10	2	2	3	2	2	4	2	3	2	2	4	4

Table 3 cont.

	AC	AD	AE	AF	AG	AH
Thief Lake WMA	-	-	-	1	1	-
Sand Lake NWR	-	-	-	-	1	-
North Detroit County	2	1	2	-	-	-
Rush Lake WPA	-	1	-	-	-	-
J. Clark Salyer NWR	-	-	-	-	-	-
Lake Alice NWR	-	-	-	-	-	-
Beaver Lake WPA	-	-	-	-	-	-
Knutson's Bay	-	-	-	-	-	-
Bowdoin NWR	-	-	-	-	-	-
Benton Lake NWR	-	-	-	-	-	-
Red Rock Lakes NWR	1	1	-	1	-	-
Oxford Slough WPA	-	-	-	-	-	-
Gray's Lake NWR	-	-	-	-	-	-
Bear River MBR	-	1	-	-	-	2
Total Haplotype Freq.	3	4	2	2	2	2

Table 4. Franklin's gull sampling locations (mapped in Figure 1), N(I) number of individuals, N(H) number of haplotypes, *h* haplotype diversity, π nucleotide diversity, and SD standard deviation.

Populations	Genetic Polymorphism			
	N(I)	N(H)	$h \pm SD$	$\pi \pm SD$
Thief Lake WMA, MN	14	14	1.0000 +/- 0.0270	0.2092±0.1079
J.Clark Salyer NWR, ND	18	16	0.9869 +/- 0.0229	0.28465±0.1436
Beaver Lake WPA, ND	6	6	1.0000 +/- 0.0962	0.0107± 0.0074
Rush Lake WPA, ND	10	10	1.0000 +/- 0.0388	0.2556±0.1347
Lake Alice NWR, ND	13	12	0.9872 +/- 0.0354	0.1242 ± 0.0649
Sand Lake NWR, SD	10	9	0.9778 +/- 0.0540	0.1592 ±0.0853
North Detroit Township, SD (NDT)	19	16	0.9825 +/- 0.0223	0.2763±0.1390
Knutson's Bay, MT	11	11	1.0000 +/- 0.0388	0.4189±0.2201
Bowdoin NWR, MT	16	14	0.9750 +/- 0.0348	0.3124±0.1590
Benton Lake NWR, MT	14	11	0.9341 +/- 0.0611	0.11747±0.0610
Red Rock Lakes NWR, MT	19	14	0.9591 +/- 0.0307	0.1579±0.0799
Grays Lake NWR, ID	19	17	0.9883 +/- 0.0210	0.1607±0.0814
Oxford Slough WPA, ID	19	17	0.9883 +/- 0.0210	0.3182±0.1599
Bear River MBR, UT	20	15	0.9684 +/- 0.0254	0.2082±0.1046
TOTAL	208	182		

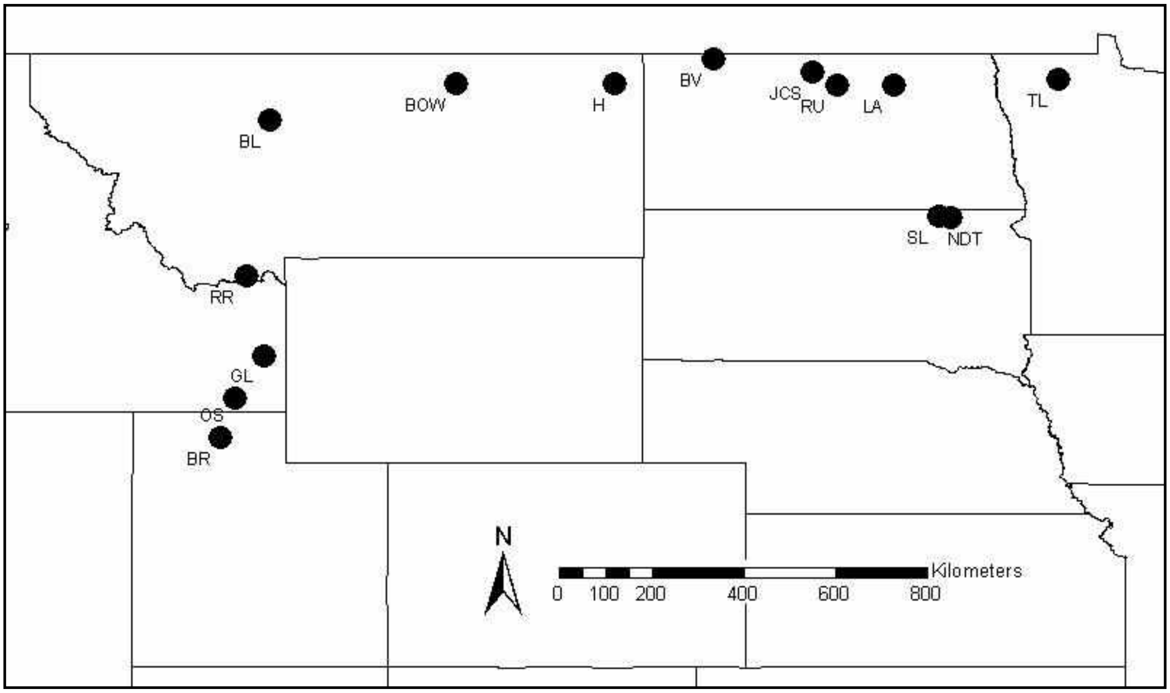

Figure 1. Sampling locations from the breeding range of the Franklin's gull. The letter code corresponds to sampling information (Table 2) for each colony location included in genetic analyses.

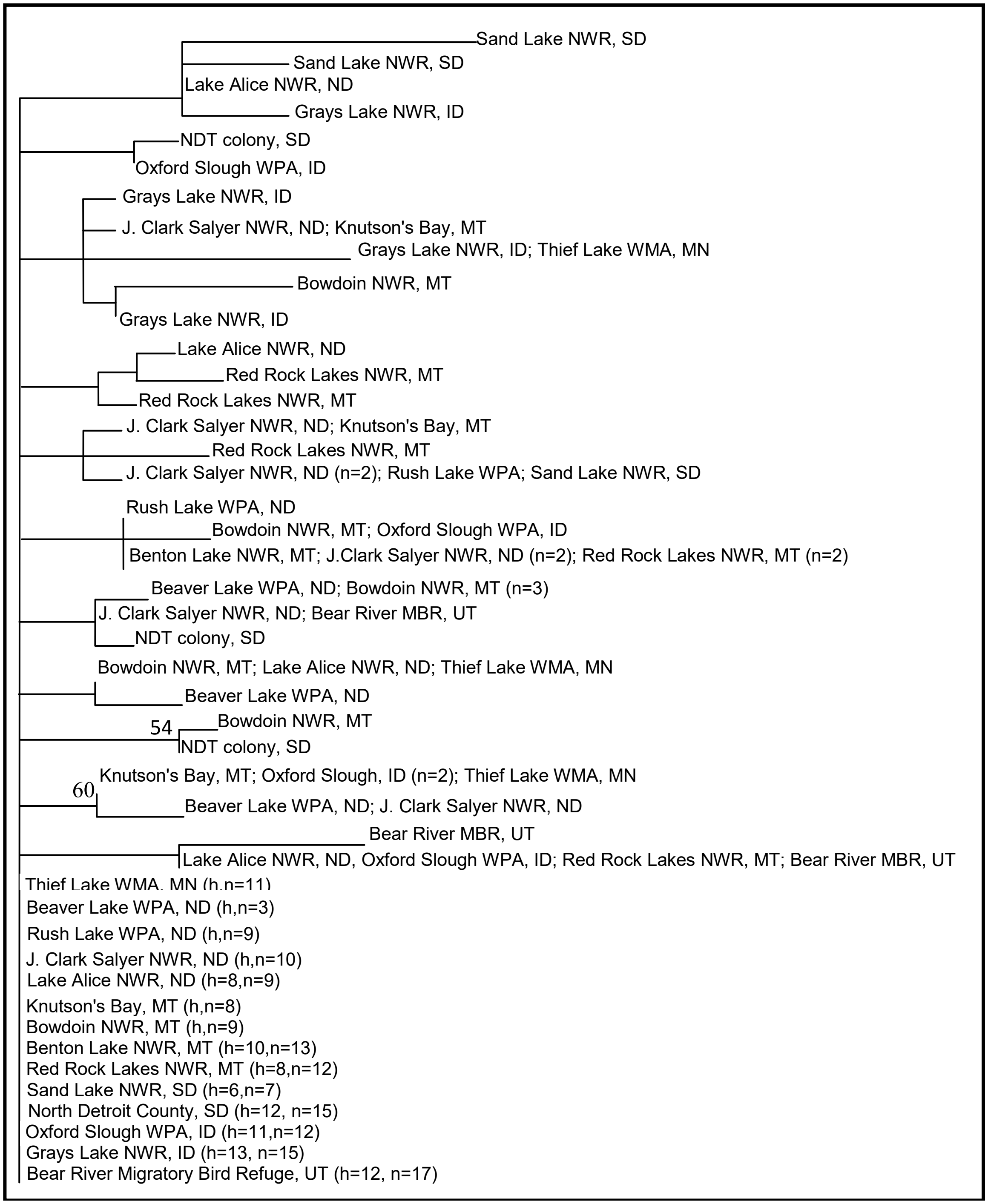

Figure 2. Phylogenetic tree produced using maximum likelihood criteria in GARLI -*ln* likelihood = 1701.10512. Bootstrap support is given at appropriate nodes. Each branch represents one haplotype. Parentheses prior to nodes indicate posterior probabilities. Parentheses in the resolved portion following location represent number of individuals that share the haplotype represented. The remaining 150 Franklin's gulls compose a polytomy of individuals represented among all colonies sampled. Parentheses following colony location indicate *h,* number of haplotypes, *n*, number of individuals.

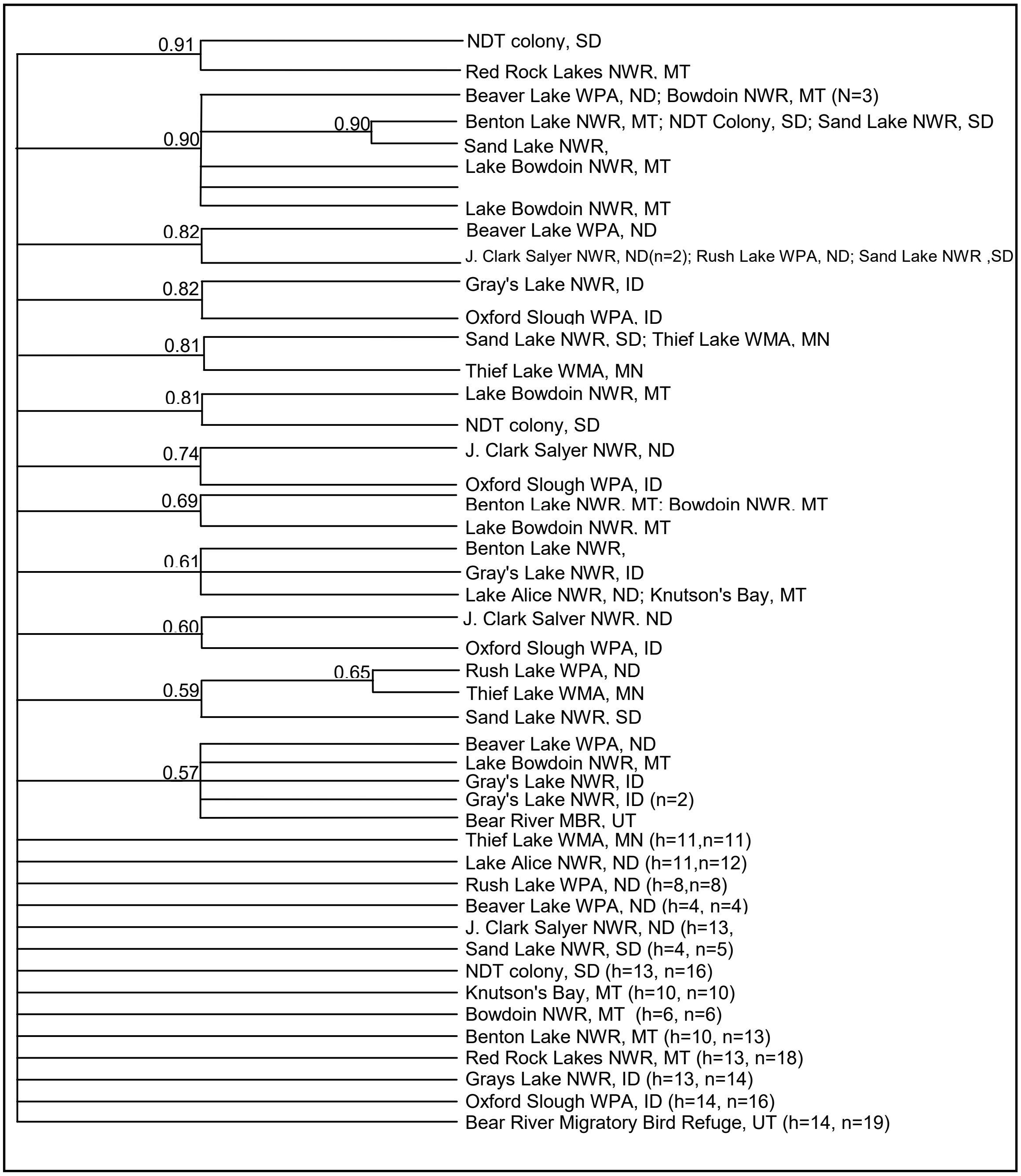

Figure 3. Phylogenetic tree produced using maximum likelihood criteria in program MRBAYES –*ln* likelihood = 1557.62088. Posterior probabilities assigned to nodes assess the strength of the relationships found at branch nodes. Each branch with resolution represents one haplotype. Parentheses in the resolved portion following location represent number of individuals that share the haplotype represented. The remaining Franklin's gulls sampled compose a polytomy of individuals represented among all colonies sampled. Parentheses following colony location indicate *h,* number of haplotypes *n*, number of individuals.

Table 5. Results of Analysis of molecular variance (AMOVA) based on Franklin's gull mitochondrial D-loop control region.

Source of Variation	*df*	Variation (%)	FST
Among populations	13	0.60	
Within populations	195	99.40	
TOTAL	208	100	0.00597

CHAPTER III

ASSESSMENT OF HABITAT ASSOCIATIONS OF FRANKLIN'S GULLS IN THE PRAIRIE POTHOLE REGION USING GIS AND REMOTE SENSING TECHNOLOGIES

Abstract

The success of any avian management strategy relies on a firm understanding of factors affecting habitat use. This is of even greater importance when key habitats are limited across the landscape and even more central when substantial numbers of a given species are reliant on only a few sites. Franklin's gulls are one of many colonial-nesting waterbird species designated as a species of conservation concern (e.g. American white pelican, Forster's Tern, Elegant Tern, California Gull; Kushlan et al., 2002), having a large proportion of North America's population concentrated in a select few sites. To assess habitat use of Franklin's gulls within their breeding grounds, I used geographical information systems (GIS) and remote sensing technologies to compare habitat characteristics between wetlands used and unused by Franklin's gulls. Specifically, I created a GIS database and produced digital maps in ESRI ArcGIS 9.3 based on georeferenced Airborne Environmental Research Observation Camera (AEROCam) imagery taken to evaluate land cover at colonial nest sites. Paired t-test results indicate the percent of emergent vegetation land cover was significantly different between sites, being higher in all wetland basins compared to the control wetlands, suggesting the

persistence of Franklin's gulls on wetlands in the PPR depends on a minimum percentage of wetland basins need to be emergent vegetation stands.

Introduction

Wetland degradation and loss impacts many biological organisms (Rahel 2002); most notably amphibians (Cushman, 2006), wetland birds (Johnson, 2001), and aquatic vegetation (Lougheed et al. 2008), which supports many taxa during some point in their life cycle (Mitsch & Gosselink, 1986; Bryan & Scarnecchia, 1992). For example, the prairie pothole region (PPR) possess rich, fertile soils dotted with many wetlands creating both a biologically productive area that provides optimal breeding habitat for wildlife, and simultaneously, a valuable commodity for high-production agriculture (Sugden & Beyersbergen, 1984; Dahl, 1990) which generates a conflict between conservation and economic development.

As a result of economic development, only a small portion of the original PPR wetland base remains due to deliberate drainage (surface and tile) designed to enhance agricultural production (Dahl, 2000). At the time of early settlement (1600s) it is estimated that 90 million hectares of wetlands existed in the U.S. By the mid 1980s less than half (42 million ha) remained in the lower 48 states (Dahl & Johnson, 1991), with more than 90% lost within the Midwestern states (Dahl, 1990). Such losses leave the remaining wetland habitats in great need of protection. Despite federal legislation and policies to protect and restore wetlands (e.g. The Clean Water Act, 1972; Food Security “Swampbuster” Act, 1985; No Net Wetland Loss policy, 1989), wetlands are still being lost at alarming rates (Dahl, 2000). For example between 2004 and 2009 over 25,200 hectares (ha) were lost (Dahl, 2011).

Wetland loss and degradation results in isolated habitat patches, that can have significant impacts on wildlife at the population level (Funk et al., 2010). Management of species possessing small, isolated populations requires identification of essential habitat requirements for continued success. The Franklin’s gull is a colonial waterbird species that breeds in large marshes of North America. Because of the ephemeral nature of prairie marshes and their vulnerability to drought and drainage, breeding colonies often shift localities from year to year; in some years local populations are suspected to forego annual breeding opportunities altogether (Burger & Gochfeld, 2009) in response to changing water levels and vegetation structure. Breeding populations have become reduced and segregated over time, likely due to habitat loss and increased disturbance at the breeding sites (Burger & Gochfeld, 1994).

During the Dust Bowl era (1930s) most of the Franklin’s gull historic breeding sites were lost due to large-scale drainage projects (Burger and Gochfeld 1994). More recently, the creation of large wetlands, mainly on state and federally protected wildlife refuges, has allowed population expansion. The Franklin's gull breeds almost exclusively on these types of wetlands within the U.S. Even with recent population expansion, there exist fewer than 20 breeding colony locations in the U.S. in a given year, with most of the population concentrated within a portion of the available locations (Burger & Gochfeld, 1994). For example, since the 1900s, the largest Franklin’s gull breeding colonies (more than 100,000 breeding pairs) have been located on marshes in the PPR, primarily in North Dakota and Minnesota (Burger & Gochfeld, 1994). Because of their threatened habitats, disjunct populations, and few, but dense nesting colonies, the Franklin's gull is designated a species of high conservation concern in Minnesota, North Dakota, South Dakota, Montana, and Idaho (Table 1).

The Franklin’s gull is a long-distance migrant that winters primarily along the western portion of coastal South America with a small isolated inland population in Peru (Burger & Gochfeld, 1994). The combination of decreasing wetland habitats in the contiguous U.S. and few breeding locations makes protection of wetlands critical to the species success. Given that the northern U.S. is at the southern extent of the Franklin's gull breeding range, it is important that habitat sites are maintained. If the trend of wetland loss continues, the Franklin's gull may be forced to breed at higher latitudes, exclusively in Canada, increasing the migration distance and associated survival stressors. Understanding their habitat needs and preferences is essential to maintain quality nesting areas within the U.S.

Previous habitat use studies indicate that the structure and cover of local vegetation may be more important than the plant species composition in selection of desirable sites for breeding wetland-dependant bird species (VanRees-Siewert & Dinsmore, 1996). The Franklin's gull uses aquatic vegetation to build nest structures and as protection from predators (Burger, 1974). The documentation of the amount of emergent vegetation stands, marsh habitat, and other cover types influencing wetland site selection is useful to inform management decision. These factors have not been examined for the Franklin’s gull.

I used site-specific geospatial data in the form of aerial imagery taken at the time of nesting to assess the habitat use within Franklin's gull nesting colonies. The objective of this study is to evaluate land cover at established nesting colonies, as well as comparable nearby wetland sites not used by Franklin's gulls using a GIS framework. Two study sites were located in North Dakota, one in South Dakota, and one in Minnesota. These sites were compared to sites with similar wetland characteristics, which were not used as nesting colonies by Franklin's gulls to evaluate habitat selection. Results of this research will provide a better assessment of Franklin's gull occupancy at nesting locations where they are successful. I predicted the percent cover of emergent vegetation and wetland basin size would be the two factors most important to Franklin's gulls for nest site selection. Reasons for my predictions include their site choice is most likely based on needs for nesting, which emergent vegetation is used as building material, stabilization, and cover from predators; a wetland must provide adequate cover and protection which is likely within a preferred range for the Franklin's gull and I predicted larger sites to be occupied with colonies.

Methods

Wetland Habitat Selection

Franklin's gull habitat use was investigated by analyzing wetlands with established nesting colonies ('used' sites) then pairing each wetland basin with a control site with no nesting Franklin's gulls (control sites). I assessed used sites to determine wetland basin size (ha) and class (Table 6, Stewart & Kantrud, 1971). I then used the U.S. Fish and Wildlife Service (USFWS) National Wetlands Inventory (NWI) database to determine control sites. I queried the NWI wetland database to find the basin nearest to each used site, which was of similar size and class (regime). I set the smallest "used" wetland size as the minimum and the largest "used" as a maximum (size in acres). The paired wetland that 1) fell between the smallest and largest wetland used by Franklin's gulls and 2) within the wetland basin size and class restrictions was selected. A GIS with all of these selected wetland basins were sent to the Upper Midwest Aerospace Consortium (UMAC) at the University of North Dakota (UND) in the form of shapefiles for requested image collection. Aerial imagery was taken of four used wetlands (Figure 4) and paired control sites between June and August, 2010.

Study Area

Thief Lake Wildlife Management Area contains 22,240 hectares in Marshall County in northwestern Minnesota and is managed by the Minnesota Department of Natural Resources (MNDNR). Thief Lake itself is Class V (permanent wetland, Stewart & Kantrud, 1971) impoundment of approximately 2,870 hectares, and the surrounding area of the WMA comprises a variety of habitat types (e.g. emergent wetland, conifer and deciduous trees, grasses and hay land, and crop land). The control site for Thief Lake WMA is Nelson Slough found within East Park WMA located approximately 29 kilometers west of Thief Lake WMA. This WMA is approximately 4,220 hectares; it too is an impounded wetland, Class V, managed by the MNDNR. The land cover at this site consists of a mosaic of habitat types similar to the Thief Lake WMA site but with more woody vegetation including conifer and deciduous trees.

J. Clark Salyer National Wildlife Refuge (NWR) is 23,755 hectares and located along the Souris River in north central North Dakota. It is managed by the USFWS primarily for migratory birds species as a breeding or stop-over site for more than 300 species, which contributed to its designation as a Globally Important Bird Area (GIBA) by the U.S. IBA Technical Committee. The Refuge encompasses a high diversity of habitat types from prairie and riverine to forest systems. Common management of these diverse ecosystems includes prescribed fire and intensive water-level management. In 2010, the wetland impoundment (Class V) at approximately 610 hectares, with nesting Franklin's gulls was located in McHenry and Bottineau counties between Dam # 326 to the north and Dam #320 to the south. This nesting site was compared with Round Lake, a Class V lake at approximately 1,010 hectares, which was located approximately 40 kilometers southeast of the J. Clark Salyer wetland in Pierce County.

Lake Alice NWR is a 4,650-hectare habitat complex, comprised mainly of wetland, marsh, and grassland habitats. It is located in Ramsey and Towner counties, North Dakota. Continuous flooding from the nearby Devils Lake basin challenges optimal water level management for wetland-dependant wildlife at this Refuge. Despite periodic flooding, Lake Alice NWR regularly hosts one of the world's largest Franklin's gull breeding colonies in the U.S. (Burger & Gochfeld, 2009). During the nesting season of 2010, the colony breeding on this Class V wetland utilized much of the northern portion of the Refuge. Cranberry Lake was used as a comparison site for Lake Alice NWR. It too is a Class V lake, 930 hectares in size, and is located approximately 48 kilometers southwest of the Lake Alice pool in Benson County.

Sand Lake NWR is 8,620 hectares of diverse wildlife habitat, located in Brown County in northeastern South Dakota. The James River runs through this area allowing refuge management to manipulate water levels, optimizing wetland objectives for fish, wildlife, and recreation. The Refuge impoundment that is used by nesting Franklin's gulls is, Mud Lake, a Class IV-V wetland, approximately 2,145 hectares in size. Sand Lake NWR is listed as a GIBA and is also designated as a Wetland of International Importance by the Ramsar Convention (Convention on Wetlands of International Importance 1971).

In 1994, Sand Lake NWR hosted the world's largest breeding colony of Franklin's gulls with 150,000 breeding nesting pairs (USFWS 2005). Sand Lake NWR was paired with Lake St. John, a 565 hectare, Class V wetland located approximately 145 kilometers southeast in Hamline County.

Imagery Acquisition and Processing

UND developed an airborne multispectral digital imaging system in 2001 called Airborne Environmental Research Observational Camera (AEROCam). AEROCam was developed through a unique partnership with several UND departments, including the UMAC, the School of Engineering & Mines, and the flight operations at the John D. Odegard School of Aerospace Sciences. UMAC is led by UND, and covers the states of North Dakota, South Dakota, Montana, Wyoming, and Idaho with partners from academia, industry, and the government to provide services for farmers, ranchers, educators, researchers, and natural resource managers. Capabilities of AEROCam include providing imagery taken within a desired time frame in visible and near-infrared bands at a higher resolution than can be offered by alternative imagery sources in a short period of time and at no cost to selected users.

The imaging system includes a Redlake MS4100 area-scan multi-spectral digital camera that features a 1920 x 1080 CCD array (7.4-micron pixels), with 8-bit quantization. Images were delivered in TIFF format along with tabular flight, camera, and GIS data with the approximate GPS center of each image. Image processing was performed in Leica Photogrammetry Suite and ERDAS IMAGINE 2010 (Norcross, GA). All images were registered to Universal Transverse Mercator (UTM) projections, North American Datum1983 (NAD 83), with corresponding zones 12-15 (north). Digital

Elevation Models (DEM) were used at a 30-m resolution provided by NASA's Shuttle Radar Topography Mission (SRTM), downloaded from the U.S. Department of Agriculture and Natural Resource Conservation Service (NRCS) website Datagateway (http://datagateway.nrcs .usda.gov/). DEMs provided vertical information required for triangulating ground control points (GCPs) during referencing. The most recent National Agriculture Imagery Program (NAIP) orthophotos available (2009 or 2010) were used to provide the horizontal referencing information needed in triangulation. NAIP images were also acquired from the USGS Datagateway.

Sites were flown in lines from north to south and south to north to capture the full extent of each study area including some overlap for ease in the georeferencing process. It typically took three to five flight lines per wetland site to capture enough aerial images to cover the site. Each of these flight lines had a range of six to 40 images. During image processing, clearly identifiable GCPs (e.g. road and dike intersections, trees, permanent structures) with associated geographic coordinates were selected in each image. A minimum of 6-8 control points per corner per image were used. Once each flight line had all GCPs, triangulation was performed before moving on to the subsequent flight line images.

To determine referencing accuracy, a root mean squares error (RMSE) report was computed to measure the differences between our predicted value of 1-m resolution and the values of the control points within the flight line. Maximum RMSE of rectification was calculated for each site. Calculated RMSE values were used to characterize the strength of a registration and measure spatial accuracy. Lower RMSE values suggest greater spatial accuracy; the predicted data are closer to the observed data

(Grapentine, 2010). A 2.0 RMSE was chosen given the difficulty of referencing wetlands and the challenges of finding quality and quantity GCPs and was acceptable for obtaining the level of detail needed for assessing land cover. All flight line images had an accuracy measure of < 2.0 RMSE from the triangulated GCP's before being accepted as accurate and processing as an orthorectified image. Orthophotos were then mosaiced into a single TIFF image file. Georeferenced AEROcam images were uploaded and archived on the Digital Northern Great Plains (DNGP) geospatial data archive (www.dngp.umac.org).

Photo Interpretation

I followed land use classification categories as guidelines to determine classifications, descriptions of wetland basins, and land directly adjacent to the basin using the USGS National Land Cover Database (Homer et al., 2004, Table 7). This classification was a newer version using the U.S. Department of Agriculture (Anderson et al., 1976) classification system. The data were collected at a low altitude (< 3,100 m), which allowed determination of land-cover categories. Shapefiles were created in ArcGIS 9.3 for each land cover category (layer) and digitized at appropriate scales to delineate boundaries of habitats. Habitat boundaries were digitized based on specific color and texture patterns in the orthorectified mosaic. Cover class color schemes and coding followed the USGS Level I land use color code (Anderson et al. 1976) to represent land cover corresponding to habitat types. Examples include: water is dark blue, wetland is light blue, barren land is gray, agricultural land is brown, and rangeland is light orange. Colony sites were delineated by the natural wetland basin perimeter or by the impoundment of water in which the colony was located. For example, if a wetland was managed separately by intentionally controlling the water levels in a designated area, the boundary of the colony site would be delineated by the structures (dams, road, etc.). In natural, un-impounded wetland basins, the natural perimeter of the wetland basin was determined as the colony site. Land cover classification was restricted to the area directly surrounding the colony sites and control sites (30 m buffer) to determine land use, specifically presence of crop and roads as this may introduce disturbance caused by humans, vehicles, or machinery noise. Also, during the breeding season Franklin's gull activities remain within 30m of the colony (Beyersbergen, 2004).

Land Cover Analysis

To analyze the resulting database, GIS techniques assessed the heterogeneity of land-classification parameters (Jain et al., 2010). ArcGIS 9.3 (ESRI, 2009) was used to create and analyze land-cover layers. Referenced images of wetland basins were classified into various land-cover categories and used to quantify and compare the following variables: 1) cover type within the colony and in surrounding wetland habitats, 2) percent emergent vegetation, 3) basin size (ha), 4) edge to water ratio, and 5) presence of roads around the wetland perimeter. The cover type within the wetland sites was investigated to determine if there was a preference between vegetative characteristics at sites with colonies compared to wetlands with similar features. Emergent vegetation is known to be an important component to the nesting phase in Franklin's gull breeding sites as they are dependent on vegetation for nest building. Investigating the difference between sites would provide valuable information on the quantity preferred at nesting locations. Determining if there is a size criterion among wetland basins is important information since most of the nesting colonies are on managed wetland impoundments. If wetland basin size is an important variable used in site selection for nesting Franklin's gulls it is possible to manage water levels during the breeding season to provide desired habitat at that specific time. Many species are sensitive to edge effects, as more edge increases the chance of predation, disturbance, and lesser quality habitat (Yahner, 1988). Investigating the relationship among edge to water ratios of wetland basins will provide information on sensitivity levels of edge to colonies. Disturbance is another variable analyzed at these sites in the form of roads to determine if the presence or the quantity is affecting colony site locations. Examination of these five variables will provide an understanding of what habitat requirements are needed by Franklin's gull to select a site for breeding colonies.

After digitizing was complete, each of the land-cover categories (layers) was calculated (Table 8) and converted from area (ha) to percent cover of the wetland basin (Table 9) for comparison. Characteristics of the wetland sites were examined using the mean and standard deviations along with paired t-tests to determine if there was a difference between land-cover types for breeding sites versus control areas. For each land cover category the four control sites were averaged as were the four used sites to obtain mean and standard deviation calculations to objectively determine the difference among land-cover categories between sites with and without Franklin's gull colonies. A paired t-test was performed among the landscape characteristics to determine significant differences ($\alpha = 0.5$) between wetlands with nesting Franklin's gull colonies and without nesting colonies. Small sample size can influence the power of the *t*-test therefore alpha values up to 0.15 were considered and may indicate a trend for the data described in this study (if $p \leq 0.15$).

Results

Detecting a significant difference from mean and standard deviation results was hard to interpret, however they indicate there may be a difference among some land-cover variables at wetland sites with and without Franklin's gull nesting colonies (Table 10). Paired *t*-test results revealed a significant difference between the amount of emergent vegetation at wetland nesting sites (p=0.03) compared to sites that did not have nesting colonies of Franklin's gulls (Table 11). Relaxing the alpha value for small sample size to 0.15 detects a significant difference for the amount of wetland edge ("perimeter", p= 0.14) and the amount of marsh habitat between wetland sites (p = 0.12, Table 11). Among wetlands with Franklin's gull colonies (J. Clark Salyer NWR, Sand Lake NWR, Thief Lake WMA, and Lake Alice NWR), all had emergent vegetation dispersed throughout the wetland basin, not exclusively at the perimeter as seen in their paired control wetland basins (Appendix B). Breeding sites also had a dominance of certain land-cover types within all the wetland basins; all had a combination of open water, deep marsh emergent vegetation stands (cattail/bulrush), and/or marsh habitat that comprised almost the total percent land cover (> 94%). Although present, non-aquatic vegetation species (woody, herbaceous, and crop) as well as exposed ground contributed little to the percent cover (<3% average across sites).

Discussion

I predicted that the amount of emergent vegetation and wetland basin size would be key variables which influenced the occupancy of wetland basins by nesting Franklin's gulls. The results from this study found the amount of emergent vegetation was significantly different compared to the paired (control) wetland basins; however wetland basin size along with all other variables measured did not show a significant difference between sites. Because of the complexity of these wetland systems and specific nesting needs of Franklin's gulls, it seems that multiple variables may be contributing to wetland site selection for nesting (implications from this study, Burger and Gochfeld, 1994, Burger 1974). An alternative approach may be to quantify not only wetland cover classes, but also surrounding cover across the landscape to determine presence of alternative nesting sites. Examination of wetland complexes, rather than individual wetland sites may be more appropriate for a species like the Franklin's gull; in this species, wetland use for rearing young is not based on site fidelity but rather an opportunistic event based on characteristics of ephemeral habitats. Habitat heterogeneity for wetland dependant bird species has been recognized as the basic component to increasing waterbird species diversity (Fairbairn & Dinsmore, 2001). Though not significant, data indicated that a higher portion of nesting colonies occurred on sites with large amounts of open water and emergent vegetation, or at sites where open water and marsh habitat dominated the cover type. These combined factors may indicate that the presence of hemi-marsh (Weller & Spatcher, 1965) may be the key factor in Franklin's Gull habitat use. Other possible colony site selection factors (e.g., degree of wetland isolation) are likely tied to a larger geographic scale. Understanding local factors, as was the case in this investigation, is important for managing and conserving individual wetlands, but larger-scale perspectives are critical for understanding and managing populations in fragmented landscapes.

This project had several inherent limitations that had a notable effect on the significance (or lack thereof) of these results. Franklin's gull nesting sites in the U.S. rarely exceed 20 locations (Burger & Gochfeld, 1994); therefore, sample size was limited for the habitat analysis portion of this study. Sample size was further limited based on limited resources (e.g. imagery taken by AeroCam was not received georeferenced, as a result additional sites were unable to be manually referenced for difficulty of obtaining adequate GCPs in water images; funds and time to support manually referencing images were insufficient). Lack of identifiable ground features and abundant open water made georeferencing of some sites difficult (Grapentine & Kowalski, 2010). In addition, spring flooding occurred throughout the PPR during 2010 further reduced the number of nesting colonies ($n = 7$ pairs of breeding colonies and associated controls). Another factor that decreased our sample size was changes in colony nest locations of basins which were pre-determined for aerial imagery acquisition, which reduced the final sample size to four sites and their paired control wetlands.

The information provided by the AEROCam aerial images captured at colonial nest sites for the Franklin's gull provided a valuable assessment of habitat features present during colonial nest site selection for the Franklin's gull compared to other sources of aerial imagery which are not captured at the time of nesting for this species. Additionally, the resolution of the imagery acquired was 1-m, which allowed a much more detailed and accurate assessment of the land cover, quantifying the variables in this study with more precision and accuracy compared to other sources of remotely sensed aerial imagery at no cost. Despite the limitations imposed by small sample sizes, trends in results provided valuable information for wetland managers.

In conclusion, I recommend that the above approaches to investigating habitat use of Franklin's gulls be further developed. Future work should be adapted to include measuring interspersion (water-vegetation) metrics, and should also consider issues encountered during wetland geo-referencing such as shifts in nesting locations and ability to reference landscapes without identifiable ground control points (water). To ensure that quality habitat is provided for breeding, a myriad of habitat features are necessary: emergent vegetation representing a hemi-marsh condition (Weller & Spatcher, 1965), appropriate water levels, and more importantly, is the proper timing for the occurrence of these ephemeral features. Franklin's gull colonies have been known to have multiple species intermixed during nesting such as White-faced Ibis (*Plegadis chihi*), Pied-billed Grebe (*Podilymbus podiceps*), American Coot (*Fulica americana*), and Redhead (*Aythya americana*) (Burger, 1974). Thus, the management for breeding colonies of Franklin's gulls will provide suitable habitat for these other over-water nesting, wetland-dependant birds. Further, the selection of wetland sites changes between years based on fluctuating water levels and vegetative cover (Burger, 1974). Habitat features should be managed to meet these needs at a landscape level (multiple basins, wetland complexes) at the time of breeding pair arrival on the site. A comprehensive ecosystem approach will protect vital habitat for Franklin's gulls and other wetland species, maintaining the heterogeneity of these biological systems.

Remote sensing and the resulting images are important tools to provide information for decisions in wetland management by elucidating the role of individual biotic and abiotic factors. This study aimed to investigate habitat parameters found at nesting locations during the nest initiation stage through quantifying real-time land-cover data. Though the scope of this study is limited by the small sample size of nesting (used) and corresponding number of control sites, the results provide trends that can be used to better manage wetlands for use as Franklin's gull nesting sites.

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Table 6. Description of wetland classifications measured to evaluate Franklin's gull use of wetland basins in the PPR of the U.S. in 2010; adopted from Stewart & Kantrud, 1971.

Wetland Class	Wetland Name	Description
I	ephemeral ponds	The wetland-low-prairie zone dominates the deepest part of the pond basin.
II	temporary ponds	The wet-meadow zone dominates the deepest part of the wetland area. A peripheral low-prairie zone is usually present.
III	seasonal ponds and lakes	The shallow-marsh zone dominates the deepest part of the wetland area. Peripheral wet-meadow and low-prairie zones are usually present.
IV	semi permanent ponds and lakes	The deep-marsh zone dominates the deepest part of the wetland area. Shallow-marsh, wet-meadow, and low-prairie zones are usually present, and isolated marginal pockets of fen zones occasionally occur.
V	permanent ponds and lakes	The permanent-open-water zone dominates the deepest part of the wetland area. Peripheral deep-marsh, shallow-marsh, wet-meadow, and low-prairie zones are often present, and isolated marginal pockets of fen zone occasionally occur.
VI	alkali ponds and lakes	The intermittent-alkali zone dominates the deepest part of the wetland area. Peripheral shallow-marsh, wet-meadow, and low-prairie zones are usually present. A deep-marsh zone is normally absent except occasionally for isolated patches near marginal seepage areas. A few isolated pockets of fen zone are normally present along the margins.
VII	fen (alkaline bog) ponds	The fen zone dominates the deepest part of the wetland area. Peripheral wet-meadow and low-prairie zones are often present.

Table 7. Description of land cover classifications measured to evaluate Franklin's gull use of wetland basins in the PPR of the U.S. in 2010 (Homer et al., 2004).

Land Cover Class	Description
Open water	Wetland area dominated by open water > 75%
Emergent herbaceous wetland	Perennial herbaceous vegetation is >80% of vegetative cover and soil or substrate is periodically saturated or covered with water; usually found in dense stands
Marsh - aquatic bed	Intermittent area of wetland between open water and emergent vegetation stands; dominated by plants that grow and form a continuous cover on at the surface water, water <25% cover
Upland - herbaceous vegetation	Areas dominated by graminoid or herbaceous vegetation
Woody	Areas dominated by woody vegetation
Crop	Areas used for production of annual crop; all land being actively tilled
Bare land & soil	Areas with little to no vegetation, exposed land is typically bedrock, soil, accumulations of earthen soils
Roads	Roads including paved, unpaved, and two-track trails
Boundary	Perimeter of wetland basin

Table 8. Land cover (ha) by site of four wetland basins with Franklin's Gull nesting colonies paired with four non-use wetlands.

Site	Basin	Perimeter (m)	Open water	Emergent Stands	Marsh	Woody	Bare ground	Upland	Crop	Roads (m)
Thief Lake	2820.0	22147.0	0.0	251.0	2569.0	0.0	0.0	0.0	0.0	0.0
Thief Lake Control	877.7	15817.0	179.0	42.3	649.0	7.3	0.0	0.0	0.0	4034.0
Mud Lake	1603.0	19657.0	921.4	266.8	415.2	0.0	0.0	0.0	0.0	3549.7
Mud Lake Control	568.0	10293.0	534.5	1.7	0.0	17.7	0.0	4.2	8.5	2963.0
Lake Alice	5010.0	46342.0	4510.0	252.2	30.7	14.0	37.8	26.2	140.4	0.0
Lake Alice Control	920.5	15635.0	869.5	0.0	11.4	11.8	0.0	27.6	0.0	0.0
J.Clark Salyer	1839.0	17423.0	359.6	44.1	1350.0	6.9	0.0	67.4	6.3	2985.0
J.Clark Salyer Control	1033.0	14063.0	1023.7	2.2	0.8	1.5	0.0	1.2	3.5	291.0

Table 9. Description of habitat cover of wetland basins in the PPR of the U.S measured to evaluate Franklin's gull use in 2010.

Site	OPEN WATER%	EMERG%	MARSH%	WOODY%	BARE GROUND %	UPLAND %	CROP %
Thief Lake	0	9	91	0	0	0	0
Thief Lake Control	20	5	74	1	0	0	0
Mud Lake	57	17	26	0	0	0	0
Mud Lake Control	94	0	0	3	0	1	1
Lake Alice	90	5	1	0	1	1	3
Lake Alice Control	94	0	1	1	0	3	0
J.Clark Salyer	20	2	73	0	0	4	0
J.Clark Salyer Control	99	0	0	0	0	0	0

Table 10. Characteristics of wetland study sites: mean percentages (± standard deviation), mean road length, mean cover to water and edge to water ratios from wetland basins with (used) and without (unused) Franklin's gull nesting colonies across the PPR of the U.S. in 2010.

	Used	Unused
N	4	4
Size (ha)	2818.0 ± 1553.4	850.0 ± 199.0
% Open water	42.0 ± 40.0	77.0 ± 37.8
% Emergent vegetation stands	8.2 ± 6.2	1.3 ± 2.3
% Marsh/aquatic bed	47.8 ± 41.8	18.8 ± 36.7
% Woody vegetation	0.0 ± 0.0	1.3 ± 1.3
% Bare ground/soil	0.2 ± 0.4	0.0 ± 0.0
% Upland	1.0 ± 1.7	1.8 ± 1.3
% Crop	0.8 ± 1.3	0.5 ± 0.7
Roads (m)	1634.0 ± 1900.0	1822.0 ± 1988.0
Mean cover: water ratio	1295 : 1448	177 : 652
Mean edge: water ratio	26392 : 1448	13952 : 652

Table 11. Paired t-tests results for land cover characteristics comparing wetland sites with and without nesting Franklin's gull colonies during the 2010 breeding season across the PPR.

Variable	df	t	p-value
Perimeter (edge of wetland basin)	3	2.00	0.14
Open water		0.82	0.47
Marsh		2.14	0.12
Emergent vegetation		3.73	0.03
Woody vegetation		-0.84	0.46
Bare ground		1.00	0.39
Upland/herbaceous vegetation		0.89	0.44
Crop		0.94	0.41
Roads		-0.13	0.90

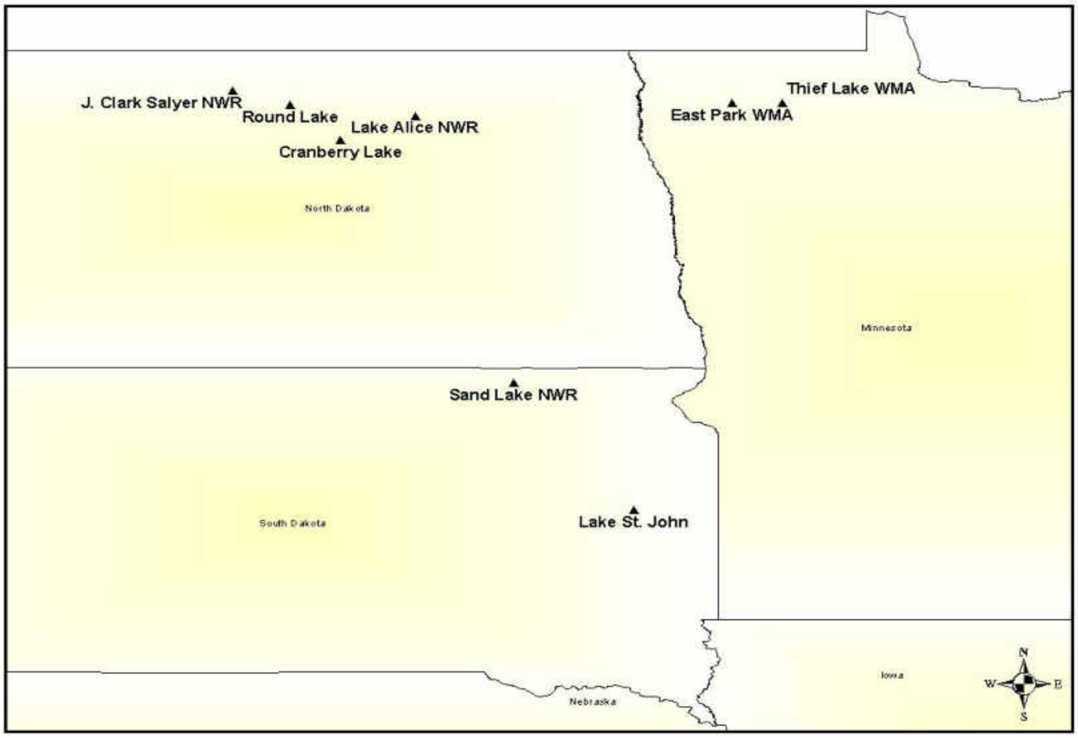


Figure 4**.** Wetland location sites with established Franklin's gull colonies (*n*=4) paired with control sites (wetlands, *n*=4).

CHAPTER IV

CONCLUSIONS

The Franklin's gull is one of many colonial-nesting waterbird species identified as a high priority species for conservation throughout their range. Nesting colonies are located at only a handful of locations throughout the U.S.; these are geographically separated from one another. Conceptions prior to this study were isolated nesting locations may prevent gene flow and limit genetic diversity (Avise, 2004). Isolation of these populations is also a symptom of a second key issue: ongoing habitat fragmentation. It is necessary to provide adequate habitat to maintain genetic diversity for any species of concern. Identifying and quantifying desirable habitat traits for a species is critical to sound management. A combination of both genetic and GIS approaches provided unique insights into the ecology of Franklin’s gulls.

This research was the first attempt to assess relationships of Franklin’s gull colonies breeding across the U.S. through DNA analysis and additionally quantified habitat use at sample colonies. Results of this investigation indicate that Franklin's gull nesting colonies in the Midwestern portion of the U.S., although geographically segregated, are panmictic. In addition my research shows high levels of genetic diversity among individuals in all colonies sampled. Given the levels of genetic diversity documented in this study there is less concern in the event of colony abandonment or collapse; if suitable habitat is made available in subsequent years shortly following the event of non-use they will return and genetic diversity will remain stable.

Because composition of individuals within colonies varies from year to year, management should focus on ecosystem strategies. In the U.S., Franklin's gull nesting colonies occur almost exclusively on state and federal wildlife areas. This is beneficial to the Franklin's gull given most wetlands are impoundments with abilities to manage habitat at optimal locations and times therefore increases the chance of successfully establishing a nesting site. In any given year there are typically less than 20 colonies in the United States. High levels of mating interaction among individuals, which was revealed through genetic analysis, indicates a low level of natal site fidelity, which is likely due to the ephemeral nature of wetlands in the PPR. Thus, it is important to ensure adequate habitat, though composition of breeding pairs will change from year to year.

The driving force of colony site selection is unknown, but is clearly important. I did not find significant evidence to predict nesting locations from each year, there were identifiable trends. Results of this study suggest that a balance of open water, marsh habitat, and emergent vegetation stands is desirable for Franklin’s gull breeding pairs. We know hemi-marsh (equal amounts of emergent vegetation and water in an interspersed pattern) wetlands support high numbers of bird species diversity (Weller & Spatcher, 1965; Rehm & Baldassarre, 2007), likely to enhance prey diversity such as invertebrates for Franklin’s gull and other species of wetland birds. It is important for future studies to further quantify these habitat parameters for the success and proper management of Franklin’s gulls given that this study failed to detect significance for individual characteristics involved with wetland site selection.

This study, while limited in scope, provides an important starting point for management of Franklin’s gulls, and serves as a model for integrating information from population genetic and geographic approaches. Additional research is needed to determine whether genetic structure of the remaining breeding range (i.e. Canada) corroborates this study's findings. Further, genetic studies of Franklin’s gulls on the wintering grounds would provide valuable insights into the breeding biology of these colonial waterbirds. Information about pair formation as well as determining recruitment factors for spring migration would allow a total evidence approach to ensuring the presence of the species in U.S. ecosystems.

Literature Cited

Avise, J.C. 2004. Molecular Markers, Natural History, and Evolution, 2nd Edition. Sinauer, Sunderland, MA. 684 pp

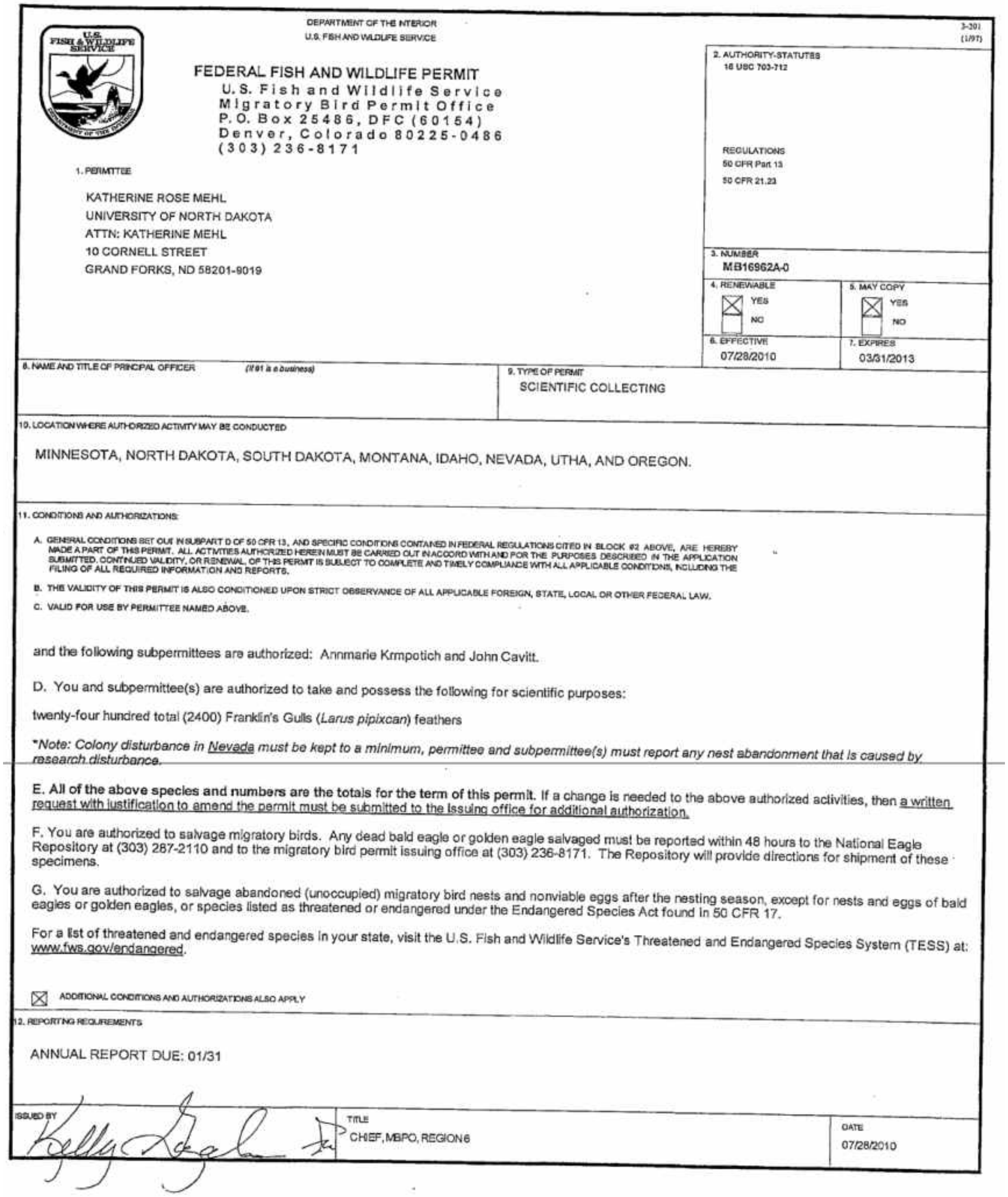
Rehm, E. M., and G. A. Baldassarre. 2007. The influence of interspersion on marsh bird abundance in New York. Wilson Journal of Ornithology 119:648–654.

Weller, M. W., and C. S. Spatcher. 1965. Role of habitat in the distribution and abundance of marsh birds. Iowa State University of Science and Technology, Ames, Iowa, USA.

APPENDICES

Appendix A
Permits

Federal Permit: United States



DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE

3-201
(1/97)

FEDERAL FISH AND WILDLIFE PERMIT

U.S. Fish and Wildlife Service
Migratory Bird Permit Office
P.O. Box 25486, DFC (60154)
Denver, Colorado 80225-0486
(303) 236-8171

1. PERMITTEE

KATHERINE ROSE MEHL
UNIVERSITY OF NORTH DAKOTA
ATTN: KATHERINE MEHL
10 CORNELL STREET
GRAND FORKS, ND 58201-9019

2. AUTHORITY-STATUTES
16 USC 703-712

REGULATIONS
50 CFR Part 13
50 CFR 21.23

3. NUMBER
MB16962A-0

4. RENEWABLE
☒ YES
☐ NO

5. MAY COPY
☒ YES
☐ NO

6. EFFECTIVE
07/28/2010

7. EXPIRES
03/31/2013

8. NAME AND TITLE OF PRINCIPAL OFFICER (If #1 is a business)

9. TYPE OF PERMIT
SCIENTIFIC COLLECTING

10. LOCATION WHERE AUTHORIZED ACTIVITY MAY BE CONDUCTED

MINNESOTA, NORTH DAKOTA, SOUTH DAKOTA, MONTANA, IDAHO, NEVADA, UTHA, AND OREGON.

11. CONDITIONS AND AUTHORIZATIONS:

A. GENERAL CONDITIONS SET OUT IN SUBPART D OF 50 CFR 13, AND SPECIFIC CONDITIONS CONTAINED IN FEDERAL REGULATIONS CITED IN BLOCK #2 ABOVE, ARE HEREBY MADE A PART OF THIS PERMIT. ALL ACTIVITIES AUTHORIZED HEREIN MUST BE CARRIED OUT IN ACCORD WITH AND FOR THE PURPOSES DESCRIBED IN THE APPLICATION SUBMITTED. CONTINUED VALIDITY, OR RENEWAL, OF THIS PERMIT IS SUBJECT TO COMPLETE AND TIMELY COMPLIANCE WITH ALL APPLICABLE CONDITIONS, INCLUDING THE FILING OF ALL REQUIRED INFORMATION AND REPORTS.

B. THE VALIDITY OF THIS PERMIT IS ALSO CONDITIONED UPON STRICT OBSERVANCE OF ALL APPLICABLE FOREIGN, STATE, LOCAL OR OTHER FEDERAL LAW.

C. VALID FOR USE BY PERMITTEE NAMED ABOVE.

and the following subpermittees are authorized: Annmarie Krmpotich and John Cavitt.

D. You and subpermittee(s) are authorized to take and possess the following for scientific purposes:

twenty-four hundred total (2400) Franklin's Gulls (*Larus pipixcan*) feathers

**Note: Colony disturbance in Nevada must be kept to a minimum, permittee and subpermittee(s) must report any nest abandonment that is caused by research disturbance.*

E. All of the above species and numbers are the totals for the term of this permit. If a change is needed to the above authorized activities, then a written request with justification to amend the permit must be submitted to the issuing office for additional authorization.

F. You are authorized to salvage migratory birds. Any dead bald eagle or golden eagle salvaged must be reported within 48 hours to the National Eagle Repository at (303) 287-2110 and to the migratory bird permit issuing office at (303) 236-8171. The Repository will provide directions for shipment of these specimens.

G. You are authorized to salvage abandoned (unoccupied) migratory bird nests and nonviable eggs after the nesting season, except for nests and eggs of bald eagles or golden eagles, or species listed as threatened or endangered under the Endangered Species Act found in 50 CFR 17.

For a list of threatened and endangered species in your state, visit the U.S. Fish and Wildlife Service's Threatened and Endangered Species System (TESS) at: www.fws.gov/endangered.

☒ ADDITIONAL CONDITIONS AND AUTHORIZATIONS ALSO APPLY

12. REPORTING REQUIREMENTS

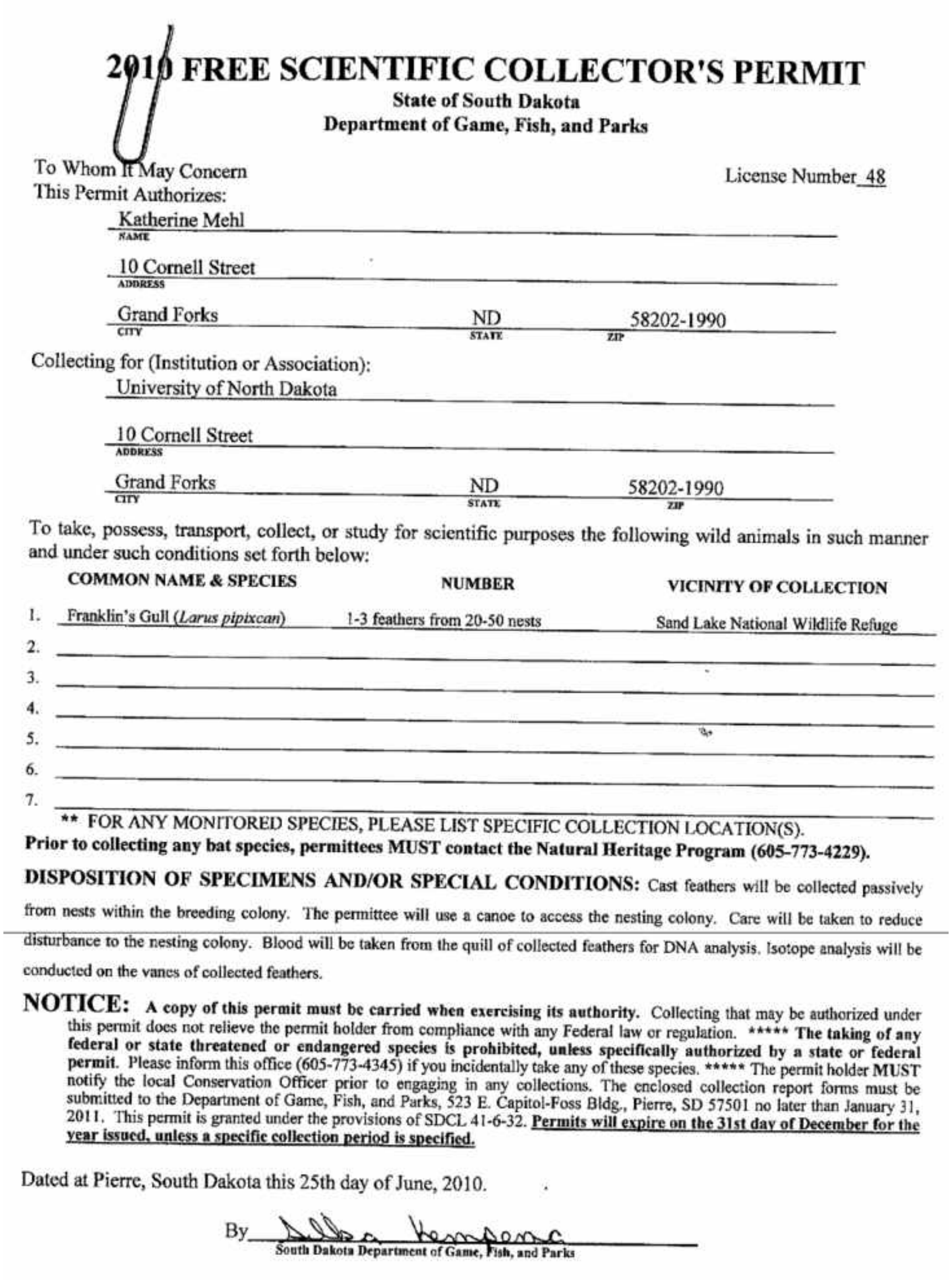
ANNUAL REPORT DUE: 01/31

ISSUED BY

TITLE
CHIEF, MBPO, REGION 6

DATE
07/28/2010

State Permit: South Dakota



2010 FREE SCIENTIFIC COLLECTOR'S PERMIT

State of South Dakota
Department of Game, Fish, and Parks

To Whom It May Concern
This Permit Authorizes:

License Number 48

Katherine Mehl
NAME

10 Cornell Street
ADDRESS

Grand Forks ND 58202-1990
CITY STATE ZIP

Collecting for (Institution or Association):
University of North Dakota

10 Cornell Street
ADDRESS

Grand Forks ND 58202-1990
CITY STATE ZIP

To take, possess, transport, collect, or study for scientific purposes the following wild animals in such manner and under such conditions set forth below:

	COMMON NAME & SPECIES	NUMBER	VICINITY OF COLLECTION
1.	Franklin's Gull (*Larus pipixcan*)	1-3 feathers from 20-50 nests	Sand Lake National Wildlife Refuge
2.			
3.			
4.			
5.			
6.			
7.			

** FOR ANY MONITORED SPECIES, PLEASE LIST SPECIFIC COLLECTION LOCATION(S).

Prior to collecting any bat species, permittees MUST contact the Natural Heritage Program (605-773-4229).

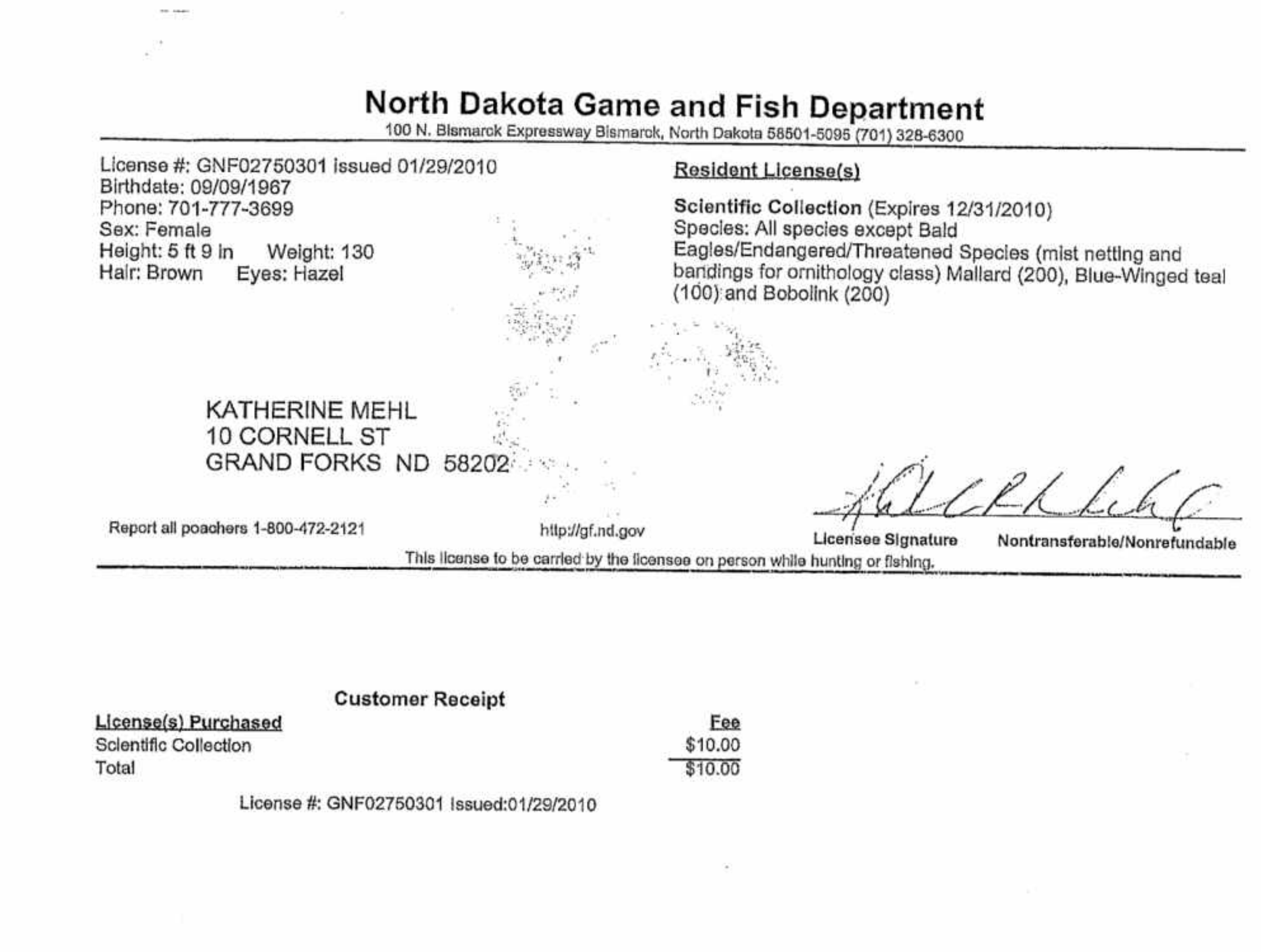
DISPOSITION OF SPECIMENS AND/OR SPECIAL CONDITIONS: Cast feathers will be collected passively from nests within the breeding colony. The permittee will use a canoe to access the nesting colony. Care will be taken to reduce disturbance to the nesting colony. Blood will be taken from the quill of collected feathers for DNA analysis. Isotope analysis will be conducted on the vanes of collected feathers.

NOTICE: **A copy of this permit must be carried when exercising its authority.** Collecting that may be authorized under this permit does not relieve the permit holder from compliance with any Federal law or regulation. ***** **The taking of any federal or state threatened or endangered species is prohibited, unless specifically authorized by a state or federal permit.** Please inform this office (605-773-4345) if you incidentally take any of these species. ***** The permit holder MUST notify the local Conservation Officer prior to engaging in any collections. The enclosed collection report forms must be submitted to the Department of Game, Fish, and Parks, 523 E. Capitol-Foss Bldg., Pierre, SD 57501 no later than January 31, 2011. This permit is granted under the provisions of SDCL 41-6-32. **Permits will expire on the 31st day of December for the year issued, unless a specific collection period is specified.**

Dated at Pierre, South Dakota this 25th day of June, 2010.

By
South Dakota Department of Game, Fish, and Parks

State Permit: North Dakota



North Dakota Game and Fish Department

100 N. Bismarck Expressway Bismarck, North Dakota 58501-5095 (701) 328-6300

License #: GNF02750301 issued 01/29/2010
Birthdate: 09/09/1967
Phone: 701-777-3699
Sex: Female
Height: 5 ft 9 in Weight: 130
Hair: Brown Eyes: Hazel

Resident License(s)

Scientific Collection (Expires 12/31/2010)
Species: All species except Bald Eagles/Endangered/Threatened Species (mist netting and bandings for ornithology class) Mallard (200), Blue-Winged teal (100) and Bobolink (200)

KATHERINE MEHL
10 CORNELL ST
GRAND FORKS ND 58202

Report all poachers 1-800-472-2121 http://gf.nd.gov **Licensee Signature** **Nontransferable/Nonrefundable**

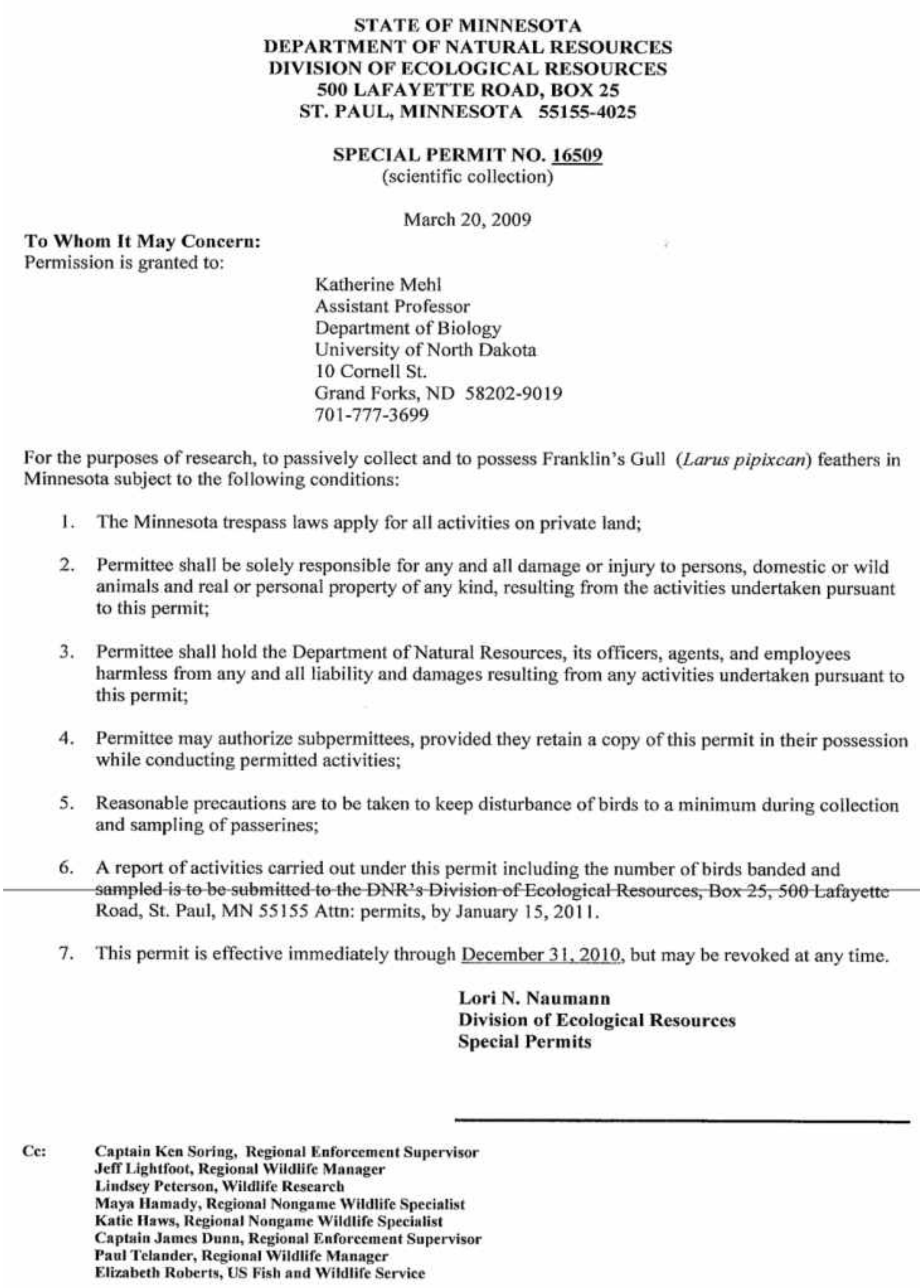
This license to be carried by the licensee on person while hunting or fishing.

Customer Receipt

License(s) Purchased	Fee
Scientific Collection	$10.00
Total	$10.00

License #: GNF02750301 Issued:01/29/2010

State Permit: Minnesota



STATE OF MINNESOTA
DEPARTMENT OF NATURAL RESOURCES
DIVISION OF ECOLOGICAL RESOURCES
500 LAFAYETTE ROAD, BOX 25
ST. PAUL, MINNESOTA 55155-4025

SPECIAL PERMIT NO. 16509
(scientific collection)

March 20, 2009

To Whom It May Concern:
Permission is granted to:

Katherine Mehl
Assistant Professor
Department of Biology
University of North Dakota
10 Cornell St.
Grand Forks, ND 58202-9019
701-777-3699

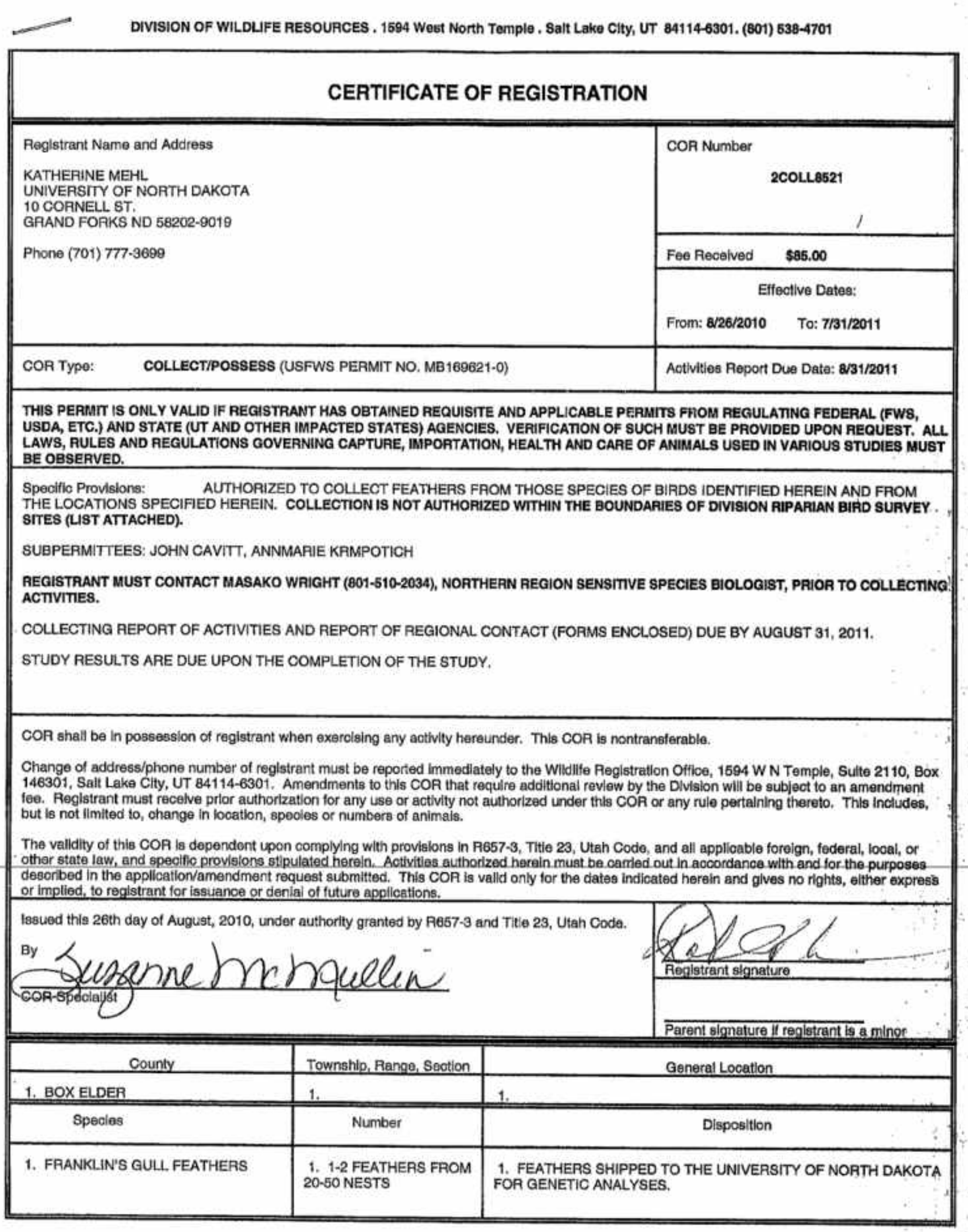
For the purposes of research, to passively collect and to possess Franklin's Gull (*Larus pipixcan*) feathers in Minnesota subject to the following conditions:

1. The Minnesota trespass laws apply for all activities on private land;
2. Permittee shall be solely responsible for any and all damage or injury to persons, domestic or wild animals and real or personal property of any kind, resulting from the activities undertaken pursuant to this permit;
3. Permittee shall hold the Department of Natural Resources, its officers, agents, and employees harmless from any and all liability and damages resulting from any activities undertaken pursuant to this permit;
4. Permittee may authorize subpermittees, provided they retain a copy of this permit in their possession while conducting permitted activities;
5. Reasonable precautions are to be taken to keep disturbance of birds to a minimum during collection and sampling of passerines;
6. A report of activities carried out under this permit including the number of birds banded and ~~sampled is to be submitted to the DNR's Division of Ecological Resources, Box 25, 500 Lafayette~~ Road, St. Paul, MN 55155 Attn: permits, by January 15, 2011.
7. This permit is effective immediately through December 31, 2010, but may be revoked at any time.

Lori N. Naumann
Division of Ecological Resources
Special Permits

Cc:
Captain Ken Soring, Regional Enforcement Supervisor
Jeff Lightfoot, Regional Wildlife Manager
Lindsey Peterson, Wildlife Research
Maya Hamady, Regional Nongame Wildlife Specialist
Katie Haws, Regional Nongame Wildlife Specialist
Captain James Dunn, Regional Enforcement Supervisor
Paul Telander, Regional Wildlife Manager
Elizabeth Roberts, US Fish and Wildlife Service

State Permit: Utah



DIVISION OF WILDLIFE RESOURCES . 1594 West North Temple . Salt Lake City, UT 84114-6301. (801) 538-4701

CERTIFICATE OF REGISTRATION

Registrant Name and Address

KATHERINE MEHL
UNIVERSITY OF NORTH DAKOTA
10 CORNELL ST.
GRAND FORKS ND 58202-9019

Phone (701) 777-3699

COR Number **2COLL8521**

Fee Received **$85.00**

Effective Dates:

From: **8/26/2010** To: **7/31/2011**

COR Type: **COLLECT/POSSESS** (USFWS PERMIT NO. MB169621-0)

Activities Report Due Date: **8/31/2011**

THIS PERMIT IS ONLY VALID IF REGISTRANT HAS OBTAINED REQUISITE AND APPLICABLE PERMITS FROM REGULATING FEDERAL (FWS, USDA, ETC.) AND STATE (UT AND OTHER IMPACTED STATES) AGENCIES. VERIFICATION OF SUCH MUST BE PROVIDED UPON REQUEST. ALL LAWS, RULES AND REGULATIONS GOVERNING CAPTURE, IMPORTATION, HEALTH AND CARE OF ANIMALS USED IN VARIOUS STUDIES MUST BE OBSERVED.

Specific Provisions: AUTHORIZED TO COLLECT FEATHERS FROM THOSE SPECIES OF BIRDS IDENTIFIED HEREIN AND FROM THE LOCATIONS SPECIFIED HEREIN. **COLLECTION IS NOT AUTHORIZED WITHIN THE BOUNDARIES OF DIVISION RIPARIAN BIRD SURVEY SITES (LIST ATTACHED).**

SUBPERMITTEES: JOHN CAVITT, ANNMARIE KRMPOTICH

REGISTRANT MUST CONTACT MASAKO WRIGHT (801-510-2034), NORTHERN REGION SENSITIVE SPECIES BIOLOGIST, PRIOR TO COLLECTING ACTIVITIES.

COLLECTING REPORT OF ACTIVITIES AND REPORT OF REGIONAL CONTACT (FORMS ENCLOSED) DUE BY AUGUST 31, 2011.

STUDY RESULTS ARE DUE UPON THE COMPLETION OF THE STUDY.

COR shall be in possession of registrant when exercising any activity hereunder. This COR is nontransferable.

Change of address/phone number of registrant must be reported immediately to the Wildlife Registration Office, 1594 W N Temple, Suite 2110, Box 146301, Salt Lake City, UT 84114-6301. Amendments to this COR that require additional review by the Division will be subject to an amendment fee. Registrant must receive prior authorization for any use or activity not authorized under this COR or any rule pertaining thereto. This includes, but is not limited to, change in location, species or numbers of animals.

The validity of this COR is dependent upon complying with provisions in R657-3, Title 23, Utah Code, and all applicable foreign, federal, local, or other state law, and specific provisions stipulated herein. Activities authorized herein must be carried out in accordance with and for the purposes described in the application/amendment request submitted. This COR is valid only for the dates indicated herein and gives no rights, either express or implied, to registrant for issuance or denial of future applications.

Issued this 26th day of August, 2010, under authority granted by R657-3 and Title 23, Utah Code.

By Suzanne McMullin
COR-Specialist

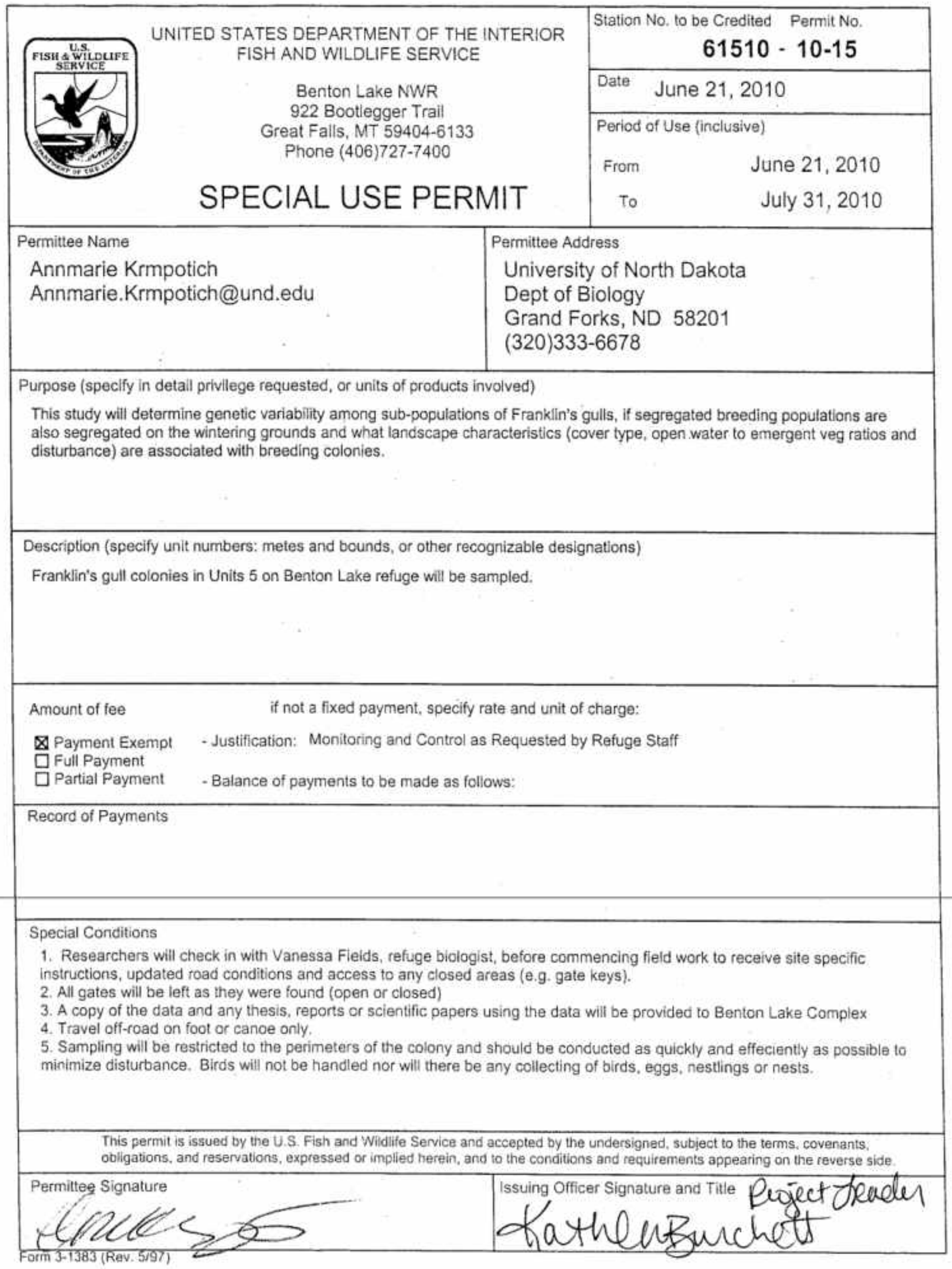
Registrant signature

Parent signature if registrant is a minor

County	Township, Range, Section	General Location
1. BOX ELDER	1.	1.

Species	Number	Disposition
1. FRANKLIN'S GULL FEATHERS	1. 1-2 FEATHERS FROM 20-50 NESTS	1. FEATHERS SHIPPED TO THE UNIVERSITY OF NORTH DAKOTA FOR GENETIC ANALYSES.

Special Use Permit: Benton Lake National Wildlife Refuge, Montana



U.S. FISH & WILDLIFE SERVICE

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Benton Lake NWR
922 Bootlegger Trail
Great Falls, MT 59404-6133
Phone (406)727-7400

SPECIAL USE PERMIT

Station No. to be Credited Permit No. **61510 - 10-15**

Date June 21, 2010

Period of Use (inclusive)
From June 21, 2010
To July 31, 2010

Permittee Name
Annmarie Krmpotich
Annmarie.Krmpotich@und.edu

Permittee Address
University of North Dakota
Dept of Biology
Grand Forks, ND 58201
(320)333-6678

Purpose (specify in detail privilege requested, or units of products involved)

This study will determine genetic variability among sub-populations of Franklin's gulls, if segregated breeding populations are also segregated on the wintering grounds and what landscape characteristics (cover type, open water to emergent veg ratios and disturbance) are associated with breeding colonies.

Description (specify unit numbers: metes and bounds, or other recognizable designations)

Franklin's gull colonies in Units 5 on Benton Lake refuge will be sampled.

Amount of fee

if not a fixed payment, specify rate and unit of charge:

☒ Payment Exempt - Justification: Monitoring and Control as Requested by Refuge Staff
☐ Full Payment
☐ Partial Payment - Balance of payments to be made as follows:

Record of Payments

Special Conditions

1. Researchers will check in with Vanessa Fields, refuge biologist, before commencing field work to receive site specific instructions, updated road conditions and access to any closed areas (e.g. gate keys).
2. All gates will be left as they were found (open or closed)
3. A copy of the data and any thesis, reports or scientific papers using the data will be provided to Benton Lake Complex
4. Travel off-road on foot or canoe only.
5. Sampling will be restricted to the perimeters of the colony and should be conducted as quickly and effeciently as possible to minimize disturbance. Birds will not be handled nor will there be any collecting of birds, eggs, nestlings or nests.

This permit is issued by the U.S. Fish and Wildlife Service and accepted by the undersigned, subject to the terms, covenants, obligations, and reservations, expressed or implied herein, and to the conditions and requirements appearing on the reverse side.

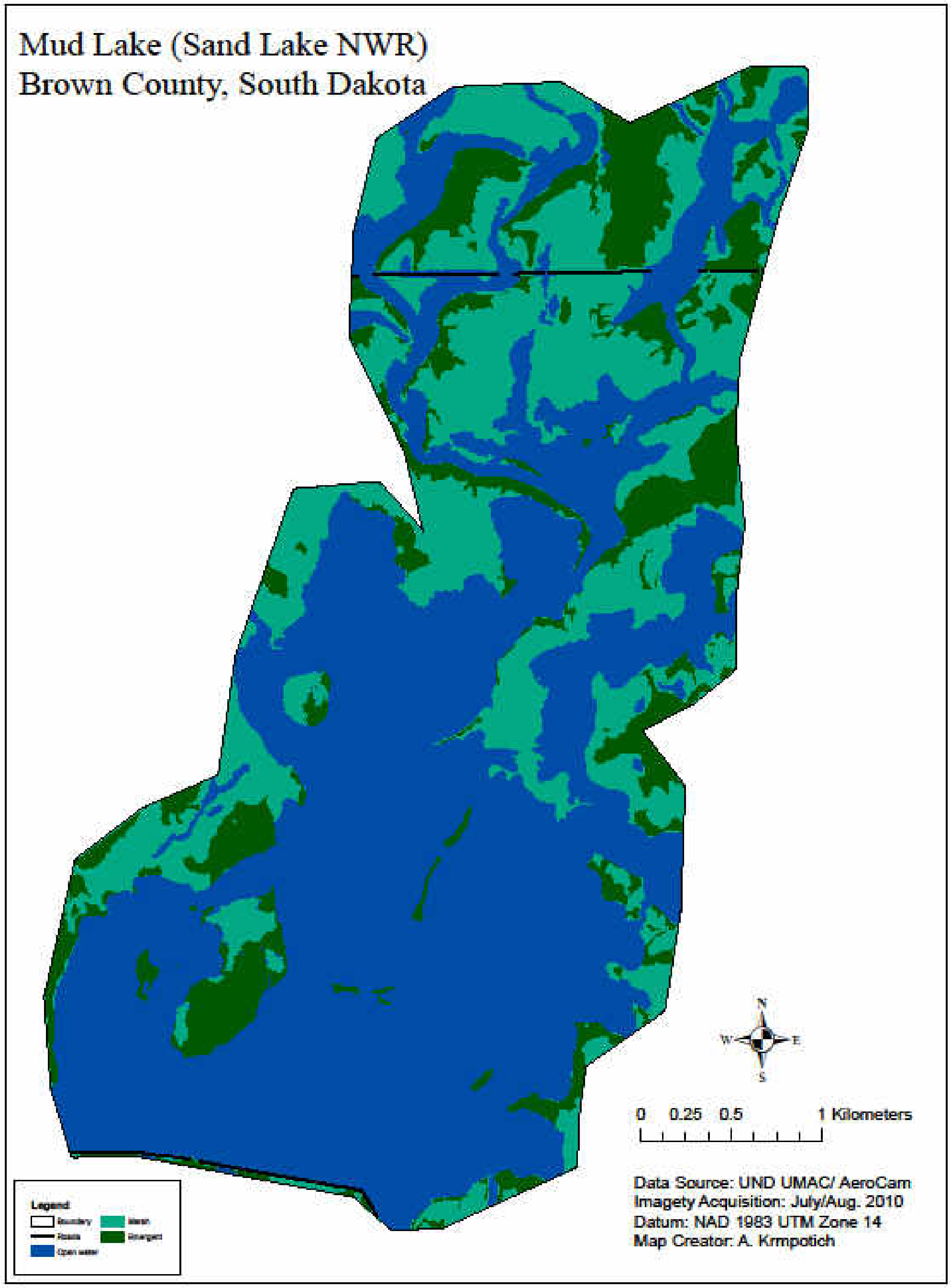
Permittee Signature

Issuing Officer Signature and Title Project Leader
Kathleen Burchett

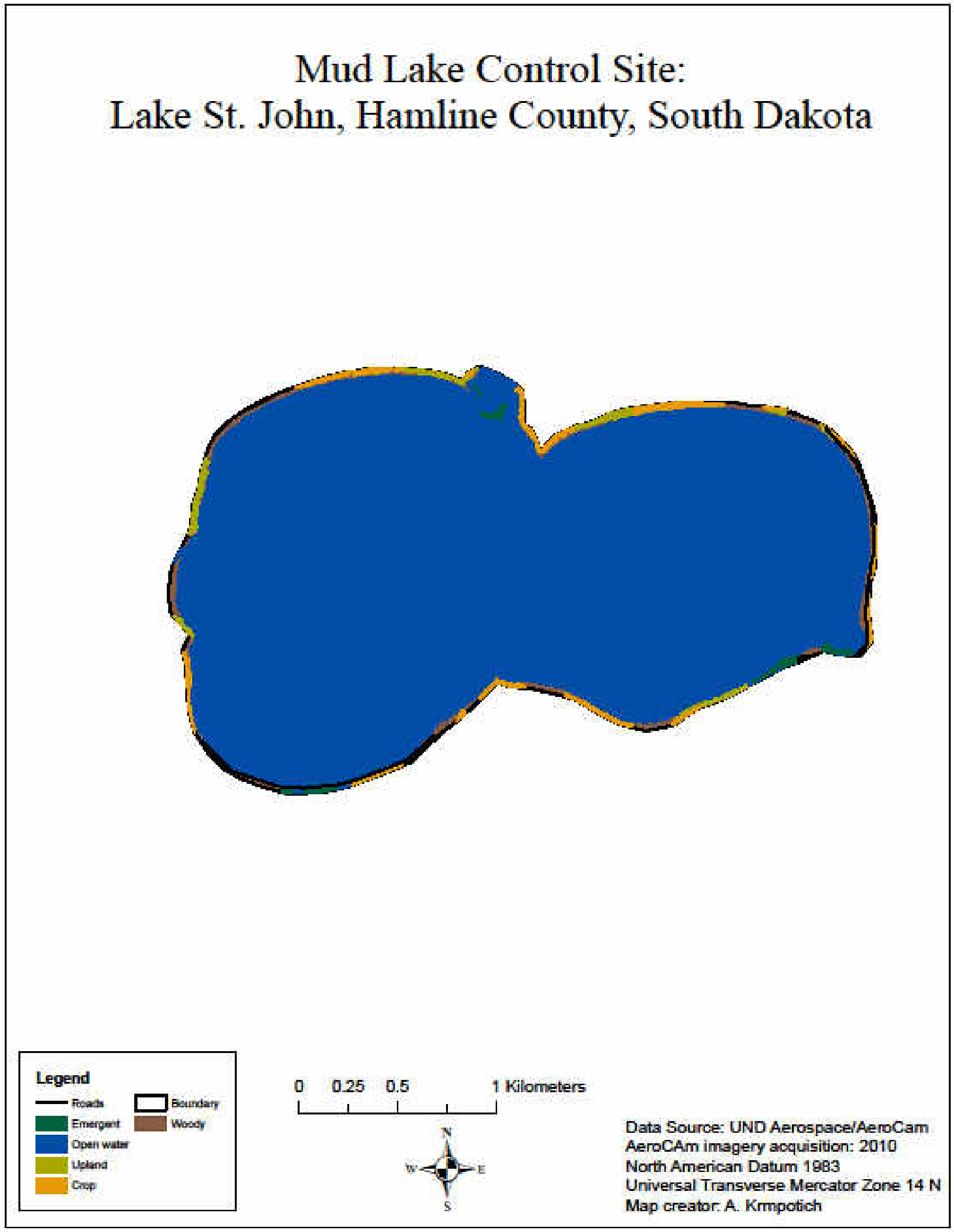
Form 3-1383 (Rev. 5/97)

Appendix B
Digitized Maps

Mud Lake at Sand Lake National Wildlife Refuge, SD

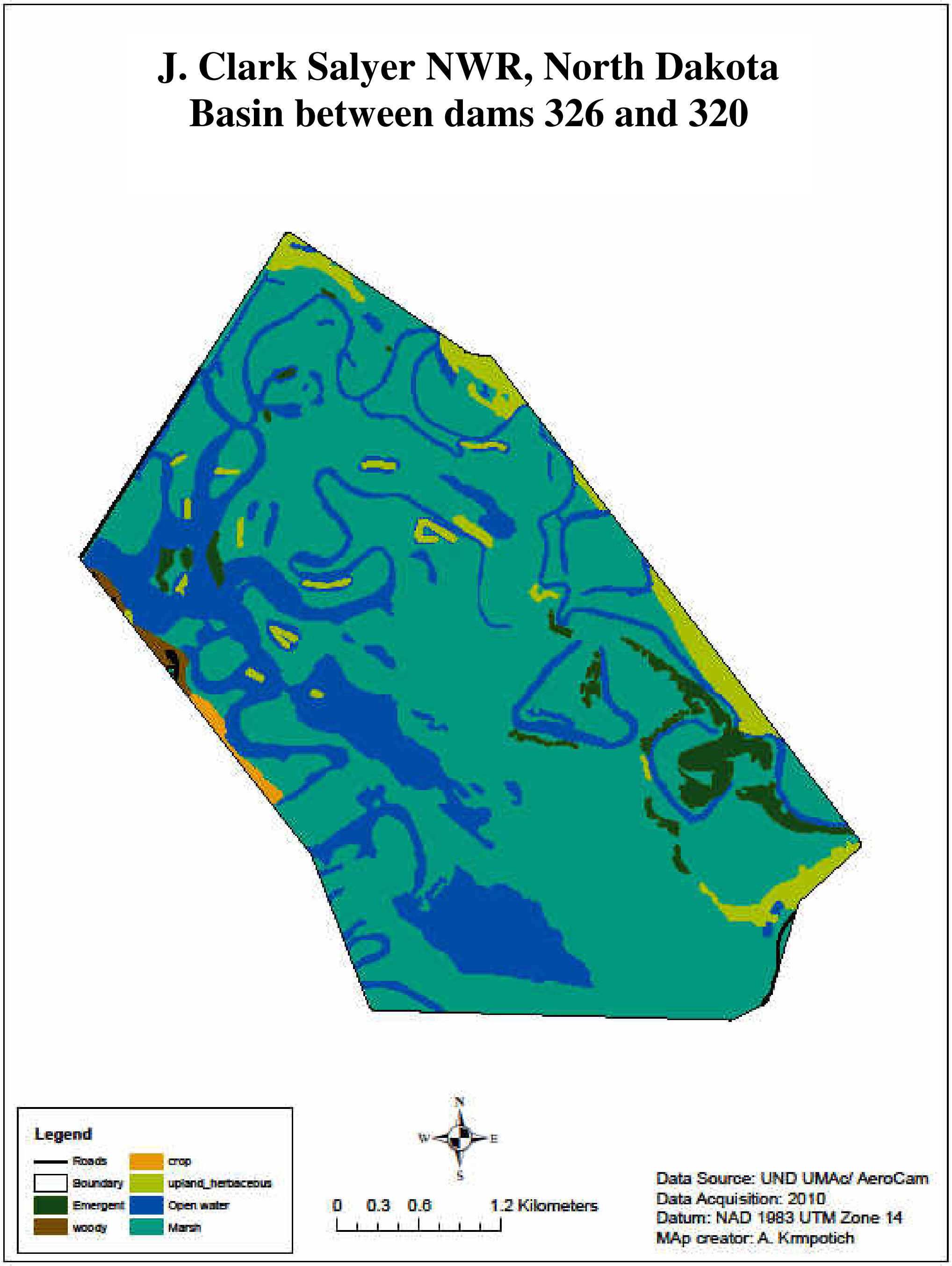


Lake St. John (Mud Lake Control Site), SD



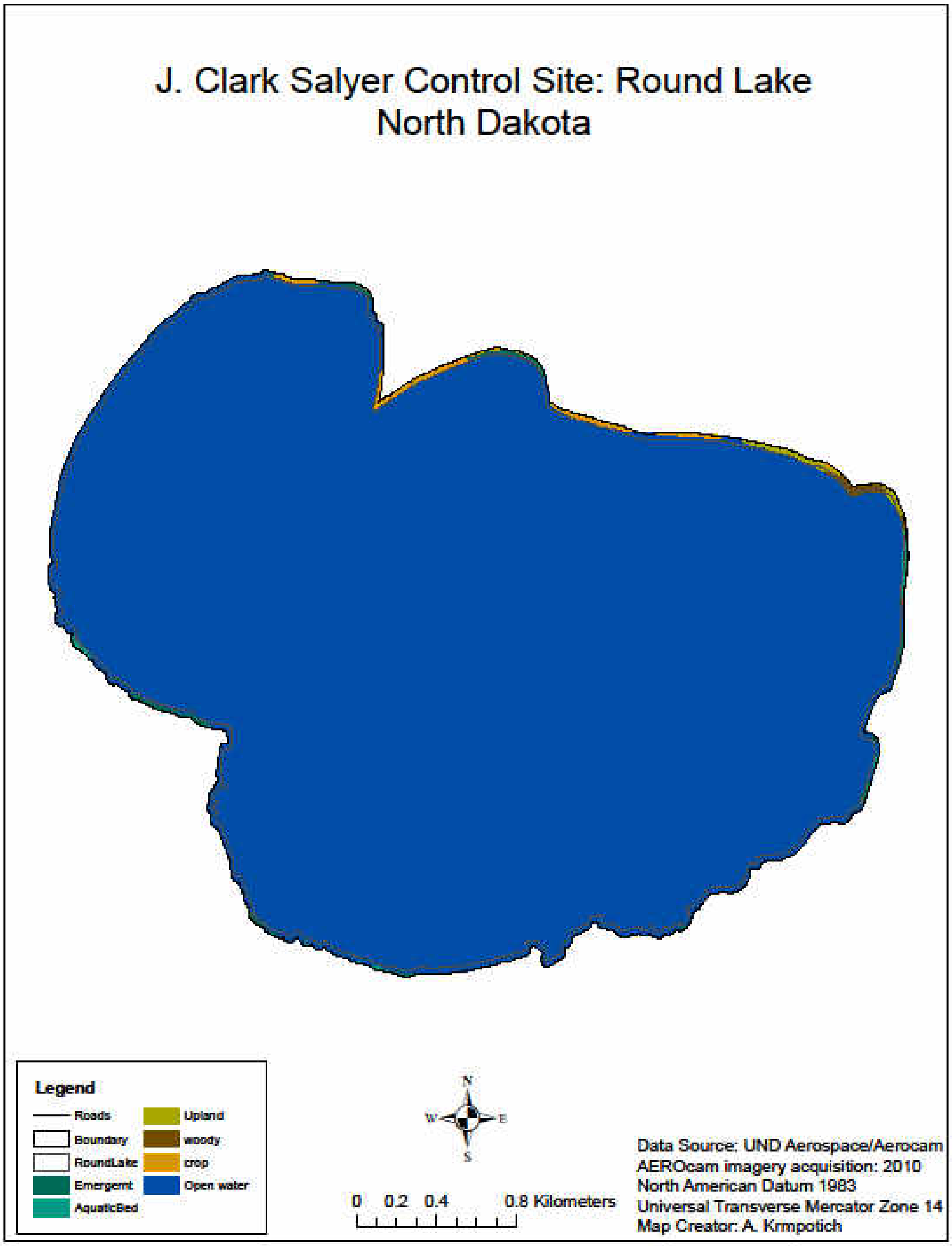
Data Source: UND Aerospace/AeroCam
AeroCAm imagery acquisition: 2010
North American Datum 1983
Universal Transverse Mercator Zone 14 N
Map creator: A. Krmpotich

J. Clark Salyer National Wildlife Refuge, ND

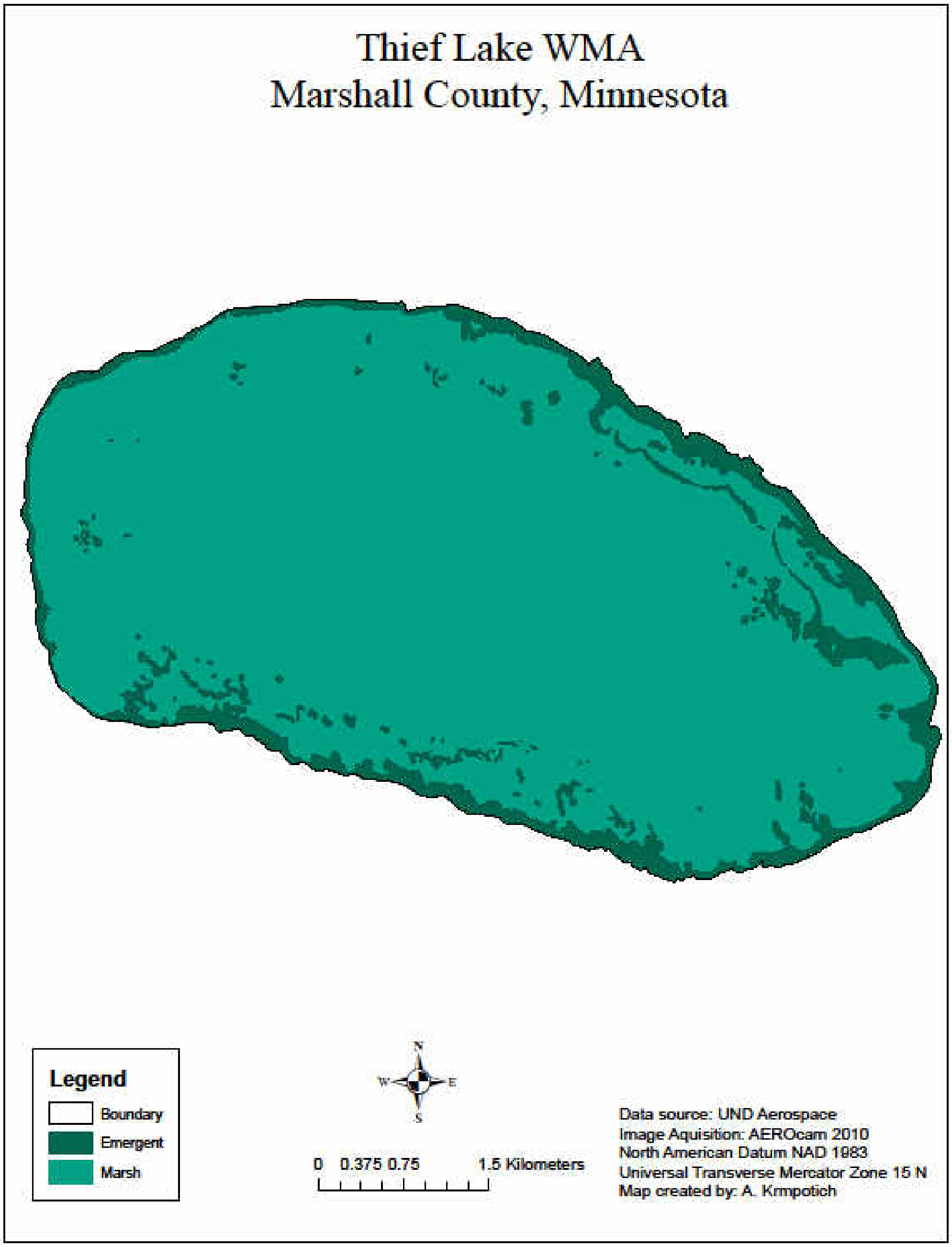


Data Source: UND UMAc/ AeroCam
Data Acquisition: 2010
Datum: NAD 1983 UTM Zone 14
MAp creator: A. Krmpotich

Round Lake (J.Clark Salyer Control), ND

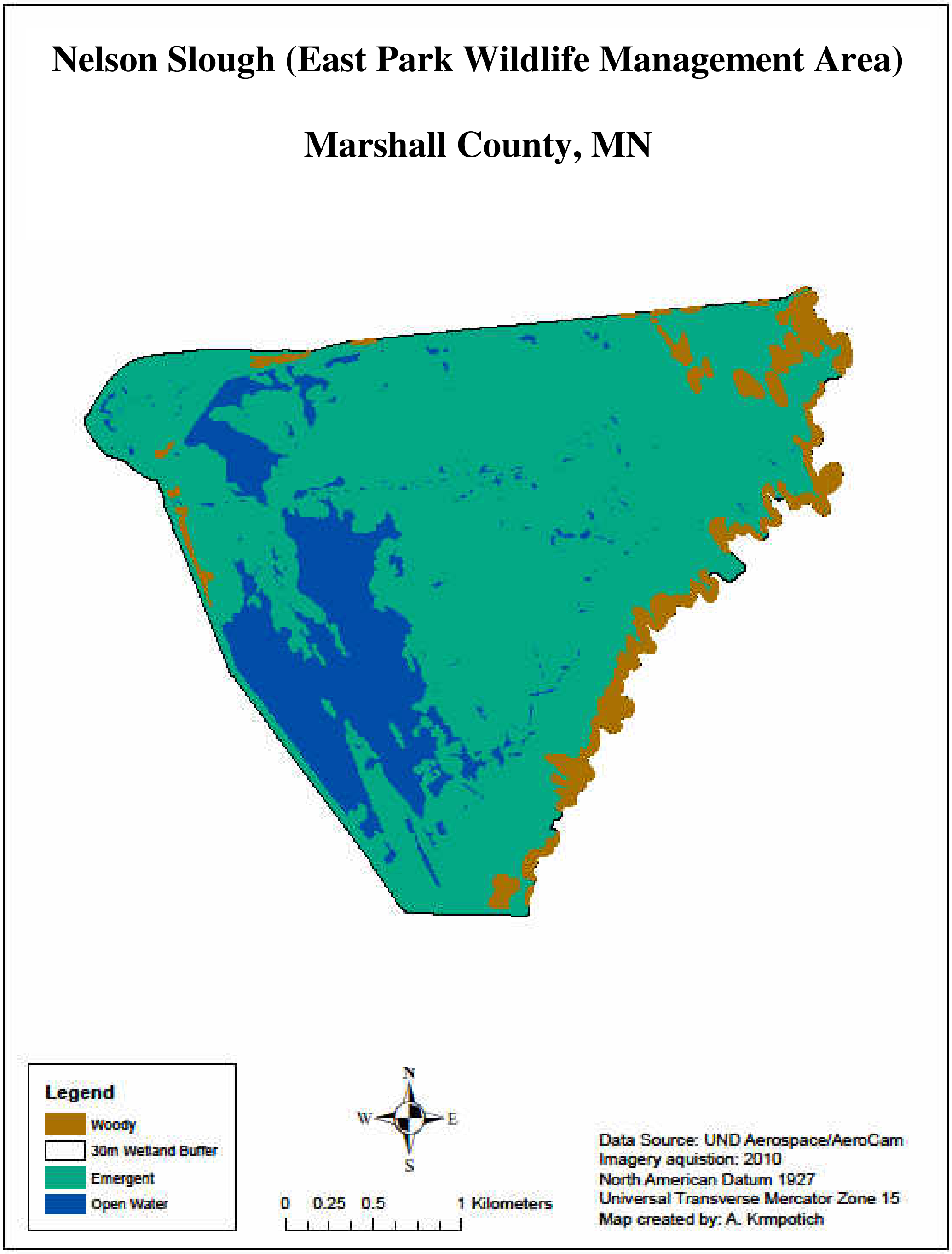


Thief Lake Wildlife Management Area, MN



Data source: UND Aerospace
Image Aquisition: AEROcam 2010
North American Datum NAD 1983
Universal Transverse Mercator Zone 15 N
Map created by: A. Krmpotich

Nelson Slough (Thief Lake Control), MN



Data Source: UND Aerospace/AeroCam
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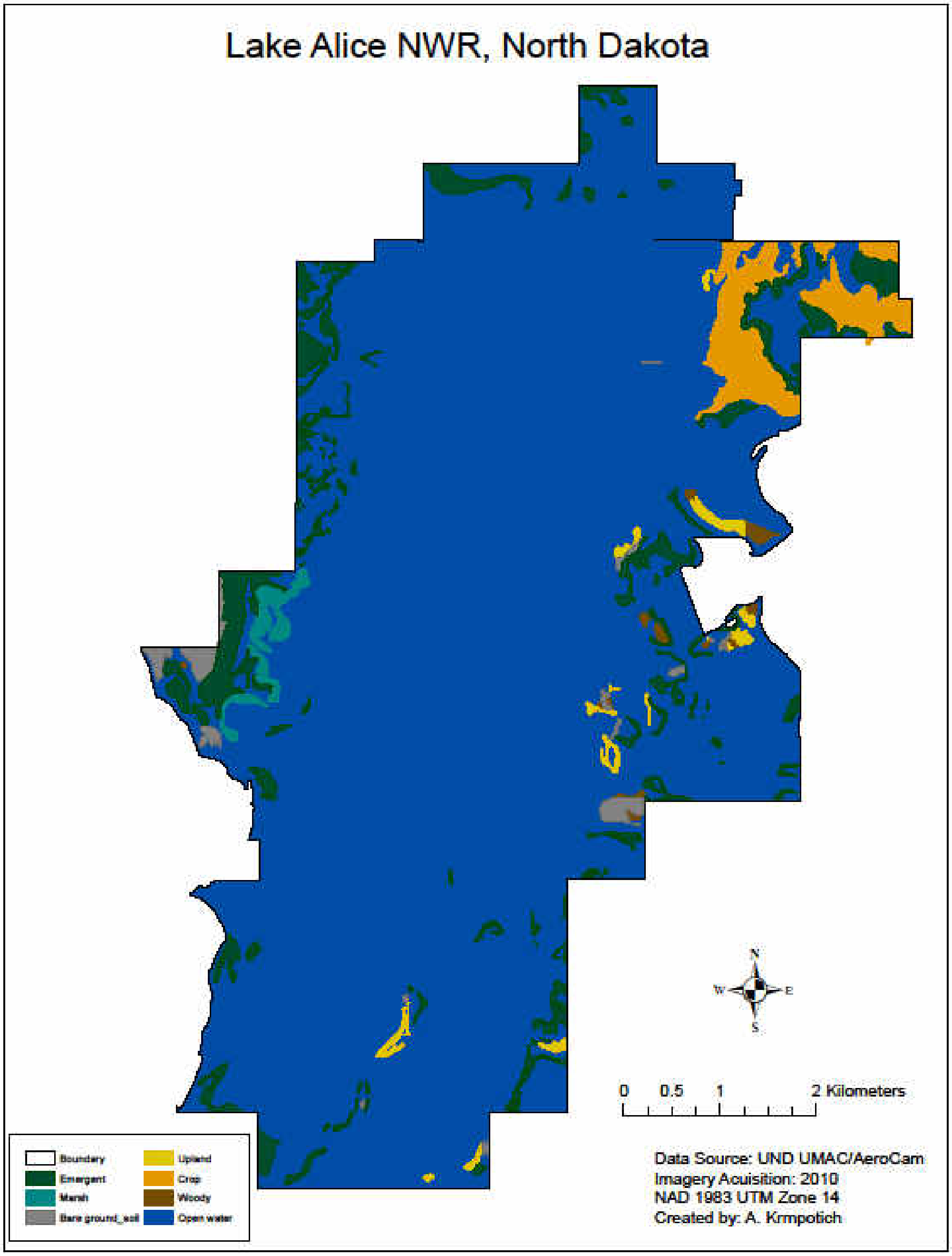
North American Datum 1927
Universal Transverse Mercator Zone 15
Map created by: A. Krmpotich

Lake Alice National Wildlife Refuge, ND


Lake Alice NWR, North Dakota

Data Source: UND UMAC/AeroCam
Imagery Acuisition: 2010
NAD 1983 UTM Zone 14
Created by: A. Krmpotich

Cranberry Lake (Lake Alice Control), ND