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River otter (*Lontra canadensis*) use of *Phragmites australis* and density estimation using genetic techniques

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RIVER OTTER (*LONTRA CANADENSIS*) USE OF *PHRAGMITES AUSTRALIS* AND DENSITY ESTIMATION USING GENETIC TECHNIQUES

by

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RIVER OTTER (*LONTRA CANADENSIS*) USE OF *PHRAGMITES AUSTRALIS* AND DENSITY ESTIMATION USING GENETIC TECHNIQUES

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River otters were extirpated in Nebraska in the early 1900's. In 1986 Nebraska Game and Parks Commission began reintroducing otters. In support of developing an otter management plan, I conducted research on two aspects of otter ecology in Nebraska. First, I examined otter use of habitats dominated by the non–native aquatic plant, *Phragmites australis*, in the Big Bend reach of the Platte River. Sixteen otters were trapped, radio–tagged, and tracked between 2006 and 2009. I identified 517 den/resting locations, 127 of which were unique locations. I compared den/resting site habitat use to availability to determine if otters were using *Phragmites* in proportion to availability. Females use unique sites in *Phragmites* more than expected but males do not. However, the frequency of use of *Phragmites* for both males and females was in proportion to availability. *Phragmites* provides cover for females and is likely used for resting locations when traveling with pups.

Second, I used non–invasive genetic techniques to estimate river otter density in the Big Bend reach of the Platte River and the feasibility of using this technique across Nebraska. Density was estimated using DNA from scat and mark-recapture methods. Otter scat was collected along 29 kilometers of the Platte River during two independent sampling sessions in fall of 2009. DNA was extracted from the scats and genotyped at 10 microsatellite loci. Unique individuals were identified for both sampling sessions, noting

recaptures between sessions. River otter density was 0.99-1.13 otters/kilometer. The density is higher than previously reported for otters in North America. The complexity of the central Platte River, the prevalence of sand pits, and the fact that this population has been unexploited since its initial reintroduction, likely accounts for the relatively high density.

Otter populations in the central Platte River are high and not negatively impacted by the invasion of *Phragmites*. Results from this study will support the creation and of an otter management plan and ensure the persistence of otters in Nebraska.

DEDICATION

To the charismatic and enchanting river otters of Nebraska.

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CHAPTER 1: INTRODUCTION

The North American river otter (*Lontra canadensis*) was historically abundant in all major watersheds of the United States and Canada (Lariviere and Walton 1998). By the early 1900's river otter populations had declined significantly across North America due to unregulated trapping, pollution, loss of habitat, draining of marshes and channelization of streams (Melquist and Hornocker 1983). The current distribution of the river otter extends across New England, Great Lakes regions, Gulf coast, and Pacific and Atlantic coasts (Larivière and Walton 1998). As of 1998 river otters were considered rare or absent in Arizona, Indiana, Iowa, Kansas, Kentucky, Nebraska, New Mexico, North Dakota, Ohio, Oklahoma, South Dakota, Tennessee and West Virginia (Larivière and Walton 1998). The North American river otter is currently listed as threatened in Colorado, Nebraska and South Dakota. Due to trapping, incidental harvest, vehicle collisions and habitat destruction and alterations, humans remain the largest source of river otter mortality (Melquist et al. 2003). However, the geographical range of rivers otter is expanding through reintroductions and other conservation efforts focusing on wildlife habitat.

River otters were rare in Nebraska as early as 1908, and it is thought that they were extirpated not long after that (Jones 1964). Between 1986 and 1991 the Nebraska Game and Parks Commission (NGPC) reintroduced 159 river otters from Alaska, British Columbia, Idaho, Louisiana, Michigan, Ontario, and Wisconsin into five of Nebraska's rivers at seven release sites (Bischof 2003). Since these reintroductions the only data collected concerning otter status in Nebraska has been from recovered carcasses (Bischof

2003) and annual bridge surveys, conducted by NGPC since 2000, documenting otter sign following snow falls. Due to the absence of sufficient quantitative data, much remains unknown about river otter ecology in Nebraska, or the success of the reintroductions. River otters continue to be a state threatened species and are protected from hunting and trapping; however, incidental harvest persists as the largest source of mortality in Nebraska. Between 1987 and 2001 approximately 86% of 104 confirmed otter mortalities were due to accidental trapping (Bischof 2003). The need for quantitative data in Nebraska, especially regarding habitat use, has increased in the last decade due to the dramatic changes to many of Nebraska's riparian habitats such as those found along the Platte River.

River otters can occupy a variety of habitats ranging from marine environments to mountain streams to desert canyons (Larivière and Walton 1998). They are most abundant in areas with ample food and the least amount of human disturbance (Polechla 1990), including coastal marshes, estuaries, and streams. Preferred interior (non–coastal) habitats include lowland marshes and swamps interconnected with lakes and streams (Reid et al. 1994). There is a strong correlation between beaver populations and otter presence (Polechla 1989), because otters often use beaver structures for dens (Melquist et al. 2003). Melquist and Hornocker (1983) found that 38% of the 1,283 den and resting sites used by otters in central Idaho were in beaver bank dens or lodges. The allure of beaver structures to otters is hypothesized to be a combination of availability, adequate shelter, and the presence of an underwater escape route (Melquist and Hornocker 1983).

Foraging and dietary habits of river otters vary depending on the type of habitat occupied. Otters are considered piscivores, and thus specialists, though they will eat a

variety of other animals (Melquist et al. 1981; Cooley 1983). Otters occupying interior stream habitats forage for fish in areas of the stream or pond where fish seek cover such as deep, low current areas or near logjams (Melquist and Hornocker 1983). The species of fish taken by otters is usually in proportion to availability and in inverse proportion to the swimming ability of the fish (Ryder 1955; Toweill 1974). Diets are often supplemented by other available resources such as crustaceans, insects, amphibians, small mammals and birds (Melquist et al. 2003).

River otters are often considered a "flagship species" for the conservation of wetlands and aquatic habitats (Foster–Turley 1996). A "flagship species" is one which is highly visible and charismatic to the general public that effectively illustrates the importance of an ecosystem. Otters are also a "keystone species" (a species that has a disproportionate effect on the health and function of the ecosystem to which they belong) in the wetlands they occupy due to their role in facilitating nutrient transport between terrestrial and aquatic habitats and their role as a top predator (Bowyer et al. 2003). Otter presence in an ecosystem tends to indicate an environment with ample, high quality water (Polechla 2000). They are sensitive to pollution and rapidly accumulate high levels of chemicals such as mercury and organochlorine compounds (Duffy et al. 1996; Larivière and Walton 1998). Habitat destruction from development and agriculture practices, including the use of pesticides, often result in the accumulation of contaminates and declines in water quality (Deems and Pursley 1978). Bowyer et al. (2003) described river otters as a "sentinel species" (a species that is highly susceptible to environmental contaminants and pollution) making them an indicator of a water system's environmental health.

The importance of river otters to Nebraska's riparian ecosystems, both intrinsically and as a species indicative of a healthy, functioning ecosystem, makes understanding river otter ecology vital. Rapidly changing riparian landscapes, affected by the spread of invasive species such as common reed (*Phragmites australis*), have recently made the need to fill this information gap even more necessary.

The following chapters describe my research on two aspects of river otter ecology in Nebraska. First, I examined river otter use of habitats dominated by the non–native aquatic plant, *Phragmites australis*, in the Big Bend reach of the Platte River. There have been no quantitative studies on river otter ecology in Nebraska since they were reintroduced in the late 1980's. Furthermore, no research has been done on how river otters use *Phragmites australis*, a highly invasive reed that is of increasing concern throughout the Great Plains. Second, I used non–invasive genetic techniques to estimate river otter density in the Big Bend reach of the Platte River and the feasibility of using this technique across Nebraska. This research was performed as a part of a broader study that focuses on estimating home range size and overall habitat use for river otters in Nebraska (Sam Wilson, unpublished data). These two chapters address questions that are necessary for the development and implementation of a statewide river otter management plan.

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CHAPTER 2: NORTH AMERICAN RIVER OTTER (LONTRA CANADENSIS) USE OF COMMON REED (PHRAGMITES AUSTRALIS) IN THE BIG BEND REACH OF THE PLATTE RIVER

INTRODUCTION

River otters (*Lontra canadensis*) were extirpated in Nebraska in the early 1900's as a result of unregulated trapping and habitat loss (Jones 1964; Melquist et al. 2003). Between 1986 and 1991 the Nebraska Game and Parks Commission (NGPC) reintroduced 159 otters from five states (Alaska, Idaho, Louisiana, Michigan, and Wisconsin) and two Canadian provinces (British Columbia and Ontario) into five rivers in Nebraska at seven release sites (Bischof 2003). Sightings of otters and their sign have been observed since the release in all seven watersheds, indicating the successful establishment of otters in Nebraska, though little is known of otter habitat use in Nebraskan rivers.

River otters can occupy a wide range of habitats. However, within interior habitats they prefer lowland marshes, swamps and bogs interconnected with slow moving streams and lakes (Melquist and Hornocker 1983; Reid et al. 1994). Otters may use a variety of habitat features for den and resting sites. A single otter may use as many as 88 different den sites (Melquist and Hornocker 1983). Typically, otters will use areas based on convenience and availability but prefer sheltered or secluded sites that provide protection (Melquist et al. 2003). Otters are adaptable in their habitat use. In northern Idaho along the Clearwater River canyon, where rock cavities were the most available den type, otters used these more than any other den type (Mack et al. 1994). In central Idaho where slow–moving streams and extensive riparian habitat were more common, 67% of den sites utilized by otters were beaver bank dens, lodges, logjams, and riparian

vegetation (Melquist and Hornocker 1983). Female otters do not excavate their own natal dens, but modify dens from existing structures created by other animals or natural shelters, and have been documented building a nest–like structure in aquatic vegetation (Lowery 1974; Grinnell et al. 1937; Liers 1951). In the arid Western United States, suitable otter habitat consists of areas with permanent water sources, high pool to riffle rates, high beaver density, and adequate vegetative cover (Christensen 1984; Bradley 1986; Allen 1987; Malville 1990). Many of these key habitat features are common throughout the central Platte River in Nebraska.

The Platte River begins in Colorado and flows through Wyoming into eastern Nebraska where it drains into the Missouri River. The Big Bend reach is an area of the Platte River between the cities of Lexington and Chapman in central Nebraska (Sidle et al. 1989). This area is of conservation concern and is classified as a biologically unique landscape (Schneider et al. 2005). It is critical habitat for a number of threatened and endangered species including river otters, whooping cranes (*Grus americana*), the interior least tern (*Sterna antillarium athalassos*), the piping plover (*Charadrium melodus)* and pallid sturgeon (*Scaphirhynchus albus*) (Sidle and Faanes 1997). Prior to European settlement, this area of the Platte River was characterized by its wide, shallow channel with un–vegetated sandbars with high flows in the winter and low flows in the summer. Since the early twentieth century the Platte River hydrology and habitat have been greatly altered. Flows have been reduced by up to 70% along the Big Bend reach, primarily from water development projects including canals and dams (Sidle et al. 1989). Dams have also reduced the amount of sediment that reaches the Big Bend reach. Reduced flows have led to a decrease in scouring of vegetation within the river channel

and as a result riparian forests along the Platte River have increased by 29 to 75% since 1939. Wetland meadows adjacent to the river have been converted to croplands, sand and gravel mines, and roads resulting in a 23 to 45% loss of wetland meadows since 1939 (Sidle et al. 1989). Although habitat changes have been occurring on the Platte River for over a century, a more recent concern is the habitat alterations resulting from the invading species, *Phragmites australis* (Cav.) Trin. ex Steudel (hereafter referred to as *Phragmites*).

Phragmites, a large perennial, rhizomatous wetland grass, is of increasing concern throughout North America (Marks et al. 1993). A non–native genotype of *Phragmites* has spread extensively over the last 200 years and it is currently present in all of the contiguous 48 states and Canada (Chambers et al. 1999). *Phragmites* is characterized by its dense, nearly impenetrable stands consisting of stalks up to 20 feet tall. The Big Bend reach of the central Platte River has been invaded in recent years by *Phragmites*, aided by drought conditions (Brei and Bishop 2005). Although the native *Phragmites* genotype is likely present in the central Platte River, it is morphologically indistinguishable from the non–native genotype that is thought to be far more abundant.

The rapid spread of *Phragmites* in the United States has led it to encroach on many native plant communities and wildlife habitats (Marks et al. 1993). Areas where *Phragmites* is dominant tend to have both lower plant and animal species richness, especially in freshwater marshes (Meyerson et al. 2000). Within a stand detritus tends to accumulate resulting in reduced light and lower temperatures, which may prevent many other plant species from germinating. Information regarding the impact of *Phragmites* on wildlife is scarce. Little information is available on mammal use of *Phragmites*, though

muskrats (*Ondatra zibethicus*) have been documented foraging on the rhizomes and clipping the stalks for use in building their lodges (Daiber 1982). The presence of muskrats in areas with abundant *Phragmites* may benefit ducks, rails, and other wetland birds and song birds by removing patches of *Phragmites* which establishes small, secluded pools (Benoit and Askins 1999).

Riparian vegetation is a vital component of river otter habitat and changes in vegetation can impact river otter populations (Melquist and Dronkert 1987; Newman and Griffin 1974). Decreased structural diversity of vegetation on shorelines can decrease the prey and cover available to otters (Allen 1987). Dense riparian vegetation is often used by dispersing otters for den and resting sites. However, this is likely a result of the otter being unfamiliar with the area or a lack of suitable den sites immediately accessible (Melquist and Hornocker 1983). Riparian vegetation is also more frequently used during the spring, likely due to flooding of bank dens (Melquist and Hornocker 1983). The importance of riparian vegetation for otters coupled with rapid transformation of riparian habitat in Nebraska makes understanding otter use of non–native riparian vegetation critical to a management plan for river otters in Nebraska.

Because of this, my research objectives were to determine if: 1) the number of unique den sites utilized by river otters in *Phragmites* was in proportion to availability and 2) the frequency of den sites utilized by river otters in *Phragmites* was in proportion to availability.

MATERIALS AND METHODS

Study area

My study area was the stretch of the Platte River and its associated channels and lakes located in central Nebraska known as the Big Bend reach (Sidle and Faanes 1997). This area of the river was chosen because the majority of otter sightings in Nebraska since reintroduction have been along the Platte River. Fifty–three percent of all river otter sightings in Nebraska between 1987 and 2002 occurred within one kilometer of the Platte River and 63% occurred within three kilometers (Bishof 2003). This reach has been designated a priority ecosystem by the United States Geological Survey (USGS), has undergone severe landscape and vegetative changes in the past decade, and has become the primary focus of many conservation and restoration organizations including The Nature Conservancy, NGPC, The Whooping Crane Habitat Maintenance Trust, and the United States Fish and Wildlife Service. The extensive research being conducted in the area and the habitat restoration efforts underway make this an ideal study location for comparing river otter habitat use and the effects of changes in habitat. The majority of the study area is owned by private landowners, but other sections are owned by The Nature Conservancy and The Whooping Crane Habitat Maintenance Trust, or are part of state recreation areas or state wildlife management areas. Otter trapping occurred primarily along a 13 mile stretch of the Platte River between the Shelton and Alda exits of Interstate 80 in the fall of 2006, 2007, and 2008.

Land cover

I utilized a 2005 land cover map created by the Great Plains GIS Partnership to determine habitat available to otters. The map was classified from aerial imagery taken

in August and September in 2004 and 2005. The original file had a one meter spatial resolution that was converted to ten meters to reduce file size for use in analyses (Brei and Bishop 2005). The overall accuracy associated with the land cover is 98.7%, however when agriculture, water, and developed land cover types are removed, the accuracy decreases to 82.7%. The *Phragmites* cover class has an accuracy of 80.6%. The distribution of *Phragmites* changes seasonally as the river floods and dries. The lower accuracy for *Phragmites* can be attributed to this seasonal change, as well as the lapse in time between the initial data collection and imaging.

All spatial analyses were performed using the 2005 land cover data in vector format (the initial land cover was produced in both raster and vector formats) in ArcGIS 9.3 (ESRI Redlands, CA; Brei and Bishop 2005). The original land cover file contained 27 land cover types. I reclassified these into six land cover types (Table 2.1): agriculture, *Phragmites*, roads, riparian vegetation (purple loosestrife, *Lythrum salicaria*; riparian shrubland, river early successional, riparian woodland, river shrubland, and un–vegetated sandbar), water (canal/drainage, irrigation reuse pit, lagoon, reservoir, river channel, sandpit, stock pond, and floodplain marsh), and other (bare ground/sparse vegetation, meadow sand ridge, mesic wet meadow, undisturbed grassland, upland shrubland, upland woodland, rural developed, upland grassland, urban/suburban, and xeric wet meadow).

Capture and Radio–tagging

River otter movement and location data were collected through radio telemetry of radio tagged otters. Trapping occurred during the fall and early winter months to ensure that no females with dependent young were captured and to prevent heat exhaustion. Trapping locations were determined by tips from landowners as well as the identification

of sites with extensive otter sign. Sleepy Creek #11 double long–spring foothold traps with a double jaw (Sleepy Creek Manufacturing, Berkley Springs, WV) modified with shock springs and three swivels and Oneida Victor #1.5 soft–catch padded coil spring foothold traps (Oneida Victor Inc. Cleveland, OH) modified with shock springs and three swivels were used for capture (Oneida Victor #1.5 soft–catch padded coil spring foothold trap was only used briefly at the beginning of the study). When these traps are used correctly there is minimal risk of serious damage to the captured animal (Blundell et al. 1999).

River otters cannot be baited to traps, so traps were set in areas where river otter presence was determined by the presence of tracks and scat. Depending on the location and concentration of this sign it was determined subjectively by the trapper where the otter was most likely to "haul out" onto land. Traps were set both on land and in water. Land traps were usually placed around otter latrine sites which are repeatedly used by one or more otters, and at the beginning and end of crossover trails between the river and a lake or slough. River otters tend to emerge from the water in the easiest and most accessible location. Attempts were made to funnel otters to traps by creating a ramp in the sand. Traps were then set and placed in the water where the otter was likely to step. Suitable trapping locations had no logs or deep rooted vegetation that the trap's chain could become tangled in. Traps were anchored on land so that when the chain was stretched out it barely reached the water where the trap was to be set. This ensures that the otter has the option of being in the water or on land to thermoregulate.

Trapped otters were put into a transport carrier that consisted of a 20 gallon smooth plastic barrel that was modified with a sliding Plexiglas door (similar to method

described by Serfass et al. 1993) and driven to Lincoln, NE. Under the supervision of a veterinarian, otters were restrained using a squeeze box as described by McCullough (1986) and anesthetized with ketamine and midazolam at a rate of 20 mg/kg and 0.25 mg/kg respectively. A sterilized radio transmitter (ATS M1250B, 3 stage or M1240, 2 stage with motion and mortality sensors) was implanted through the abdominal musculature following the procedure described by Hernandez–Divers et al. (2001). Meloxicam was administered for pain at 0.1 mg/kg and an injection of Penicillin benzathine was administered subcutaneously at 70,000 IU/kg to prevent infection.

During surgery the otter's heart rate, respiratory rate, and body temperature were closely monitored and recorded. After surgery otters received fluids (Lactated Ringer's solution) under the skin before being placed into a kennel to recover. A passive integrated transponder (PIT) tag was inserted under the skin at the base of the tail for permanent identification. After the otter recovered from the anesthesia (usually within a few hours of surgery) it was taken back to the trap site and released. All techniques for trapping and handling were approved by the University of Nebraska–Lincoln Institutional Animal Care and Use Committee (UNL–IACUC #06–09–035D and #432).

Monitoring

All animals with radio transmitters were monitored from the ground using a Communication Specialists R–1000 receiver, an omni–directional antenna, and a three element Yagi antenna. Researchers attempted to locate each animal two to four times per week, or as frequently as possible. If ground contact was lost over a long period of time, telemetry fixed–wing aircraft was used to attempt to relocate lost animals. When the actual location was accessible, a GPS location was taken at that point. Inaccessible or

distant locations were determined via triangulation or biangulation. Otters were tracked until the transmitter stopped functioning (at approximately 600 days) or until otters were located at least 50 times. No den was recorded as being used twice on the same day by the same otter. Data collection ended December 2009.

Statistical analysis

For the purposes of this study I used only exact locations (hereafter, sites are considered a place whereas locations are a place with associated otter identification). All locations that were triangulated or biangulated were removed because the error associated with these points was too large for fine scale habitat use analysis. I also removed all locations in which the otter was moving, thus remaining locations were known den or resting sites. Den sites are considered to be more permanent features that otters spend an extended amount of time in, while resting sites are sites where otters stay briefly as they travel between den sites. To identify unique den sites (sites that are spatially unique and weighted equally without regard to the number of times it was used) duplicate locations, occurring when two or more locations were within 30 meters of each other (the approximate accuracy of the handheld GPS units), were removed. The three eastern most and western most (approximately 5% of the total number of locations) outliers were removed because I cannot adequately assess habitat use in this area because it is the edge of the range of tagged individuals and is thus not primarily used by tagged otters. The same methods were applied to the unique den sites for males only and females only. To assess how frequently otters use dens in different habitats I used all the den/resting locations collected, which weighted each site based on the number of times it was used. For frequency data, outliers were any points that fell outside of the east and west

boundary that was created by the removal of outliers for unique den/resting sites. This was done to ensure the same areas were used for both the unique den analysis and the frequency of den use analysis. The same methods were applied to frequency data for males only and females only. The north channel of the Platte River between the Wood River and Alda I–80 exits was removed from the expected habitat analyses because I could not access that stretch of river.

Chi–squared goodness of fit tests were performed to test for differences in otter use of *Phragmites* relative to availability (SAS® 9.2, PROC FREQ; SAS Institute 2010). I used an alpha of 0.10 to determine significance because of the small sample size, and to reduce the likelihood of Type II errors (Holling and Allen 2002). When statistically significant results were identified, I further analyzed these using the PROC FREQ (SAS® 9.2; SAS Institute 2010) command for binomial proportions to identify where the differences were. For analyses with small sample size, chi–squared exact tests were performed. Analyses were conducted using unique den/resting sites and for the frequency of use of den/resting sites. Within these two categories I analyzed locations of both males and females, males only, and females only for a total of six different analyses.

There has been no research on river otter ecology in Nebraska. To avoid making false assumptions I varied two assumptions regarding habitat availability; the extent that otters will travel overland to reach den/resting sites and the different land cover types that are considered available habitat. River otter literature has documented otters traveling several kilometers overland, but their primary activity is usually within a few meters of the water. To account for this variation I performed the analysis using different assumptions regarding otter movements for each of the six groups, defining available

habitat by buffering the riparian habitat by 100 meters, one kilometer, and three kilometers (Figures 2.1–2.3). Within each of these buffers available den habitat was either defined as any of the six land cover types (agriculture, other, roads, water, riparian vegetation, and *Phragmites*) or as only riparian vegetation and *Phragmites*. All six land cover types were used first to confirm the assumption that otter habitat use is limited to riparian areas (riparian vegetation, *Phragmites*, and water). The analysis was then limited to only riparian vegetation and *Phragmites* as available habitat for den/resting sites (water was not included because otters do not den directly in the water, though dens are often in close proximity to water).

RESULTS

Between September 2006 and December 2009 18 otters were radio–tagged and a total of 1,030 locations recorded. Radio–tagged otters were tracked as far west as Kearney and as far east as Columbus, Nebraska. Of the 1,030 locations, 540 were exact locations and known den/resting sites (Appendix A; Appendix B). There were no exact locations recorded for two otters who's transmitter signals were quickly lost, therefore the following analyses is from location data collected from 16 otters (eight males and eight females). Otters often frequented the same den site multiple times. There were 127 unique den/resting sites used by 16 otters from a total of 517 den/resting sites (after outliers were removed). There were 64 unique den/resting sites used by males out of 191 total den/resting locations for males, and 74 unique den/resting locations used by females out of 326 den/resting locations for females.

Unique den/resting sites utilized by river otters

River otters used habitat different than expected for unique den/resting sites with all six land cover types included and at all three availabilities (100 meters, 1 kilometer, and 3 kilometers). Agriculture and other were used less than expected, roads were used in proportion to availability and riparian vegetation, water, and *Phragmites* were used more than expected. With only two land cover types (*Phragmites* and riparian vegetation) included, otters used habitat significantly different than was expected. For all three availabilities, otters used riparian vegetation less than expected and *Phragmites* more than expected. The percent cover types for both expected habitat use and observed habitat use are in Table 2.2. Chi–squared values and p–values are reported in Table 2.8.

Unique den/resting sites utilized by male river otters

Male river otters used habitat differently than expected at all three availabilities and with all land cover types included for unique den/resting sites. Agriculture and other were used less than expected, roads were used in proportion to availability, and water, riparian vegetation and *Phragmites* were used more than expected. However, with only riparian vegetation and *Phragmites* considered available, male otters used the habitat in proportion to availability at all three availabilities for unique den/resting sites. The percent cover types for both expected habitat use and observed habitat use are reported in Table 2.3. Chi–squared values and p–values are reported in Table 2.8.

Unique den/resting sites utilized by female river otters

Female river otters used habitat differently than expected with all land cover types included and at all three availabilities for unique den/resting sites. Females used agriculture and other less than expected, roads in proportion to availability, and water,

riparian vegetation and *Phragmites* more than expected. With only two land cover types included, female otters used habitat significantly different than was expected. For all three availabilities, females used *Phragmites* more than expected and riparian vegetation less than expected. The percent cover types for both expected habitat use and observed habitat use are in Table 2.4. Chi–squared values and p–values are reported in Table 2.8.

Frequency of den/resting sites utilized by river otters

River otters used habitat differently than expected with all land cover types included and at all three availabilities for the frequency of den/resting sites utilized. Otters utilized agriculture, other, and roads less frequently than expected, and water, riparian vegetation and *Phragmites* more than expected. With only two land cover types included, there was no difference at any of the three availabilities between the expected frequency of use and the observed frequency of use. The percent cover types for both expected habitat use and observed habitat use are in Table 2.5. Chi–squared values and p–values are reported in Table 2.9.

Frequency of den/resting sites utilized by male river otters

Male river otters used habitat differently than expected with all land cover types included and at all three availabilities for the frequency of den/resting sites utilized. Male otters used agriculture and other less than expected and water, riparian vegetation and *Phragmites* more than expected at all three availabilities. Roads were used in proportion to availability when the river was buffered by 100 meters, but were used less for the 1 kilometer and 3 kilometer availabilities. With only riparian vegetation and *Phragmites* considered available, there was no difference between the expected frequency of use and the observed frequency of use for male otters. The percent cover types for both expected

habitat use and observed habitat use are in Table 2.6. Chi–squared values and p–values are reported in Table 2.9.

Frequency of den/resting sites utilized by female river otters

Female river otters used habitat differently than expected with all land cover types included and at all three availabilities for the frequency of den/resting sites utilized. Female otters used agriculture, other, and roads less than expected and water, riparian vegetation and *Phragmites* more than expected at all three availabilities. With only riparian vegetation and *Phragmites* considered available, there was no difference between the expected frequency of use and the observed frequency of use for female otters. The percent cover types for both expected habitat use and observed habitat use are in Table 2.7. Chi–squared values and p–values are reported in Table 2.9.

DISCUSSION

In order to understand river otter use of riparian habitat invaded by *Phragmites*, I performed multiple levels of analysis to increase the robustness of the results and diminish the likelihood of making Type I errors. The most conservative method used for estimating habitat available to otters is the 100 meter buffer around the river channel, as otters do not frequently travel much further overland (Melquist and Hornocker 1983). However, because otters have been documented traveling greater distances, the Platte River channel is very complex and braided, and little is known about the habits of otters in Nebraska, analyses using a one kilometer buffer of the river channel might also be appropriate. I performed these analyses where all six defined land cover types were included and with only two land cover types included in the analyses (*Phragmites* and riparian vegetation). All analyses that included all land cover classes indicated that otters

prefer riparian areas (riparian vegetation, *Phragmites*, and water). My conclusions are based on the results of the analyses that used the 100 meter buffer for available habitat and with two land cover classes.

Otters strongly selected riparian habitats. This is evident from analyses that included all six land cover types, indicating that agriculture, other, and roads were used less than expected and *Phragmites*, riparian vegetation and water were used more. Because otters do not den directly in the water the apparent increased use of water supports previous research that otters most often den in very close proximity to the water (Melquist and Hornocker 1983). This is also supported by observations that many of the dens that the otters occupied were on the bank in beaver lodges or dens.

Riparian vegetation adjacent to wetland areas are an essential habitat component for river otters, as they not only provide cover, but are also attractive to beavers. Otters tend to be more abundant in areas of high beaver density, because beaver dens are numerous and provide shelter with an underwater escape route (Melquist and Hornocker 1983). Otters have also been documented using resting or den sites in dense riparian vegetation, though this has been most commonly documented for dispersing otters or those otherwise unfamiliar with the area (Melquist and Hornocker 1983; Dubec et al. 1990). Melquist and Hornocker (1983) observed an increase in use of riparian vegetation in the spring and hypothesized that this was a result of the flooding of bank dens.

Otters prefer *Phragmites* for unique den/resting sites but, this appears to be a result of female use; males showed no preference for either *Phragmites* or riparian vegetation, but used them in proportion to their availability. However, frequency of use

of each den habitat for females, showed no difference for female otter use of *Phragmites* and riparian vegetation. *Phragmites* can spread rapidly, and it likely expanded its range between image acquisition and the collection of otter data. However, by including land cover types susceptible to invasion such as un–vegetated sandbars with riparian vegetation, my results are conservative because this potentially over estimates the use of riparian vegetation by otters.

Den and resting sites are not a limited resource for otters in the study area. Thus, female use of *Phragmites* is not likely due to resource co–option by males. The characteristically dense stands of *Phragmites* may be more important for females with young because it provides adequate cover for resting sites. Since it is unknown how much time was spent at each location it is possible that less time was spent in areas with *Phragmites*. During data collection, a female with pups was observed resting in a dense stand of *Phragmites*. Alternatively, *Phragmites* is abundant throughout the study area and provides the same quality of cover in all locations. Beaver dens and logjams however, may be more difficult to locate. Therefore, when these types of shelter are found, the otter may repeatedly use that structure because its quality and availability are already known and a similar structure may not be easily located.

Habitat features used for den and resting sites is determined by availability. Otters seek areas for den and resting sites that are secluded and provide adequate cover and protection (Melquist and Hornocker 1983; Melquist and Dronkert 1987). The predominate geographic feature that meets these requirements will be the most utilized. Otters in central Idaho most often used logjams and beaver bank dens, as these were the most available features (Melquist and Hornocker 1983). Conversely, the coastal river

otters of Alaska preferred old growth forests where the root masses of large conifers provided cavities and crevices in which to hide (Bowyer et al. 1995).

Although this analysis is robust enough to draw the conclusion that there is some preference for *Phragmites* for otters, there are limitations to the analysis created by the land cover data. Errors associated with the land cover data could arise because of the time lapse between image acquisition for the land cover map and when the otter location data were collected. Otters were tracked from the fall of 2006 to the winter of 2009. There is annual and seasonal variation that occurs with *Phragmites* as a result of flooding and drought. *Phragmites* removal programs began in 2007 when parts of the study area were sprayed with herbicide and later disked in 2008. Since more broad range eradication efforts did not begin until 2009, these changes likely had a negligible effect on the data analysis.

Future studies should examine general otter habitat use both on the Platte River and other rivers throughout the state as well as the most commonly used den types. As *Phragmites* eradication efforts continue throughout the study area, otters should be monitored to detect changes in habitat use for den/resting sites when *Phragmites* is no longer available. The invasive genotype of *Phragmites* was not historically present in Nebraska while river otters were. The apparent preference that female otters show for *Phragmites* for den and resting sites is probably a result of its availability. As habitat alterations currently underway on the central Platte River intensify, it is important to recognize the effects this might have on river otters. Riparian vegetation has long been recognized as a vital component of otter habitat (Melquist and Dronkert 1987). Therefore, the absence of *Phragmites* as a result of eradication efforts is not likely to

negatively impact otters in the future; though a failure to encourage dense, native growth along the banks may decrease the available habitat. To ensure the continued success of the river otter reintroduction in Nebraska it is imperative that managers take into account the importance of riparian vegetation for river otter habitat.

Table 2.1. The 2005 Platte River land cover developed by the Great Plains GIS Partnership originally contained 27 land cover types. To evaluate river otter movements in relation to *Phragmites* these 27 land covers were reclassified by grouping similar land covers together into six new land cover types.

Land cover type reclassification	Original land cover type
Agriculture	Agriculture
Phragmites	*Phragmites*
Roads	Roads
Riparian vegetation	Purple loostrife Riparian shrubland River early successional Riparian woodland River shrubland Un–vegetated sandbars
Water	Canal/drainage Irrigation reuse pit Lagoon Reservoir River channel Sand pit Floodplain marsh
Other	Bare ground/ sparse vegetation Meadow sand ridge Mesic wet meadow Undisturbed grassland Upland shrubland Upland woodland Rural developed Upland grassland Urban/suburban

Table 2.1. Continued.

Land cover type reclassification	Original land cover type
	Xeric wet meadow

Table 2.2. The distribution of unique den/resting sites for all otters with the percent of locations present in each land cover type with all cover types included and with two cover types (*Phragmites* and riparian vegetation) included.

	Locations with 30m buffer (%)	Available habitat (%)		
Land cover types		100m	1km	3km
All land cover types				
Agriculture	1	17	32	51
Other	17	42	41	32
Phragmites	12	3	2	1
Riparian vegetation	53	30	18	10
Roads	1	2	3	3
Water	17	6	4	2
Two land cover types				
Phragmites	18	10	11	10
Riparian vegetation	82	90	89	90

Table 2.3. The distribution of unique male otter den/resting sites with the percent of locations present in each land cover type with all cover types included and with two cover types (*Phragmites* and riparian vegetation) included.

	Locations with 30m buffer (%)	Available habitat (%)		
Land cover types		100m	1km	3km
All land cover types				
Agriculture	2	17	32	51
Other	16	42	41	32
Phragmites	8	3	2	1
Riparian vegetation	55	30	18	10
Roads	1	2	3	3
Water	18	6	4	2
Two land cover types				
Phragmites	13	10	11	10
Riparian vegetation	87	90	89	90

Table 2.4. The distribution of unique female otter den/resting sites with the percent of locations present in each land cover type with all cover types included and with two cover types (*Phragmites* and riparian vegetation) included.

	Locations with 30m buffer (%)	Available habitat (%)		
Land cover types		100m	1km	3km
All land cover types				
Agriculture	1	12	28	50
Other	15	47	44	33
Phragmites	13	4	2	1
Riparian vegetation	52	30	18	11
Roads	1	1	3	2
Water	17	6	4	2
Two land cover types				
Phragmites	19	12	11	10
Riparian vegetation	81	88	89	90

Table 2.5. The distribution of all otter den/resting sites with the percent of locations present in each land cover type with all cover types included and with two cover types (*Phragmites* and riparian vegetation) included

	Locations with 30m buffer (%)	Available habitat (%)		
Land cover types		100m	1km	3km
All land cover types				
Agriculture	1	17	32	51
Other	24	42	41	32
Phragmites	6	3	2	1
Riparian vegetation	52	30	18	10
Roads	1	2	3	3
Water	17	6	4	2
Two land cover types				
Phragmites	10	10	11	10
Riparian vegetation	90	90	89	90

Table 2.6. The distribution of all male otter den/resting sites with the percent of locations present in each land cover type with all cover types included and with two cover types (*Phragmites* and riparian vegetation) included.

	Locations with 30m buffer (%)	Available habitat (%)		
Land cover types		100m	1km	3km
All land cover types				
Agriculture	1	17	32	51
Other	2	42	41	32
Phragmites	5	3	2	1
Riparian vegetation	51	30	18	10
Roads	1	2	3	3
Water	22	6	4	2
Two land cover types				
Phragmites	9	10	11	10
Riparian vegetation	91	90	89	90

Table 2.7. The distribution of all female otter den/resting sites with the percent of locations present in each land cover type with all cover types included and with only two cover types (*Phragmites* and riparian vegetation) included.

	Locations with 30m buffer (%)	Available habitat (%)		
Land cover types		100m	1km	3km
All land cover types				
Agriculture	<1	12	28	50
Other	26	47	44	33
Phragmites	6	4	2	1
Riparian vegetation	54	30	18	11
Roads	<1	1	3	2
Water	14	6	4	2
Two land cover types				
Phragmites	10	12	11	10
Riparian vegetation	90	88	89	90

Table 2.8. Chi–squared and P–values for unique den/resting sites for all otters, males only, and females only for 100 meters, 1 kilometer, and 3 kilometer availabilities for analyses with all land cover types included and with two cover types (*Phragmites* and riparian vegetation) included.

	Unique all		Unique males		Unique females	
Availabilities	χ^2	P–value	χ^2	P–value	χ^2	P–value
All land cover types						
100 meters	116.60	<0.001	51.72	<0.001	64.88	<0.001
1 kilometer	253.59	<0.001	117.33	<0.001	155.49	<0.001
3 kilometers	587.16	<0.001	272.05	<0.001	341.42	<0.001
Two land cover types						
100 meters	6.34	0.01	0.33	0.63	3.08	0.08
1 kilometer	4.41	0.04	0.11	0.82	4.27	0.04
3 kilometers	6.34	0.01	0.33	0.63	5.79	0.02

Table 2.9. Chi–squared and P–values for frequency of use of den/resting sites for all otters, males only, and females only for 100 meters, 1 kilometer, and 3 kilometer availabilities for analyses with all land cover types included and with two cover types (*Phragmites* and riparian vegetation) included.

	Frequency all		Frequency males		Frerquency females	
Availabilities	χ^2	P–value	χ^2	P–value	χ^2	P–value
All land cover types						
100 meters	335.54	<0.001	165.83	<0.001	166.13	<0.001
1 kilometer	805.6	<0.001	362.32	<0.001	455.47	<0.001
3 kilometers	1915.56	<0.001	844.12	<0.001	1019.1	<0.001
Two land cover types						
100 meters	0.06	0.80	0.01	0.93	0.91	0.34
1 kilometer	0.70	0.40	0.19	0.67	0.26	0.61
3 kilometers	0.06	0.80	0.01	0.93	0.00	0.98

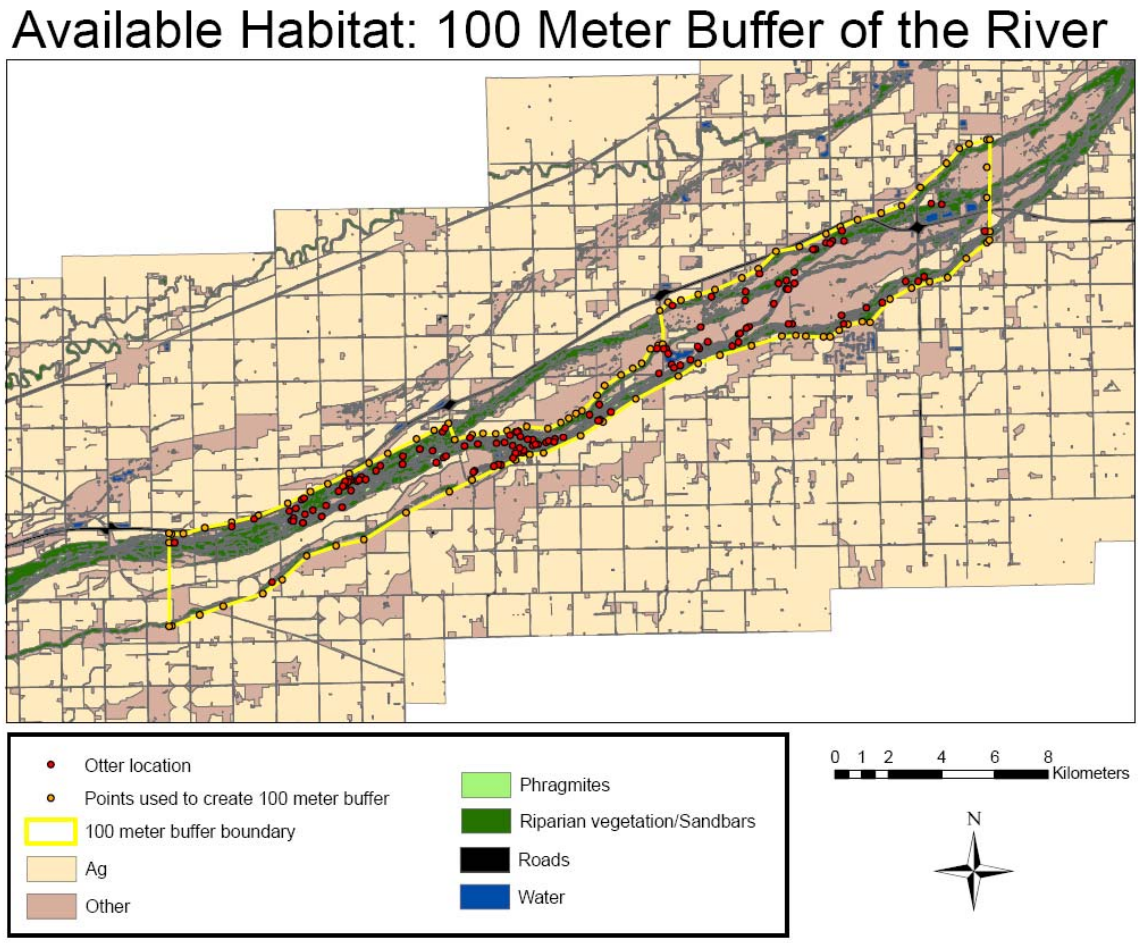

Figure 2.1. The area used to determine expected habitat use by river otters when the river channel was buffered by 100 meters.

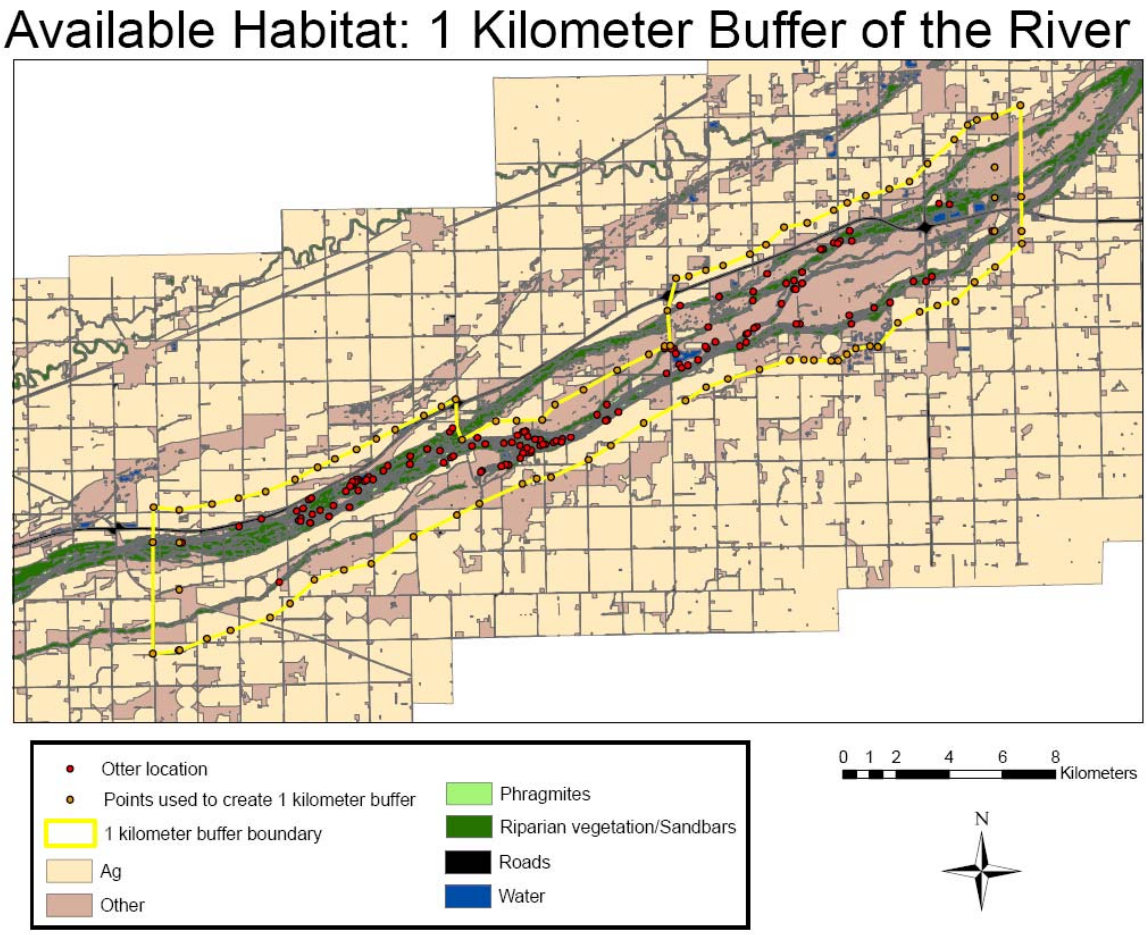

Figure 2.2. The area used to determine expected habitat use by river otters when the river channel was buffered by 1 kilometer.

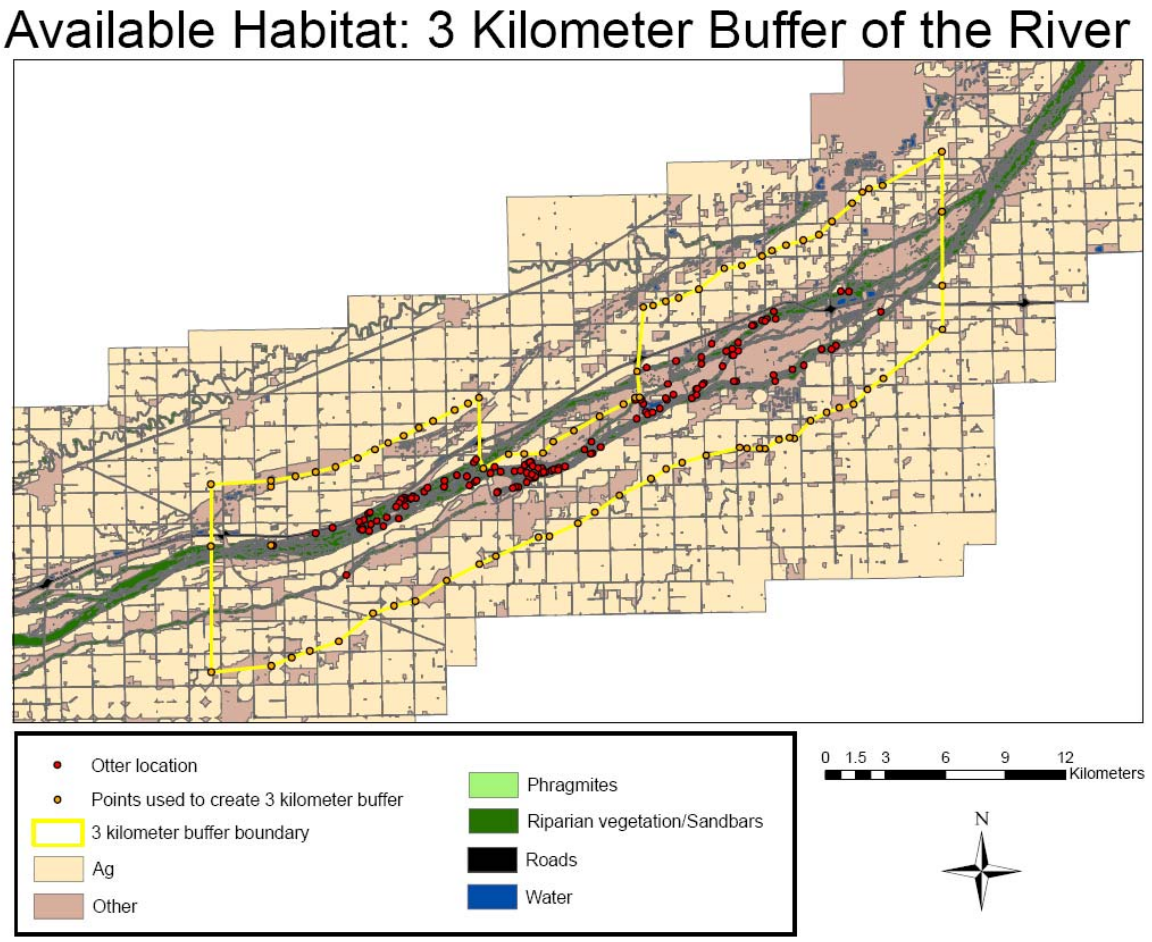

Figure 2.3. The area used to determine expected habitat use by river otters when the river channel was buffered by 3 kilometers.

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CHAPTER 3: ESTIMATING RIVER OTTER (*LONTRA CANADENSIS*) DENSITY USING NON–INVASIVE GENETIC TECHNIQUES

INTRODUCTION

River otters were rare in Nebraska as early as 1908, and they were probably extirpated not long after that (Jones 1964). Between 1986 and 1991 the Nebraska Game and Parks Commission (NGPC) reintroduced 159 river otters, originating primarily from Alaska and Louisiana, into five of Nebraska's rivers (Bischof 2003). Following these reintroductions the Nebraska Game and Parks Commission (NGPC) has collected carcasses from roadsides and incidental harvests by fur trappers (Bischof 2003) and since 2000 has performed annual bridge surveys documenting otter sign. Due to the lack of quantitative data, much remains unknown about river otter ecology and population status in Nebraska. River otters are a state threatened (Tier 1) species and are currently protected from hunting and trapping. The need for quantitative information for otters in Nebraska, especially regarding population size, has increased in the last decade due to dramatic changes to many of Nebraska's riparian habitats, including core habitat such as that found along the Platte River. Population data is critical and necessary for the development of a scientifically sound Nebraska river otter management plan.

The rare and elusive nature of river otters makes population size difficult to estimate. Typical mark–recapture estimates are time consuming and costly. Even extensive trapping can result in few captures, and once trapped otters can become trap shy making recaptures unlikely (Melquist and Hornocker 1979). Otters are also primarily nocturnal so daytime observations are rare. Tracks and scat are the most reliable way of determining river otter presence.

Recent developments in wildlife genetic analyses have made it possible to estimate population size without handling the target species. DNA analysis of scat samples can identify the species, gender, and the individual that deposited the sample (Taberlet et al. 1996). Thus, DNA from collected scat can be used to estimate population size using mark–recapture methods. This method has no effect on the target species and is not biased by trap avoidance or trap happy behaviors, whereas trapping poses risks to the animals and the researcher. It also is relatively inexpensive and less time consuming. The use of scat as a source of DNA can also provide information regarding diet, gender and genetic diversity within a population (Schwartz and Monfort 2008).

Although other biological substances such as hair are commonly used for genetic analysis, given the life history and behavior of river otters, scat is the most logical and reliable source of genetic samples. Scat contains epithelial cells sloughed from the intestines and samples are generally large enough to perform multiple DNA extractions (Schwartz and Monfort 2008). In contrast, hair samples contain relatively few cells and are often consumed after one DNA extraction. River otter life histories create two challenges for using scat: otters tend to use communal latrines which can potentially result in cross–individual contamination and there are chemical inhibitors to DNA amplification in scat. The success of obtaining viable DNA from samples and determining individual identity varies greatly among species as well as among specific studies. The quality of the DNA depends heavily on field conditions, preservation of the sample, and handling in the lab (Schwartz and Monfort 2008). Prigioni et al. (2006) used fecal DNA to estimate population size of the European otter (*Lutra lutra)* in Italy and

successfully genotyped 41% of collected samples, whereas 65% of the samples collected from European otters in Kinmen by Hung et al. (2004) were successfully genotyped.

The successful use of noninvasive genetic analysis from scat can allow researchers to more safely and efficiently study a species that has traditionally been difficult to study, especially in regards to sample size and the spatial extent of the study. This method is particularly important for studying rare carnivores. Otter populations are recovering from near extirpation and are otherwise difficult to sample using traditional techniques. Here I report on the reliability and success of using DNA from scat to estimate river otter density for a biologically unique segment of the Platte River in Nebraska.

Results from this project will inform the creation of a management plan for river otters in Nebraska. In order to effectively and efficiently manage for this species in Nebraska there must be a reliable population estimate. Using DNA from scat will yield this information as well as explore a new technique of studying wildlife that will be useful for future research of river otters as well as other rare or elusive species.

MATERIALS AND METHODS

Study area

River otter scat was collected along a 29 kilometers of the Big Bend reach of the Platte River between Gibbon and Alda exits south of Interstate 80 in Nebraska. A total of 14 volunteers in 11 kayaks and canoes were divided into three groups and assigned to three river segments (determined by bridge crossings with river access). The river segments were designated Gibbon exit to Shelton exit (approximately eight kilometers)

Shelton exit to Wood River exit (approximately 13 kilometers), and Wood River exit to Alda exit (approximately eight kilometers).

Scat collection

Scat was collected twice from the study area, first on September 23, 2009 and then again on October 7, 2009. Equal numbers of volunteers were used for each sampling day to ensure equal effort during both collections. Prior to collection all volunteers were instructed to identify otter scat by its unique appearance and to focus searches especially on the points of sand bars and raised ground, where otter latrines are most likely to be found. To ensure efficiency and reliable sampling each stretch of river had at least one biologist experienced with river otters.

Volunteers and researchers canoed and kayaked along the river banks and sandbars searching for otter scat in open sandy areas, points on sandbars, as well as bank areas where beaver dens were visible (as often otters utilize these dens). When scat was found each sample was collected in a quart size Ziploc bag. Contamination was avoided as much as possible by turning each bag inside out and using it as a "glove" to collect individual samples (samples were determined to be deposited by different individuals or at different times based on appearance and location at the discretion of the collector). During the first collection in September all scat remaining on the ground after collection was removed to ensure that no samples were collected twice. All samples were labeled with the stretch of the river they were found on as well as the GPS coordinates for that specific location.

Scat preservation

Within one day of collecting the scat all samples were transferred from the plastic bags to paper bags. Paper bags were then folded and placed under a fume hood to dry. Samples were dried for three days and then placed in a cardboard box in a cool dry area for storage.

DNA extraction

The QIAamp DNA Stool Mini Kit (QIAGEN Sample and Assay Technologies) was used for DNA extractions and the section Protocol: Isolation of DNA from Stool for Human DNA Analysis in the QIAamp DNA Stool Handbook (QIAGEN 2007) was used for procedures with changes described below (Mowry 2010). All DNA extractions were performed in a designated room to decrease the possibility of contamination.

Each scat sample was removed from the paper bag and the outer layer of the scat was scraped into a 2ml tube (tube was filled approximately 2/3 full of dried scat). Approximately 1.6 ml of buffer ASL was added to each scat sample. Samples were vortexed continuously for one minute to homogenize the sample. Following this, samples were centrifuged for one minute so the scat particles formed a pellet. The remaining supernatant was then put into a new, labeled 2ml tube and the scat particles discarded.

One InhibitEX tablet was added to each sample (only half a tablet was added if there was not much supernatant) and the sample was then vortexed for one minute to suspend the tablet. The sample was then incubated for one minute at room temperature to allow the InhibitEX tablet to absorb the inhibitors present in the scat. After centrifuging

for five minutes the supernatant was removed and placed into a new, labeled 1.5ml tube and the pellet discarded. The sample was then centrifuged for three minutes to remove any remaining solid and was transferred to a new 2ml tube along with 25µl of proteinase K and 600 µl of Buffer AL. The sample was then vortexed for 15 seconds and placed in a dry bath incubator at 70ºC for ten minutes.

After incubating, 600 µl of 95% ethanol was added to the lysate and mixed briefly by vortexing. Then, 600 µl of the lysate was aliquoted to the 2ml spin column (a microcentrifuge tube containing a silica membrane that the DNA binds to) and centrifuged for 1 minute. The spin column was then placed in a new 2ml tube and another 600 µl of lysate was aliquoted and put in the centrifuge for 1 minute. This step was then repeated for the remaining 600 µl of lysate.

After the spin column was placed into a new 2ml tube, 500 µl of Buffer AW1 was added and centrifuged for 1 minute. The spin column was put into a new 2ml tube and Buffer AW2 was added and the tube was centrifuged for 3 minutes. Then, the spin column was placed a new 2ml tube and centrifuged for 1 minute to ensure that all of Buffer AW2 was removed. Lastly, the spin column was placed into a 1.5ml tube and 200 µl of Buffer AE was added. The sample was incubated for 10 minutes at room temperature and then centrifuged for 1 minute in order to elute the DNA. The final 1.5ml tube containing the DNA elution was then stored at -20 ºC.

PCR to test for DNA extraction success

Polymerase chain reaction (PCR) was performed, following the protocol designed by Mowry (2010) at the RIO07R and RIO16R microsatellite loci (as defined by Mowry

2010) to evaluate the success of the DNA extraction. These two loci were chosen as test loci because they have been shown to have high amplification success (Mowry 2010).

First a cocktail of reagents for each of the loci was mixed in a 1.5 ml tube. The total volume of each cocktail is dependent on the number of samples to be tested. Per reaction, 10.9 µl of ultra pure PCR water, 2.5 µl of buffer, 2.5 µl of 2 mM dNTP's (deoxyribonucleotide triphosphate), 1 µl forward primer (locus specific), 1 µl reverse primer (locus specific), 2 µl of $MgCl_2$ (Magnesium Chloride), 2 µl BSA (bovine serum albumin), and 0.1 µl TaqGold was added to the 1.5ml tube. The cocktail was then briefly mixed by vortexing.

PCR reactions were performed in 96–well plates. Twenty–two microliters of cocktail (either RIO07R cocktail or RIO16R cocktail) was placed in each well. Three microliters of template DNA were added to each reaction. In addition to the samples being tested a positive (positive samples were only run on the first few plates to ensure samples with otter DNA were amplifying) and a negative PCR sample was also prepared to identify any contamination. Positives were obtained using tissue samples from carcasses collected by NGPC.

Finally, plates were sealed with PCR sealing film and placed in the centrifuge for 15 seconds to ensure no drops remained on the edges of the wells. The plates were then put into the thermal cycler and the following PCR profile was run:

95 ºC for 10 min

45 cycles of:

95 º C for 1 min

56 ºC for 1 min

72 ºC for 1 min

1 cycle of:

72 º C for 10 min

After the PCR was completed the samples were run through an electrophoresis agarose gel to visualize the presence of DNA. A 2% super–fine gel (NuSieve 3:1) was prepared with TBE buffer and GelStar (Lonza) loading dye, and poured into an agarose gel mold to set. Once the gel had set it was placed in the electrophoresis apparatus filled with 0.5X TBE buffer. Approximately 5 µl of the PCR product from each sample was mixed with approximately 1 µl of loading buffer. The first well in the gel was loaded with a ladder and all subsequent wells were filled with PCR product mixed with loading buffer. Gels were run for approximately 30 to 45 minutes at 130 volts or until the dye appeared to be about half way down the gel. Gels were then placed in a spectrophotometer to visualize the DNA. If the positive sample amplified well and the negative sample was clean then the samples that were positive for otter DNA and those that were negative were recorded for each locus tested.

Multiplex PCR

Multiplex PCR was performed on the samples that yielded DNA following the protocol of Mowry (2010). Multiplexes are a combination of primers for the microsatellite loci that are amplified all at once instead of having to perform separate, individual reactions for each sample at each locus. Two multiplexes must be run for each sample, one with five of the microsatellite loci and the other with the five remaining loci. Per sample, per multiplex, 5 µl of Master Mix (QIAGEN), 0.5 µl of diluted primer mix

(mix containing the five labeled forward primers and the five corresponding reverse primers along with 1:5 TE buffer), 0.8 µl of BSA and 2.5 µl of ultra pure PCR water was added. Finally, 1.2 µl of DNA extract was added to each reaction. A positive and negative were run with each plate. The samples were then put into the thermal cycler and the following PCR profile for multiplexes was run:

95 ºC for 10 min

40 cycles of:

94 ºC for 30 sec

56 ºC for 90 sec

72 ºC for 60 sec

1 cycle of:

60 ºC 30 min

After the PCR was complete, the samples were sent to the University of Missouri DNA Core for fragment analysis. An ABI 3730 DNA Analyzer (Life Technologies Corporation, Carlsbad, CA) was used to size DNA fragments for genotyping.

Genotyping and population estimation

The results from the fragment analysis were analyzed using GeneMarker version 1.91 (SoftGenetics LLC, State College, PA). For each sample, all microsatellite loci were examined and the individual represented by the sample was identified as being either a homozygote or heterozygote at that locus. Using the comparative method described by Frantz et al. (2003) heterozygotes were confirmed when there were at least two matching genotypes and homozygotes when there were at least three matching genotypes. Multiplex PCR was performed on some samples up to seven times so that a

consensus genotype could be reached. Samples in which a consensus genotype could not be definitively determined were discarded from further analysis. All allele scoring and genotype consensuses were performed by the same researcher to prevent individual bias. I assumed genotyping errors are comparable to that identified by (Mowery 2010) because the same microsatellites and laboratory protocols were used.

Once all samples were genotyped I compared genotypes and identified individuals. I compared matching genotypes two different ways, the liberal and conservative methods. If there was uncertainty about whether two samples represented one or two individuals, the liberal method identified the two samples as coming from two individuals. Alternatively, the conservative method identified these two samples as representing one individual. By using these two methods I increased the robustness of the analysis and accounted for any potential errors from uncertainty in the number of individuals identified.

Using the unique genotypes I performed analyses in GENEPOP 4.0.9 (Raymond and Rousset 1995) to test the assumption that the population is in Hardy–Weinberg equilibrium and that the assumption of linkage disequilibrium among microsatellite loci was valid (α=0.005 after using the Bonferroni procedure for multiple comparisons). I used Gimlet 1.3.3 (Valiere 2002) to calculate the probability of identity for siblings ($P_{(ID)sibs}$; the probability that two siblings share a genotype) that was used to determine the number of loci that needed to be genotyped to differentiate between related individuals, and the observed and expected heterozygosity values for each locus (Paetkau and Strobeck 1994; Waits et al. 2001).

For population size estimation individuals that were collected during the first sampling session on September 23, 2009 were considered "marked". If those individuals were also identified in the second sampling session on October 7, 2009 they were recaptures. Population size was estimated for the liberal method genotypes and for the conservative method genotypes using two methods. I first used the Lincoln–Peterson model adjusted for small sample size to estimate the number of individuals in the population, the 95% confidence interval, and the detection probability (Chapman 1951; Appendix C, equations 1–3). I also used Program MARK for closed captures to compare two models (White and Burnham 1999). The first model, p(t)=cN, has the detection probability for captures (p) as a function of time (t), with the detection probability for recaptures (c) equal to the detection probability for captures. The second model, p.=cN, assumes that the detection probability for captures is not a function of time but is constant, and that the detection probability for recaptures is equal to the detection probability for captures. For both models the detection probability for captures and recaptures were equal because with non–invasive collection of scat samples there is no need to account for trap–shy or trap–happy individuals. Model selection based on Akaike's information criterion (AIC) was used to determine which model was most supported by the data.

To determine whether a smaller portion of the river could be sampled and still yield the same results, (that is, to determine whether sampling 29 kilometers was too much, too little or just right) I estimated population size for each of the three stretches of river alone, Gibbon to Wood River (consisting of two stretches: Gibbon to Shelton and Shelton to Wood River), and Shelton to Alda (consisting of two stretches: Shelton to

Wood River and Wood River to Alda). For simplicity I only used data from the liberal genotyping method and the population size estimated in Program MARK.

RESULTS

Scat collection

During the first scat collection on September 23, 2009 146 otter scats were collected. There were 30 samples (from nine latrines) collected between Gibbon and Shelton, 50 samples (from 23 latrines) between Shelton and Wood River, and 66 samples (from 12 latrines) between Wood River and Alda. When scat was collected two weeks later on October 7, 2009 a total of 146 otter scats were collected. There were 28 samples (from eight latrines) collected between Gibbon and Shelton, 53 samples (from 17 latrines) between Shelton and Wood River, and 65 samples (from 15 latrines) between Wood River and Alda (Table 3.1).

DNA extraction success

Of the 292 samples collected, 229 amplified at the RIO07R locus and 124 amplified at the RIO16R locus. Only five of the samples that amplified at RIO16R did not amplify at RIO07R, therefore 234 samples amplified at least at one of the two loci yielding an overall otter DNA extraction success of 80.1%. When separated by sampling session, 117 samples from the first session amplified at one or both of the two loci (80.1%) and 117 samples from the second session amplified at one or both of the two loci (80.1%; Table 3.1).

Genotyping and population estimation

I calculated $P_{(ID)sibs}$ to be $5.40x10^{-3}$ for the liberal genotypes and $4.53x10^{-3}$ for the conservative genotypes. From this I determined that I needed at least seven loci genotyped to distinguish between related individuals. Unique individuals determined with the liberal method and conservative method did not show significant deviation from Hardy–Weinberg equilibrium or any linkage between loci (Table 3.2).

Overall genotyping success was 18.2% (samples genotyped at seven or more loci). The genotyping success for the first and second sampling sessions was 21.9% and 14.4% respectively (Table 3.1). When using the liberal method for determining unique individuals I identified 23 individuals collected during the first session and 12 individuals collected during the second session, eight of which were recaptures (27 total individuals identified). The conservative method resulted in 16 individuals collected during the first session and 11 collected during the second session, five of which were recaptures (22 total individuals identified).

When Program MARK was used to estimate population size and determine which model best supported the data, I found that for the conservative genotyping method p.=cN was the model most supported by the data and accounted for 57% of the model weight, though p(t)=cN had a ΔAIC of 0.532 making it within the confidence set (ΔAIC ≤ 2) as well (Table 3.3). Conversely, when the liberal method for genotyping was used p(t)=cN was the model most supported by the data and accounted for 92% of the model weight and p.=cN had a ΔAIC of 4.9 and was not included in the confidence set (Table 3.3). When the individuals determined by the conservative method of genotyping were used in MARK, estimated population size was 28.6 (95% C.I. = 22.9–51.7) with a

detection probability of 0.47. The population size estimated by MARK for closed captures when the liberal genotyping method was used was 32.8 otters (95% C.I. = 28.3–52.3) with a probability of detection of 0.70 for the first session and 0.37 for the second session (Table 3.4; Figure 3.1).

The Lincoln–Peterson population estimate for river otters along the 29 kilometer sampling area when the liberal genotyping method was used was 33.7 ± 10.6 otters with a detection probability of 0.38. The Lincoln–Petersen population estimate when the conservative genotyping method was used was 28.1 ± 10.0 with a detection probability of 0.35 (Table 3.4).

Using the population sizes estimated above, I calculated otter density (otters/kilometer). When the results of the conservative genotyping method was used with Program MARK and the Lincoln–Petersen model otter densities and associated 95% confidence intervals were 1.0 otters/kilometer (0.8–1.8 otters/kilometer) and 1.0 otters/kilometer (0.6–1.3 otters/kilometer) respectively. Otter density using the liberal genotyping method was 1.1 otters/kilometer (1.0–1.8 otters/kilometer) when Program MARK was used and 1.2 otters/kilometer (0.8–1.5 otters/kilometer) when the Lincoln–Petersen model was used (Table 3.5).

When each stretch of the river was examined independently there were not enough samples to estimate population size and density with a confidence interval on the Gibbon to Shelton stretch. I estimated the density from the Shelton to Wood River stretch to be 1.3 otters/kilometer (95% C.I. = 1.0–3.8), and from Wood River to Alda to be 1.5 (95% C.I. = 1.4–3.0; Table 2.6). The p(t)=cN model was most supported by data

for the Shelton to Wood River stretch, and the p.=cN model was most supported for the Wood River to Alda stretch (Table 3.7). When the results from Gibbon to Wood River stretch (the Gibbon to Shelton stretch and the Shelton to Wood River stretch combined) were used p(t)=cN was the model most supported by data (Table 3.8) and density was estimated to be 1.3 otters/kilometer (95% C.I. = 0.9–3.1; Table 3.9). Using the results from the Shelton to Alda stretch (the Shelton to Wood River stretch and the Wood River to Alda stretch compined) and the p(t)=cN model in MARK, density was estimated to be 1.4 otters per kilometer (95% C.I. = 1.1–2.3; Table 3.9).

When the results from the liberal genotyping method were doubled in program MARK and the Lincoln–Petersen model, otter density was estimated to be 1.2 otters/kilometer (95% C.I. = 1.0–1.6) and 1.2 (95% C.I. = 0.9–1.4) respectively (Table 3.10). The model p(t)=cN was model most supported by the data (Table 3.11).

DISCUSSION

The typically degraded nature of DNA extracted from otter scat has resulted in highly variable extraction and genotyping success rates among studies. Other studies (of both Eurasian otters and North American otters) have reported extraction successes ranging from 38% to 99% of samples containing otter DNA (Ben–David et al. 2004; Hung et al. 2004; Ferrando et al. 2008; Arrendal et al. 2007; Hansen et al. 2007; Prigioni et al. 2006). Most of these studies preserved collected scat in ethanol. The 81% extraction success that I obtained after samples were dried indicates that drying is a viable alternative to ethanol and can yield high percentages of samples containing otter DNA (Schwartz and Monfort 2008). Despite the relatively high proportion of samples containing otter DNA, genotyping success was low (18.2%). Genotyping successes

reported from Eurasian otter (*Lutra lutra*) studies ranged from 14% to 73% (Hánjková et al. 2009; Lanszki et al. 2008; Dallas et al. 2003; Ferrando et al. 2008; Kalz et al. 2006; Prigioni et al. 2006;Arrendal et al. 2007; Hung et al. 2004; and Janssens et al. 2008). However, studies using scat collected from the North American river otter have genotyping success as low as 8% across four loci (Hansen et al. 2007) and 33% at one locus (Ben–David et al. 2004). A recent study by Mowry (2010) in Missouri reported a genotyping success of 24% across at least seven of ten loci.

Hypotheses as to why river otter scat yields such poor quality DNA include diet, biology, and environmental conditions (Fike et al. 2004). Based on the differences in genotyping success that I observed between the first and second sampling session (21.9% and 14.4% respectively), when overall extraction success was equal between sampling sessions, suggests that environmental conditions were the most likely cause of differential degradation of DNA within samples. Hydrolysis is the most common factor of DNA deterioration (Schwartz and Monfort 2008), and the aquatic nature of otters and the proximity of their latrines to water could have affected the poor quality of otter DNA. Hydrolysis is also the most likely explanation for the difference in sample quality between sessions, because a few days prior to the second collection the study area experienced heavy rains.

The genotyping success in this study would likely have been improved if anal jellies (secretions deposited from the anal gland used for scent marking) were collected. Mowry (2010) reported a genotyping success for anal jellies of 71%. Other studies have also observed higher genotyping success from anal jellies and postulated that the absence of PCR inhibitors from prey and the presence of more sloughed cells may account for this

difference (Hánjaková et al. 2006). However, anal jelly samples are relatively rare and none were collected during my sampling sessions.

Although my overall genotyping success was low, otter abundance could still be estimated. I assumed that individuals that were "captured" but not marked (i.e. not genotyped) were randomly distributed, meaning there is no individual bias among samples that were not genotyped. Thus, these samples were not included and only genotyped samples were considered "captures". Estimates of abundance for the liberal and conservative genotyping methods were similar, though, as expected, the liberal genotyping method yielded a slightly higher abundance estimate (Figure 3.1). Similarly, there was no difference between estimates performed in MARK and those with the Lincoln–Petersen model. The primary differences between these two methods were the 95% confidence intervals (C.I.). The Lincoln–Petersen model estimates a symmetrical C.I., while MARK adjusts the C.I. to avoid zero, resulting in asymmetrical C.I.s. There were 22 known individuals for the conservative genotyping method and 27 known individuals for the liberal genotyping method. Only the Lincoln–Petersen model for the conservative genotyping method estimated a lower boundary less than that of the known minimum population size (22 otters).

The densities of otters within the study area are considerably higher than has been documented in other populations. Densities reported for Eurasian otters range from 0.18 otters per kilometer in southern Italy (Prigioni et al. 2006) to 1.8 otters per kilometers in Kinmen, China (Hung et al. 2004). However, the high density of otters reported by Hung et al. (2004) may be attributable to high genotyping errors rather than to accurate representation of otter density in the study area (Hánjaková et al. 2009). For the North

American river otter, densities have typically been much lower. Most recently, Mowry (2010) found an average otter density across rivers in central Missouri of 0.2 otters per kilometer, with the lowest density being 0.1 and the highest density being 0.5 otters per kilometer. Other reported estimates of *L. canadensis* density are 0.2 to 0.4 otters per kilometer in central Idaho (Melquist and Hornocker 1983), 0.4 otters per kilometer in Alaska (Bowyer et al. 2003), and 0.4 to 0.7 otters per kilometer in Vancouver Island, British Columbia (Guertin 2009).

The relatively high density of otters along the 29 kilometer stretch of river I sampled is consistent with observations of otters in the area. Eighteen different otters were captured between 2006 and 2008 and the telemetric movement data indicates that most did not disperse much further than the area from which scat was collected. Also, in October of 2007, approximately 14 otters were observed in the study area emerging from a single den and recorded by video camera.

Otter densities may be notably higher throughout the study area than has been documented in other areas of the North American river otter's range as a result of the nature of the Big Bend reach of the Platte River. This area of the Platte River is highly braided and consists of four channels each with multiple associated streams and sloughs. There are also a large number of sand and gravel mines resulting in dozens of small lakes immediately adjacent to the river that are commonly stocked with fish. The abundance of beaver in the area likely plays a role in the facilitation of high otter density as well. The main channels (such as the one sampled for this study) are used by most of the otters in the area for navigation and the actual linear river kilometers represented by the population estimate is much higher than 29 kilometers. The braided nature of the central

Platte River, the prevalence of sand and gravel pits, as well as the fact that this population has been unexploited since its initial reintroduction in 1986, likely accounts for the relatively high density. Thus, previously reported otter densities in North America cannot be directly compared to the otter densities reported in this study.

I estimated population densities and associated confidence intervals for each of the three river stretches independently, the Gibbon to Shelton and Shelton to Wood River stretches combined, the Shelton to Wood River and Wood River to Alda stretches combined, and hypothetically, for a segment of 58 kilometers with double the captures and recaptures, to determine if more or less area should be sampled for future studies. If only one of the stretches had been sampled there would either be too few samples genotyped for an estimate of population size (Gibbon to Shelton stretch) or the results would have overestimated the number of otters (Table 3.6). For the Gibbon to Wood River stretch or the Shelton to Alda stretches, otter densities would be close to that observed when all three stretches were sampled, however the confidence intervals would be much larger indicating an upper bound density of as much as 3.1 otters per kilometer (Table 3.9). However, when I doubled the sample area and the number of samples genotyped, there was no difference in density and confidence intervals increased slightly. If this is indicative of the number of samples that would be collected if the 58 kilometers were surveyed instead of 29, I would not have had the time nor funding to collect from those extra kilometers. However, these results suggest that it is not the length of river surveyed that is important, but the number of samples that are collected. There were too few samples collected along the Gibbon to Shelton stretch to estimate otter abundance, and with the number of samples collected along two stretches, the abundance estimate

would include large confidence intervals. If twice as many total samples were collected, however, there would not be an increase in the precision of the estimates. Therefore, it is impossible to predict the adequate study area size because it depends on the amount of samples that can be collected. However, my sample size of about 150 samples from each session was adequate to estimate abundance with reasonable 95% confidence intervals.

The additional information that the collected otter DNA can yield as well as the results of this study, present other potential research questions. Perhaps the most critical research that should be conducted as a result of this study is to estimate otter abundance and density in the other rivers in Nebraska. This would finally yield an overall assessment of the Nebraskan river otter population status. This data could also be used to assess genetic diversity and relatedness within and among populations to characterize the overall genetic health of Nebraska's otters. This additional information would be vital for the creation of an otter management plan. A controlled study that examines the effects of rain or the number of days passed since the scat was deposited would allow researchers to potentially increase the genotyping success of otter scat samples by altering scat collection to maximize the quality of the DNA extracted. Furthermore, because three subspecies (*L.c. canadensis*, *L.c. lataxina*, and *L. c. pacifica*) (Sam Wilson, personal communication) were initially reintroduced at specific release sites, studies investigating the genetic diversity of populations throughout the state could examine initial dispersal and interbreeding of the founding populations.

River otters on the Big Bend reach of the Platte River occur in relatively high densities compared to other river otter studies in North America, though the complex nature of the Platte River makes density comparisons difficult. This study has generated

previously unknown information regarding, the density of river otters in a biologically unique landscape, and a method to estimate population size of an elusive (though apparently not rare) species. Future studies should perform further genetic analyses to examine the sex ratios, relatedness, and genetic diversity of individuals in the population. There is still much to be learned from this emerging method, however, implementing genetic analyses as a part of a larger study in Nebraska will contribute valuable information necessary for the creation and implementation of a river otter management plan.

Table 3.1. The number of scat samples collected for genetic analysis during the two sampling sessions and the proportion of those samples that tested positive for otter DNA and were able to be genotyped at seven or more loci. Samples were collected between the Gibbon and Alda exits south of Interstate 80 on the Platte River in Nebraska. Session 1 occurred on September 23, 2009 and session 2 occurred on October 7, 2009.

		DNA present		Genotyped at 7+ loci	
	Number of samples collected	Number of samples	Percent of session total	Number of samples	Percent of session total
Session 1	146	117	80.1	32	21.9
Session 2	146	117	80.1	21	14.4
Total	292	234	80.1	53	18.2

Table 3.2. The number of alleles identified at each of the ten microsatellite loci used, the observed (H_o) and expected (H_E) heterozygosity values for each locus and the mean for all loci for the conservative genotyping method and for the liberal genotyping method. The conservative genotyping method is one of the two methods I used to distinguish individual genotypes. If there was uncertainty regarding the identification of the otters represented by the sample, the conservative method called them the same and the liberal method considered them to be different individuals.

	Conservative genotyping method			Liberal genotyping method		
Locus	Number of alleles	H_o	H_E	Number of alleles	H_o	H_E
RIO01R2	5	0.45	0.61	5	0.52	0.58
RIO02R	5	0.68	0.69	5	0.74	0.67
RIO04R	4	0.55	0.55	4	0.58	0.50
RIO06R	4	0.59	0.56	4	0.48	0.51
RIO07R	6	0.64	0.78	6	0.67	0.78
RIO08R	4	0.82	0.74	4	0.81	0.74
RIO11	5	0.45	0.69	5	0.41	0.70
RIO13R	9	0.73	0.74	9	0.70	0.75
RIO15R	3	0.14	0.13	3	0.11	0.11
RIO16R	2	0.55	0.49	2	0.52	0.49
Mean	4.7	0.60	0.56	4.7	0.58	0.55

Table 3.3. For each genotyping method used (conservative or liberal) two models were fit to the data to estimate otter population size in Program MARK. Model selection based on Akaike's information criterion and model weights was used to determine which model was most supported. The model p(t)=cN was the model most supported by the data when the conservative genotyping method was used. The model p.=cN was the model most supported by the data when the liberal genotyping method was used.

Genotyping Method	Model	AIC_c	ΔAIC	AIC Weight
Conservative	p.=cN	-36.872	0	0.566
	p(t)=cN	-36.339	0.532	0.434
Liberal	P(t)=cN	-66.725	0	0.92
	p.=cN	-61.851	4.874	0.08

Table 3.4. Population size estimates for otters along a 29 kilometer study area of the Platte River during the fall of 2009 (N; 95% confidence intervals) for the conservative and liberal genotyping methods using Program MARK and the Lincoln–Petersen estimator (adjusted for small samples size).

	Conservative genotyping method		Liberal genotyping method	
Population estimation method	N	95% Confidence interval	N	95% Confidence Interval
Program MARK	28.63	22.89–51.70	32.81	28.33–52.33
Lincoln–Petersen	28.14	18.14–38.14	33.67	23.07–44.273

Table 3.5. Densities of river otters (95% C.I.) along a 29 kilometer study area of the Platte River during the fall of 2009 using Program MARK and the Lincoln–Petersen estimator adjusted for small sample size. Densities were estimated using the results from the conservative genotyping method and the liberal genotyping method.

	Conservative genotyping method		Liberal genoytping method	
Population estimation method	Density (otters/km)	95% C.I.	Density (otters/km)	95% C.I.
Program MARK	0.99	0.78–1.78	1.13	0.98–1.80
Lincoln–Petersen	0.97	0.63–1.32	1.16	0.80–1.53

Table 3.6. Estimated population size (N) and density (otters per kilometer) for river otters for each of three stretches of the Platte River sampled and the associated 95% confidence intervals. The three stretches of river that were sampled were Gibbon to Shelton (G–S), Shelton to Wood River (S–WR), and Wood River to Alda (WR–A). There were not enough samples genotyped on the Gibbon to Shelton stretch to estimate population size. For the Shelton to Wood River stretch, both models were within the confidence set, however p(t)=cN was the model was most supported by the data with 68% of the model weight. The p.=cN model was the model most supported by the data for the Wood River to Alda stretch with 64% of the model weight.

	G–S			S–WR			WR–A		
Model	Estimate	Lower	Upper	Estimate	Lower	Upper	Estimate	Lower	Upper
p(t)=cN									
N (density)	6.0 (0.8)	6.0 (0.8)	6.0 (0.8)	17.0 (1.3)	12.7 (1.0)	49.0 (3.8)	12.1 (1.5)	11.1 (1.4)	24.3 (3.0)
p.=cN									
N (density)	———————	———————	———————	21.5 (1.7)	13.7 (1.1)	65.3 (5.0)	12.7 (1.6)	11.2 (1.4)	25.8 (3.2)

Table 3.7. Model selection results based on Akaike's information criteria (AIC) and model weights for estimating otter abundance for each stretch of the Platte River sampled. Models within two ΔAIC points were considered plausible and were included in the confidence set. For the Gibbon to Shelton (G–S) river stretch the p(t)=cN model was most supported by the data. The same is true for the Shelton to Wood River (S–WR) stretch, though the p.=cN model was also supported. The p.=cN model was the model most supported by the data for the Wood River to Alda (WR–A) stretch, though the p(t)=cN model was also supported.

	G–S			S–WR			WR–A		
Model	AIC_c	ΔAIC	AIC Weight	AIC_c	ΔAIC	AIC Weight	AIC_c	ΔAIC	AIC Weight
p(t)=cN	-5.35	0.00	0.998	-8.65	0.00	0.68	-2.51	1.16	0.64
p.=cN	7.37	12.72	0.002	-7.12	1.53	0.32	-3.67	0.00	0.36

Table 3.8. Model selection based on Akaike's information criteria (AIC) and model weights for estimating otter abundance for two stretches of the Platte River study area; Gibbon to Shelton and Shelton to Wood River (G–WR), and for Shelton to Wood River and Wood River to Alda (S–A). The data for both of these stretches best supported the p(t)=cN model. However, with a ΔAIC less than 2, the p.=cN was also supported for the Shelton to Alda stretch.

	G–WR			S–A		
Model	AIC_c	ΔAIC	AIC Weight	AIC_c	ΔAIC	AIC Weight
p(t)=cN	-31.06	0.00	0.86	-40.78	0.00	0.56
p.=cN	-27.39	3.68	0.14	-40.26	0.51	0.44

Table 3.9. Estimated population size and density (otters per kilometer) for river otters on the Platte River using MARK for when the Gibbon to Shelton and Shelton to Wood River stretches were combined (G–WR) and for when the Shelton to Wood River and Wood River to Alda stretches were combined (S–A), and the associated 95% confidence intervals. The p(t)=cN was the most supported by the data for both the Gibbon to Wood River stretch and the Shelton to Alda stretch. However, the p.=cN model was also supported by the data for the Shelton to Alda stretch.

	G–WR			S–A		
Model	Estimate	Lower	Upper	Estimate	Lower	Upper
p(t)=cN						
N (density)	27.0 (1.3)	19.7 (0.9)	65.1 (3.1)	28.5 (1.4)	23.6 (1.1)	48.3 (2.3)
p.=cN						
N (density)	––––––––	––––––––	––––––––	27.6 (1.3)	23.3 (1.1)	46.3 (2.2)

Table 3.10. Population size (N) and density (otters per kilometer) with the associated 95% confidence intervals when the data from the liberal genotyping method was doubled and the length of the Platte River sampled was doubled (29 kilometers to 58 kilometers) to determine if confidence intervals would improve if more river kilometers were sampled. Population size was estimated in MARK and using the Lincoln–Petersen model (L–P).

Method	N (density)	Lower	Upper
MARK	67.3 (1.2)	58.7 (1.0)	91.7 (1.6)
L–P	68.1 (1.2)	54.0 (0.9)	82.2 (1.4)

Table 3.11. Model selection based on Akaike's information criteria (AIC) and model weights for estimating otter abundance when the capture and recapture data and the length of river sampled was doubled (29 kilometers to 58 kilometers). The p(t)=cN model was most supported by the data with 99.7% of the model weight. The p.=cN model had a ΔAIC greater than two and was not supported by the data.

Model	AIC_c	ΔAIC	AIC Weight
p(t)=cN	-213.40	0.00	0.997
p.=cN	-201.63	11.77	0.002

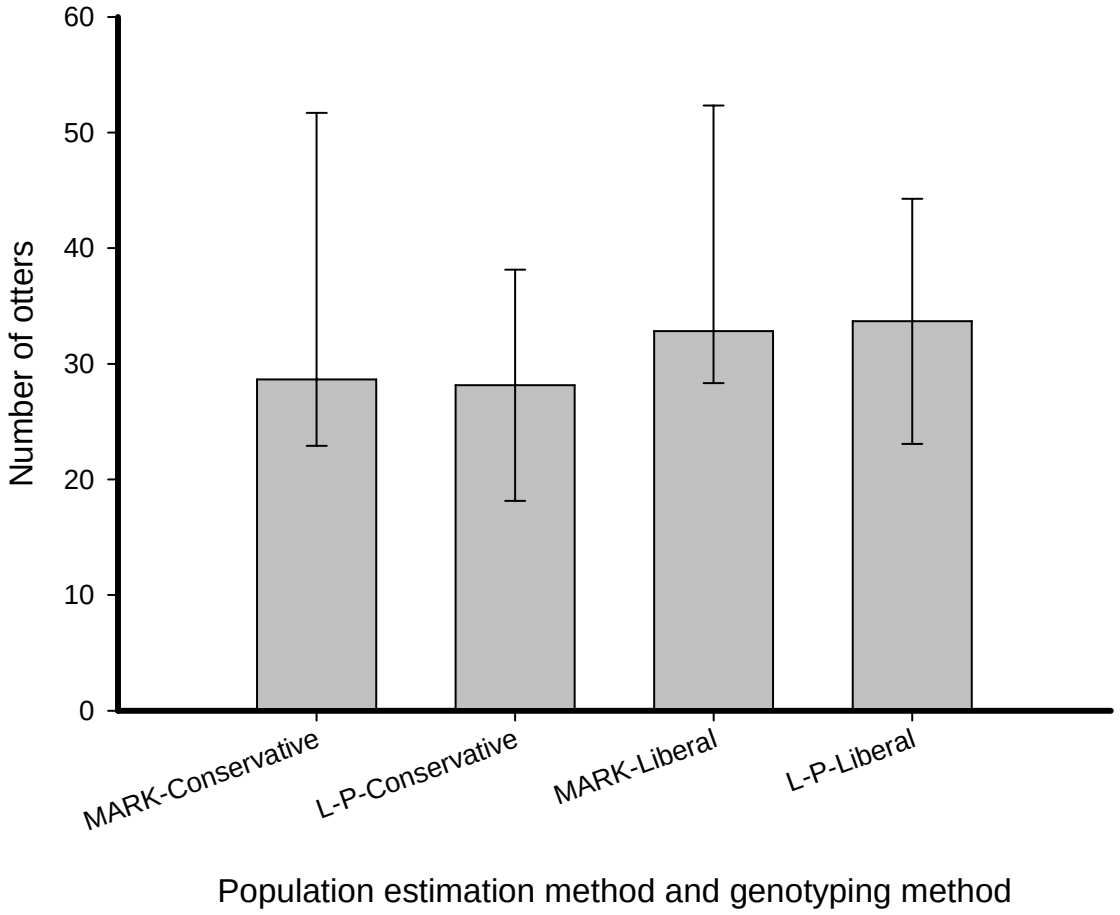

Figure 3.1. The differences in the population size estimate for otters (± 95% confidence intervals) between the conservative and liberal genotyping methods when Program MARK was used to estimate otter abundance along the Platte River study area and when the Lincoln–Petersen (L–P) model was used.

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CHAPTER 4: CONCLUSION

The lack of quantitative data collected since the reintroduction of river otters to Nebraska necessitates research into otter ecology and population dynamics in the state. It is important to fill critical information gaps so that a management plan can be created and the survival of the state population ensured. My research represents a small step towards a greater understanding of Nebraska's otters. I investigated the effects of a non–native plant, *Phragmites australis*, on river otter habitat use. I also examined the feasibility of using non–invasive genetic mark–recapture techniques, to estimate otter abundance. Though seemingly disparate, these two chapters both contribute valuable information necessary for the continued survival of a viable otter population in Nebraska.

Phragmites is of increasing concern in the Big Bend reach of the Platte River, a biologically unique landscape, because it alters plant and structural diversity, clogs river channels, alters sediment deposition and movement, and reduces flows. Although there has been extensive research into the effects of *Phragmites* on abiotic factors within an ecosystem, very little information is available on the impacts on wildlife.

For both males and female otters, and for unique den/resting sites as well as the frequency of use of den/resting sites, *Phragmites*, other riparian vegetation and water were used more than expected relative to availability, while agriculture and other were used less and roads in proportion to availability. Within used land cover types, females preferred *Phragmites* for unique den/resting sites while males used both cover types in proportion to availability. However, neither males nor females showed any difference in

the frequency of their use of *Phragmites* and other riparian vegetation relative to availability.

The significant use of *Phragmites* by females and not males indicates that *Phragmites* is likely appealing to females with pups. The characteristically dense and nearly impenetrable stands provide necessary cover for females with pups for protection from predators. The significant use of *Phragmites* by females with unique den/resting sites but not with the frequency of sites utilized is likely attributable to two reasons. First, females may be using *Phragmites* for temporary cover for resting sites when beaver dens or logjams are unavailable, thus spending less time in *Phragmites* and reducing their likelihood of detection in this cover type. Second, because *Phragmites* is distributed widely over the study area and all stands provide the same amount of cover, every site with *Phragmites* is equally adequate, whereas beaver dens and lodges or logjams may be more difficult to locate. Thus, when these sites are found otters are more likely to return to those specific dens that are not as easy to locate as *Phragmites*.

The important message is not that *Phragmites* is beneficial for river otters and it needs to be managed for. *Phragmites* is a non–native species, and because otters were previously abundant throughout the Platte River prior to the *Phragmites* invasion, it is not the plant itself which is important for otters, but the cover it provides. The current *Phragmites* eradication efforts on the Platte River could have unintended consequences to river otters in the area. *Phragmites* removal is, in and of itself, a difficult, time consuming and expensive venture and it should not be removed from banks without replacing it with native vegetation that provides similar cover. By establishing new,

native plant communities, the likelihood of *Phragmites* re–establishing is reduced, as is any costs incurred by the potential negative effects on river otters.

Chapter 3 estimated the density of otters in the Chapter 2 study area, and the feasibility of using non–invasive genetic techniques on a larger scale in rivers across the state. The fundamental idea behind non–invasive genetic analyses is to use sources of DNA left by the target species that can be collected after that individual has left. Hair and scat are the two most common sources of non–invasive DNA. For river otters, their latrine use and scent marking behaviors make scat the best source of DNA. Through individual identification of each DNA sample, traditional mark–recapture techniques can be used to estimate abundance.

Conservative genotyping estimated otter density to be 0.99 otters/kilometer (95% C.I. =0.78–1.78), while liberal genotyping estimated otter density to be 1.13 otters/kilometer (95% C.I. =0.98–1.8). The overlapping confidence intervals suggest that these estimates are not different and that the river otter density within the study area is between 0.78–1.80 otters/kilometer.

Additional tests to determine if fewer river kilometers could have been used to yield similar results indicated that, at this density of otters, if the scat collected from only one or two of the river segments had been used, the results would not have been the same. Alternatively, if we had collected twice as many samples from twice as many river kilometers, the density estimate would be similar, as would the size of the confidence intervals. This indicates that it is not the amount of area sampled that is important, but the number of samples collected. Thus, for the density of otters in this study area, 29

kilometers was adequate to estimate density because enough scat samples were collected to yield a density estimate with reasonable confidence intervals.

Previous studies of river otters in North America have typically reported much lower densities. However, the apparently high density of otters in the area may be a result of the nature of the Platte River. This area of the river is wide, highly braided, and is in close proximity to multiple sloughs, sand and gravel pits, and ponds routinely stocked with fish. Therefore, though we collected scat from a 29 kilometer stretch of river, the area is probably representative of otters using all of these various other surrounding habitats giving the illusion of a high density, when actually much more than 29 linear kilometers of habitat was available. This population also continues to be unexploited, and remains protected from intentional harvest.

Much remains unknown about river otters in Nebraska. However, these two studies have contributed to the information necessary for an otter management plan. Not only does this thesis provide specific information about otters and their ecology in an important ecosystem in Nebraska, but it also provides managers with some of the tools necessary to further this research and apply it to otter populations across the state. This information is just a small sample of what is needed before a management plan can be created and implemented, including more ecological data such as home range size and general habitat use across Nebraska. Specific aspects of life histories including movement, response to climate and land use changes, survival and reproduction are also critical to management and population persistence. Finally, as advancements continue to be made in wildlife genetics, questions concerning relatedness within and among populations, interbreeding and distribution of the three subspecies originally released, and

genetic diversity can be answered. With continued research and support from state and non–profit agencies as well as the public, we can ensure, not only the continued survival of otters in Nebraska, but thriving populations that persist for future generations to enjoy.

Appendix A. Den/resting sites for all otters (n=540) collected between 2006 and 2009 along the Big Bend reach of the central Platte River.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
14	29-Jun-09	40.78784	-98.41199	1901	11:25
14	13-Jul-09	40.80195	-98.38401	1858	12:09
14	25-Aug-09	40.80188	-98.37832	1902	16:46
14	2-May-08	40.81833	-98.34843	1868	10:22
14	8-May-08	40.81833	-98.34840	1867	12:58
14	14-May-08	40.81835	-98.34840	1862	10:05
14	15-May-08	40.81831	-98.34845	1915	11:00
14	19-May-08	40.81836	-98.34845	1855	13:57
14	17-Sep-08	40.79486	-98.39471	1886	11:13
14	18-Sep-08	40.80344	-98.37569	1894	11:13
14	23-Sep-08	40.79306	-98.40161	1977	13:21
14	25-Sep-08	40.79968	-98.35768	1873	12:19
56	9-Oct-08	40.72416	-98.65833	1942	14:26
56	20-Oct-08	40.73766	-98.63483	1972	11:49
56	6-Nov-08	40.73767	-98.63467	2043	15:43
56	7-Nov-08	40.73796	-98.63215	1991	10:29
56	2-Apr-09	40.73809	-98.63287	2004	14:28
56	28-Apr-09	40.73767	-98.62965	1968	11:20
56	11-Feb-09	40.73797	-98.63253	1972	14:05
56	17-Feb-09	40.73788	-98.63258	2035	11:53
56	18-Feb-09	40.73796	-98.63251	1978	15:16
56	19-Feb-09	40.73809	-98.63279	2002	11:49
56	20-Feb-09	40.73767	-98.63234	2001	15:53
56	24-Feb-09	40.73796	-98.63256	2060	9:44
56	27-Feb-09	40.73793	-98.62882	1983	9:49
56	2-Mar-09	40.73590	-98.63613	1970	10:23
56	11-Mar-09	40.73414	-98.63805	1972	14:47
56	19-Mar-09	40.37818	-98.63274	1981	14:48
56	23-Mar-09	40.73796	-98.63238	2039	11:37
56	6-Apr-09	40.73815	-98.63274	1973	10:51
56	7-Apr-09	40.73190	-98.65360	2001	10:12
56	28-Dec-07	40.72785	-98.64973	1976	13:45
56	22-Feb-08	40.72876	-98.63663	1979	11:20
56	4-Mar-08	40.73810	-98.63327	1983	14:22
56	5-Mar-08	40.73811	-98.63277	1968	14:15
56	10-Mar-08	40.73809	-98.63326	1979	13:08
56	13-Mar-08	40.73767	-98.63238	1984	11:30

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
56	16-Mar-08	40.73812	-98.63326	1981	10:52
56	17-Mar-08	40.73816	-98.63274	1975	10:36
56	14-Apr-08	40.73757	-98.62982	1982	9:51
56	28-May-08	40.72937	-98.64379	2010	15:05
56	19-Sep-08	40.72446	-98.65934	1974	15:13
56	23-Sep-08	40.72442	-98.65942	1980	15:19
56	1-Dec-08	40.73761	-98.63229	1983	10:14
56	3-Dec-08	40.73761	-98.63229	1983	9:47
56	6-Dec-08	40.73761	-98.63229	1983	9:41
56	8-Dec-08	40.73771	-98.63233	1976	9:51
56	16-Dec-08	40.73749	-98.63519	2058	12:51
56	17-Dec-08	40.73753	-98.63524	2046	12:37
56	13-Nov-08	40.73784	-98.63235	2069	12:50
113	11-Feb-08	40.74236	-98.56693	1953	14:22
113	5-Dec-06	40.73772	-98.63226	No Data	14:45
113	6-Dec-06	40.74947	-98.54549	No Data	9:20
113	6-Dec-06	40.74951	-98.54558	No Data	14:10
113	6-Dec-06	40.74952	-98.54543	No Data	15:20
113	11-Dec-06	40.74948	-98.54543	No Data	15:25
113	28-Dec-06	40.74947	-98.56740	No Data	13:20
113	18-Jan-07	40.74859	-98.56033	No Data	11:35
113	19-Jan-07	40.74859	-98.56033	No Data	7:35
113	22-Jan-07	40.74947	-98.54549	No Data	9:05
113	23-Jan-07	40.74859	-98.56033	No Data	8:20
113	24-Jan-07	40.74746	-98.56681	No Data	8:50
113	30-Jan-07	40.74699	-98.55538	No Data	9:35
113	5-Feb-07	40.74947	-98.54549	No Data	11:22
113	7-Feb-07	40.74200	-98.56572	No Data	11:55
113	8-Feb-07	40.74200	-98.56572	No Data	8:15
113	9-Feb-07	40.74200	-98.56572	No Data	10:52
113	12-Feb-07	40.74191	-98.56591	No Data	8:33
113	19-Feb-07	40.74624	-98.55788	No Data	14:20
113	7-Mar-07	40.75316	-98.55933	1956	13:31
113	8-Mar-07	40.75317	-98.55916	1952	8:38
113	9-Mar-07	40.75311	-98.55930	1961	16:07
113	10-Mar-07	40.75317	-98.55930	1972	14:00
113	11-Mar-07	40.75311	-98.55923	1953	14:25
113	12-Mar-07	40.75309	-98.55933	1954	9:45
113	13-Mar-07	40.75314	-98.55933	1949	19:05
113	14-Mar-07	40.75315	-98.55930	1957	13:45

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
113	15-Mar-07	40.75318	-98.55930	2008	8:23
113	16-Mar-07	40.75318	-98.55930	2008	7:30
113	17-Mar-07	40.75314	-98.55926	1946	10:30
113	19-Mar-07	40.75314	-98.55926	1946	8:32
113	21-Mar-07	40.75321	-98.55936	1916	11:37
113	22-Mar-07	40.75317	-98.55931	1928	9:06
113	23-Mar-07	40.75312	-98.55931	1986	10:55
113	26-Mar-07	40.75315	-98.55934	1944	18:08
113	28-Mar-07	40.75315	-98.55934	1944	10:30
113	29-Mar-07	40.75314	-98.55934	1944	12:45
113	30-Mar-07	40.75316	-98.55933	1948	9:37
113	2-Apr-07	40.75313	-98.55932	1948	9:45
113	4-Apr-07	40.75318	-98.55932	1924	8:12
113	6-Apr-07	40.75315	-98.55926	1964	8:55
113	7-Apr-07	40.75315	-98.55926	1964	10:50
113	9-Apr-07	40.75320	-98.55941	2014	9:50
113	12-Apr-07	40.75316	-98.55930	1922	9:05
113	13-Apr-07	40.75316	-98.55930	1922	7:30
113	16-Apr-07	40.75318	-98.55935	1923	8:17
113	18-Apr-07	40.75317	-98.55936	1920	13:02
113	19-Apr-07	40.75316	-98.55929	1948	14:20
113	23-Apr-07	40.75312	-98.55923	1955	12:19
113	25-Apr-07	40.75312	-98.55923	1955	8:35
113	26-Apr-07	40.75312	-98.55923	1955	16:33
113	27-Apr-07	40.75312	-98.55923	1955	14:45
113	29-Apr-07	40.75315	-98.55930	1939	12:10
113	30-Apr-07	40.75316	-98.55936	1884	11:10
113	1-May-07	40.75320	-98.55927	1953	16:15
113	3-May-07	40.75319	-98.55901	1958	13:10
113	7-May-07	40.75321	-98.55907	1957	8:52
113	8-May-07	40.75321	-98.55907	1957	11:43
113	9-May-07	40.75311	-98.55930	1959	9:17
113	4-Jun-07	40.76214	-98.52142	1916	10:02
113	11-Jun-07	40.76214	-98.52142	1916	9:25
113	10-Jul-07	40.74999	-98.54414	1975	20:00
113	18-Jul-07	40.74887	-98.54898	1971	11:24
113	22-Aug-07	40.73758	-98.62971	1985	10:06
113	27-Aug-07	40.73746	-98.62968	1987	9:25
113	3-Sep-07	40.73885	-98.62788	1957	11:15
113	17-Sep-07	40.74950	-98.54526	1953	15:25

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
113	4-Oct-07	40.74960	-98.54185	1940	9:45
113	8-Oct-07	40.74960	-98.54185	1940	12:05
113	12-Oct-07	40.74960	-98.54185	1940	11:15
113	15-Oct-07	40.74564	-98.55931	1942	10:35
113	17-Oct-07	40.75108	-98.53770	1941	13:55
113	22-Oct-07	40.74999	-98.54403	1938	10:25
113	2-Jan-08	40.74617	-98.55584	1933	14:55
113	3-Jan-08	40.74617	-98.55585	1962	10:02
113	17-Jan-08	40.74616	-98.55588	1962	11:17
113	23-Jan-08	40.74616	-98.55588	1962	13:33
113	24-Jan-08	40.74617	-98.55584	1955	11:22
113	28-Jan-08	40.74624	-98.55791	1939	10:30
113	4-Feb-08	40.74241	-98.56699	1986	9:06
113	6-Feb-08	40.74201	-98.56568	1956	14:03
113	7-Feb-08	40.74605	-98.55914	1987	11:00
113	12-Feb-08	40.74244	-98.56702	1963	11:56
113	13-Feb-08	40.74245	-98.56702	1993	13:13
113	14-Feb-08	40.74242	-98.56686	1979	9:19
113	15-Feb-08	40.74238	-98.56683	1930	13:40
113	10-Mar-08	40.75164	-98.55661	1977	11:00
113	13-Mar-08	40.75356	-98.55753	1982	10:51
113	15-Mar-08	40.75369	-98.55754	1977	11:18
113	16-Mar-08	40.75356	-98.55754	1997	10:34
113	17-Mar-08	40.75356	-98.55754	1997	11:45
113	18-Mar-08	40.75364	-98.55758	1952	13:29
113	20-Mar-08	40.75355	-98.55750	1956	9:00
113	27-Mar-08	40.75356	-98.55750	1957	10:06
113	28-Mar-08	40.75363	-98.55753	1954	13:22
113	14-Apr-08	40.75330	-98.55831	1967	10:30
113	22-Apr-08	40.75364	-98.55749	1998	16:17
113	24-Apr-08	40.75355	-98.55749	1953	16:11
113	28-Apr-08	40.74442	-98.56023	1969	9:22
113	28-Apr-08	40.74803	-98.55688	1997	18:00
113	28-Apr-08	40.74803	-98.55688	1997	18:30
113	28-Apr-08	40.74803	-98.55688	1997	19:00
113	29-Apr-08	40.75331	-98.55825	1958	10:26
113	1-May-08	40.75327	-98.55839	1981	9:15
113	7-May-08	40.75328	-98.55840	1959	14:40
113	8-May-08	40.75333	-98.55826	1939	9:39
113	12-May-08	40.75212	-98.56143	1948	8:25

Appendix A. Continued

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
113	13-May-08	40.75212	-98.56143	1948	10:03
113	14-May-08	40.75319	-98.55904	1950	12:57
113	15-May-08	40.75321	-98.55904	1939	15:22
113	16-May-08	40.75332	-98.55833	1954	8:38
113	19-May-08	40.75061	-98.54138	1960	9:26
113	20-May-08	40.75061	-98.54138	1960	11:10
135	4-Oct-07	40.74960	-98.54185	1940	9:45
135	8-Oct-07	40.74960	-98.54185	1940	12:05
135	12-Oct-07	40.74960	-98.54185	1940	11:15
135	15-Oct-07	40.74564	-98.55931	1942	10:35
135	17-Oct-07	40.75108	-98.53770	1941	13:55
135	22-Oct-07	40.74999	-98.54403	1938	10:25
135	2-Jan-08	40.74617	-98.55584	1933	14:55
135	3-Jan-08	40.74617	-98.55585	1962	10:02
135	17-Jan-08	40.74616	-98.55588	1962	11:17
135	23-Jan-08	40.74616	-98.55588	1962	13:33
135	24-Jan-08	40.74617	-98.55584	1955	11:22
135	28-Jan-08	40.74625	-98.55586	1965	9:15
135	31-Jan-08	40.74246	-98.56694	1946	14:12
135	4-Feb-08	40.74216	-98.56548	1961	9:17
135	6-Feb-08	40.74224	-98.56549	1964	14:17
135	7-Feb-08	40.74605	-98.55914	1987	11:00
135	27-Feb-08	40.75866	-98.52587	1945	16:50
135	5-Mar-08	40.74490	-98.59107	1952	11:39
135	10-Mar-08	40.74883	-98.58186	1949	12:45
135	17-Mar-08	40.74799	-98.60171	1983	11:13
135	18-Mar-08	40.74294	-98.60954	1977	14:48
135	27-Mar-08	40.75497	-98.59018	1962	11:47
135	4-Apr-08	40.74265	-98.61917	1976	14:05
135	7-Apr-08	40.74534	-98.59005	1997	9:40
135	1-May-08	40.74552	-98.58974	1981	9:32
135	8-May-08	40.74738	-98.59589	1968	10:18
135	13-May-08	40.74855	-98.55145	1951	9:17
135	14-May-08	40.75685	-98.52113	1950	8:35
135	15-May-08	40.75146	-98.57928	1959	16:26
135	16-May-08	40.74543	-98.59000	1972	8:52
135	1-Sep-08	40.75489	-98.59007	1957	10:34
135	15-Sep-08	40.74974	-98.56390	1957	11:15
135	24-Sep-08	40.74854	-98.55170	2005	9:56
135	1-Oct-08	40.74031	-98.57740	1940	12:24

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
135	13-Oct-08	40.74955	-98.54203	1953	14:07
135	7-Dec-08	40.75058	-98.55119	1958	14:58
135	16-Dec-08	40.74995	-98.54427	1968	15:09
135	17-Dec-08	40.74997	-98.54400	1954	9:56
175	3-Jan-08	40.72654	-98.65314	2005	15:03
175	11-Jan-08	40.72785	-98.64973	1976	10:05
175	24-Jan-08	40.72655	-98.65317	1991	9:57
175	30-Jan-08	40.72786	-98.64967	1985	11:37
175	5-Mar-08	40.74878	-98.58180	1942	13:28
175	10-Mar-08	40.74553	-98.58970	1968	11:18
175	16-Mar-08	40.74545	-98.59008	2112	10:22
175	20-Mar-08	40.73192	-98.65349	1986	15:09
175	27-Mar-08	40.74799	-98.60171	1983	13:03
175	31-Mar-08	40.74799	-98.60171	1983	9:06
175	4-Apr-08	40.74799	-98.60171	1983	13:40
175	7-Apr-08	40.74269	-98.61901	1980	10:15
175	22-Apr-08	40.74860	-98.56035	1960	16:58
175	29-Apr-08	40.74856	-98.56039	1957	9:45
175	14-May-08	40.74952	-98.54532	1951	13:22
175	15-May-08	40.74952	-98.54532	1951	17:05
175	18-Aug-08	40.74855	-98.56075	1938	14:06
175	11-Sep-08	40.74932	-98.57587	1954	14:06
175	14-Sep-08	40.75372	-98.59148	2050	10:26
175	7-Oct-08	40.74700	-98.55531	1934	13:46
175	14-Oct-08	40.74944	-98.54537	1949	12:51
195	18-Dec-06	40.81539	-98.41917	No Data	14:35
212	22-Jan-07	40.73190	-98.65360	No Data	10:15
212	25-Jan-07	40.73190	-98.65360	No Data	9:30
212	5-Feb-07	40.73190	-98.65360	No Data	8:50
212	1-Apr-09	40.72291	-98.68571	2002	15:21
212	28-Oct-06	40.73775	-98.63231	No Data	9:10
212	29-Oct-06	40.73772	-98.63232	No Data	10:00
212	1-Dec-06	40.73619	-98.63522	No Data	10:35
212	1-Dec-06	40.73639	-98.63516	No Data	14:15
212	4-Dec-06	40.73628	-98.63535	No Data	14:22
212	4-Dec-06	40.73634	-98.63534	No Data	15:43
212	5-Dec-06	40.74093	-98.62122	No Data	8:55
212	6-Dec-06	40.73640	-98.63538	No Data	10:02
212	6-Dec-06	40.73632	-98.63537	No Data	12:05
212	6-Dec-06	40.73631	-98.63542	No Data	14:40

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
212	6-Dec-06	40.73610	-98.63532	No Data	15:45
212	8-Dec-06	40.73620	-98.63534	No Data	9:18
212	8-Dec-06	40.73639	-98.63545	No Data	11:28
212	8-Dec-06	40.73626	-98.63536	No Data	15:40
212	11-Dec-06	40.73628	-98.63555	No Data	14:45
212	12-Dec-06	40.73631	-98.63544	No Data	12:55
212	12-Dec-06	40.73635	-98.63540	No Data	13:15
212	12-Dec-06	40.73625	-98.63537	No Data	14:50
212	13-Dec-06	40.73627	-98.63540	No Data	13:45
212	13-Dec-06	40.73624	-98.63535	No Data	14:15
212	19-Dec-06	40.73626	-98.63538	No Data	9:40
212	12-Jan-07	40.73209	-98.65348	No Data	12:17
212	15-Jan-07	40.73190	-98.65360	No Data	13:40
212	16-Jan-07	40.73186	-98.65362	No Data	14:20
212	17-Jan-07	40.72855	-98.65727	No Data	8:20
212	18-Jan-07	40.73190	-98.65360	No Data	12:45
212	23-Jan-07	40.73190	-98.65360	No Data	9:20
212	24-Jan-07	40.73190	-98.65360	No Data	10:45
212	26-Jan-07	40.73190	-98.65360	No Data	9:40
212	29-Jan-07	40.73190	-98.65360	No Data	11:15
212	30-Jan-07	40.73190	-98.65360	No Data	13:50
212	31-Jan-07	40.73190	-98.65360	No Data	8:45
212	1-Feb-07	40.73190	-98.65360	No Data	14:12
212	2-Feb-07	40.73190	-98.65360	No Data	9:48
212	6-Feb-07	40.73190	-98.65360	No Data	8:56
212	8-Feb-07	40.73190	-98.65360	No Data	9:15
212	9-Feb-07	40.73190	-98.65360	No Data	9:20
212	12-Feb-07	40.73190	-98.65360	No Data	11:11
212	13-Feb-07	40.73190	-98.65360	No Data	8:05
212	14-Feb-07	40.73190	-98.65360	No Data	9:00
212	20-Feb-07	40.73190	-98.65360	No Data	9:32
212	21-Feb-07	40.73190	-98.65360	No Data	10:25
212	22-Feb-07	40.73190	-98.65360	No Data	10:35
212	23-Feb-07	40.73142	-98.65461	No Data	10:20
212	1-Mar-07	40.73212	-98.65309	1989	9:30
212	23-Mar-07	40.71767	-98.71136	2013	10:10
212	9-Apr-07	40.73189	-98.65356	1964	8:45
212	12-Apr-07	40.71074	-98.77984	2033	11:50
212	18-Apr-07	40.73206	-98.65324	1962	20:00
212	19-Apr-07	40.73206	-98.65324	1962	15:30

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
212	23-Apr-07	40.73203	-98.65315	1987	14:20
212	26-Apr-07	40.73203	-98.65315	1987	16:05
212	2-May-07	40.73160	-98.65402	1998	9:37
212	3-May-07	40.73160	-98.65402	1998	7:00
212	9-May-07	40.73160	-98.65402	1998	10:52
212	29-May-07	40.73200	-98.65310	2001	14:45
212	17-Jul-07	40.72562	-98.64577	2033	13:28
212	20-Jul-07	40.72562	-98.64584	1997	10:27
212	7-Aug-07	40.73210	-98.65318	2009	10:40
212	8-Aug-07	40.73201	-98.65314	1983	12:15
212	20-Aug-07	40.70370	-98.66826	2014	11:15
212	13-Mar-08	40.71640	-98.71454	1992	14:32
212	29-Apr-08	40.71564	-98.71333	2007	14:25
212	10-Sep-08	40.72253	-98.68600	2201	14:59
212	15-Sep-08	40.72283	-98.68591	2103	7:00
212	16-Sep-08	40.72290	-98.68598	2046	14:28
212	19-Sep-08	40.72516	-98.67589	1979	15:50
212	23-Sep-08	40.72514	-98.67529	1997	14:51
212	28-Sep-08	40.72512	-98.67529	2004	15:07
212	7-Oct-08	40.72523	-98.65825	1979	16:19
233	12-Feb-09	40.84968	-98.33765	1864	13:36
233	24-Feb-09	40.74255	-98.61914	1968	10:28
233	19-Mar-09	40.72281	-98.68578	2009	11:23
233	31-Mar-09	40.72281	-98.68604	1980	15:34
233	15-Jan-08	40.77210	-98.49463	1897	10:17
233	16-Jan-08	40.77216	-98.49457	1969	10:54
233	17-Jan-08	40.77217	-98.49439	1932	12:16
233	23-Jan-08	40.77216	-98.49462	1920	14:30
233	24-Jan-08	40.77223	-98.49459	1941	13:50
233	30-Jan-08	40.78758	-98.47565	1936	14:10
233	27-Feb-08	40.81603	-98.41102	1871	15:40
233	4-Mar-08	40.80537	-98.44917	1916	16:08
233	10-Mar-08	40.80568	-98.43355	1903	14:48
233	28-Mar-08	40.82773	-98.36733	1853	9:58
233	7-May-08	40.78257	-98.47247	1903	15:07
233	13-May-08	40.77900	-98.49039	1903	10:31
233	14-May-08	40.77894	-98.49052	1949	8:57
233	15-May-08	40.77888	-98.49048	1927	11:31
233	16-May-08	40.77888	-98.49048	1927	13:31
233	19-May-08	40.77888	-98.49048	1927	10:08

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
233	28-Aug-08	40.78339	-98.47175	1921	15:03
233	2-Sep-08	40.77483	-98.48507	1930	11:56
233	4-Sep-08	40.77483	-98.48516	1946	7:28
233	23-Sep-08	40.77658	-98.48029	1872	14:03
233	28-Aug-08	40.78262	-98.47270	1920	11:43
254	13-Dec-07	40.72766	-98.66010	2000	13:45
254	28-Dec-07	40.72785	-98.64973	1976	13:45
254	29-Jan-08	40.73145	-98.65451	1979	17:10
254	6-Feb-08	40.72782	-98.64970	1982	11:06
254	11-Feb-08	40.72356	-98.65425	1995	9:05
254	22-Feb-08	40.73444	-98.63245	1960	9:35
254	4-Mar-08	40.73799	-98.62600	1986	14:48
254	28-May-08	40.72937	-98.64379	2010	15:05
254	19-Sep-08	40.72446`	-98.65930	1974	15:13
254	23-Sep-08	40.72442	-98.65942	1980	15:19
254	9-Oct-08	40.72416	-98.65833	1942	14:26
275	18-Dec-06	40.79638	-98.45557	No Data	16:15
275	19-Dec-06	40.80191	-98.44063	No Data	10:30
275	28-Dec-06	40.79559	-98.44257	No Data	11:40
275	4-Jan-07	40.80194	-98.43359	No Data	11:12
275	9-Jan-07	40.80201	-98.43351	No Data	9:50
275	22-Jan-07	40.79638	-98.45557	No Data	11:20
275	23-Jan-07	40.79638	-98.45557	No Data	10:45
275	25-Jan-07	40.80201	-98.43351	No Data	12:00
275	26-Jan-07	40.78820	-98.43490	No Data	8:45
275	29-Jan-07	40.77245	-98.49508	No Data	9:00
275	30-Jan-07	40.77245	-98.49508	No Data	10:00
275	1-Feb-07	40.77517	-98.48896	No Data	13:35
275	2-Feb-07	40.77517	-98.48896	No Data	9:02
275	5-Feb-07	40.77517	-98.48896	No Data	9:34
275	6-Feb-07	40.77517	-98.48896	No Data	8:40
275	7-Feb-07	40.77517	-98.48896	No Data	13:30
275	8-Feb-07	40.77517	-98.48896	No Data	8:52
275	13-Feb-07	40.79070	-98.41249	No Data	7:30
275	14-Feb-07	40.79070	-98.41249	No Data	9:40
275	19-Feb-07	40.79078	-98.41296	No Data	9:30
275	20-Feb-07	40.79078	-98.41296	No Data	8:30
275	21-Feb-07	40.79078	-98.41296	No Data	9:30
275	22-Feb-07	40.79078	-98.41296	No Data	9:50
275	28-Feb-07	40.79427	-98.39915	No Data	11:57

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
275	1-Mar-07	40.79440	-98.39912	1909	10:25
275	12-Mar-07	40.79945	-98.45538	1931	9:02
275	13-Mar-07	40.79945	-98.45538	1931	18:45
275	14-Mar-07	40.79943	-98.45530	1942	9:20
275	15-Mar-07	40.79943	-98.45530	1942	9:24
275	19-Mar-07	40.79943	-98.45530	1942	16:30
275	21-Mar-07	40.79945	-98.45535	1904	9:00
275	22-Mar-07	40.79944	-98.45538	1927	9:37
275	23-Mar-07	40.79943	-98.45538	1909	11:23
275	26-Mar-07	40.79943	-98.45538	1909	8:35
275	28-Mar-07	40.79943	-98.45538	1909	10:55
275	29-Mar-07	40.79943	-98.45538	1909	13:50
275	30-Mar-07	40.79947	-98.45528	1897	8:55
275	2-Apr-07	40.79954	-98.45551	1867	8:40
275	6-Apr-07	40.79954	-98.45551	1867	9:20
275	7-Apr-07	40.79944	-98.45531	1911	9:35
275	9-Apr-07	40.79946	-98.45532	1927	10:20
275	12-Apr-07	40.79520	-98.44289	1927	10:30
275	13-Apr-07	40.79947	-98.45530	1904	10:52
275	14-Apr-07	40.79947	-98.45530	1904	9:07
275	16-Apr-07	40.79947	-98.45531	1935	8:48
275	18-Apr-07	40.79945	-98.45531	1923	13:55
275	19-Apr-07	40.79948	-98.45530	1881	14:45
275	23-Apr-07	40.79944	-98.45529	1903	12:55
275	25-Apr-07	40.79944	-98.45529	1903	9:05
275	27-Apr-07	40.79944	-98.45529	1903	15:15
275	30-Apr-07	40.79944	-98.45529	1903	16:00
275	1-May-07	40.79943	-98.45528	1901	14:40
275	2-May-07	40.79945	-98.45533	1906	11:40
275	3-May-07	40.79945	-98.45527	1926	13:45
275	7-May-07	40.79945	-98.45527	1926	9:45
275	8-May-07	40.79945	-98.45527	1926	11:03
275	9-May-07	40.79945	-98.45527	1926	10:03
275	16-May-07	40.79943	-98.45528	1923	10:51
275	17-May-07	40.79943	-98.45528	1923	10:15
275	24-May-07	40.80295	-98.43709	1912	11:45
275	25-May-07	40.80295	-98.43709	1912	9:15
275	29-May-07	40.81557	-98.41605	1873	11:08
275	30-May-07	40.81557	-98.41605	1873	11:50
275	4-Jun-07	40.81562	-98.41610	1913	11:41

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
275	7-Jun-07	40.81562	-98.41610	1913	8:55
275	8-Jun-07	40.81562	-98.41610	1913	13:05
275	15-Jun-07	40.81562	-98.41610	1913	13:46
275	25-Jun-07	40.81947	-98.41205	1923	10:15
275	30-Jul-07	40.81342	-98.42398	1894	11:45
275	16-Aug-07	40.81610	-98.41626	1889	10:25
275	21-Aug-07	40.81511	-98.41736	1905	9:20
275	28-Aug-07	40.81319	-98.42506	1906	11:15
275	5-Sep-07	40.78752	-98.45428	1875	10:30
275	25-Sep-07	40.80302	-98.43710	1912	15:05
275	8-Oct-07	40.80568	-98.43359	1926	12:40
275	8-Oct-07	40.80300	-98.43701	1868	19:00
275	10-Oct-07	40.80197	-98.44064	1935	14:05
294	28-Jan-09	40.87284	-98.28655	1837	13:59
294	10-Mar-08	40.81562	-98.41608	1916	15:06
395	15-Jul-09	40.83292	-98.34605	1853	15:09
395	17-Jul-09	40.83292	-98.34605	1853	11:13
395	7-Aug-09	40.83292	-98.34605	1853	10:47
395	8-Jan-08	40.77401	-98.48787	1929	13:35
395	30-Jan-08	40.73799	-98.63221	1984	10:40
395	3-Mar-08	40.78501	-98.45898	1923	10:01
395	4-Mar-08	40.78504	-98.45892	2009	9:45
395	5-Mar-08	40.77895	-98.49048	1923	9:47
395	13-Mar-08	40.77893	-98.49054	1935	9:01
395	15-Mar-08	40.78693	-98.45525	1923	11:45
395	7-May-08	40.77889	-98.49048	1929	15:27
395	20-May-08	40.77888	-98.49048	1927	11:40
395	28-May-08	40.77888	-98.49048	1927	10:45
395	10-Sep-08	40.82797	-98.37203	2031	10:49
395	25-Sep-08	40.78285	-98.47240	1965	10:33
395	27-Aug-08	40.80299	-98.43717	1918	11:04
556	18-Feb-09	40.77401	-98.48779	1928	16:59
556	23-Feb-09	40.75162	-98.55669	1957	10:55
556	24-Feb-09	40.75156	-98.55665	1968	11:31
556	4-Mar-09	40.74886	-98.55023	1983	15:07
556	11-Mar-09	40.74886	-98.55023	1983	15:23
556	31-Mar-09	40.74778	-98.55714	1951	16:47
556	7-Apr-09	40.75159	-98.55672	1980	11:22
556	16-Apr-09	40.78502	-98.45892	1901	11:28
556	20-Apr-09	40.75161	-98.55666	1950	10:24

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
556	21-Apr-09	40.75004	-98.54423	1927	10:57
556	24-Apr-09	40.78502	-98.45888	1919	13:43
556	27-Apr-09	40.78502	-98.45895	1894	12:04
556	29-Apr-09	40.74189	-98.56876	1955	14:03
556	4-May-09	40.78497	-98.45892	1935	11:35
556	12-May-09	40.74884	-98.55019	1985	15:25
556	13-May-09	40.78503	-98.45891	1909	15:50
556	27-May-09	40.78698	-98.45525	1924	11:13
556	29-May-09	40.78699	-98.45528	1931	11:01
556	5-Jun-09	40.78696	-98.45525	1905	11:32
556	15-Jul-09	40.74569	-98.60916	1955	12:16
556	21-Jul-09	40.79512	-98.48816	1909	11:33
556	23-Jul-09	40.74726	-98.59611	1983	13:26
556	10-Aug-09	40.78724	-98.45460	1927	12:37
556	11-Aug-09	40.78802	-98.43697	1909	10:32
556	12-Aug-09	40.78241	-98.45886	1904	10:03
556	24-Aug-09	40.80014	-98.43723	1898	11:30
556	14-Sep-09	40.80002	-98.43645	1904	14:52
556	5-Nov-09	40.78808	-98.43631	1927	9:30
556	18-Nov-09	40.75666	-98.52204	1932	14:24
556	4-Dec-09	40.79790	-98.47063	1946	11:30
556	16-Dec-09	40.78525	-98.45821	1908	10:56
596	25-Feb-09	40.78041	-98.47642	1916	9:17
596	3-Mar-09	40.78109	-98.47704	1927	13:33
596	12-Mar-09	40.78104	-98.47704	1946	11:47
596	13-Mar-09	40.78110	-98.47703	1917	13:48
596	23-Mar-09	40.78105	-98.47711	1907	16:34
596	20-Apr-09	40.78058	-98.49232	1946	10:56
596	8-Jun-09	40.74854	-98.55148	1959	14:48
596	18-Jun-09	40.74860	-98.55148	1955	14:15
596	15-Jul-09	40.74337	-98.59445	1970	11:22
596	14-Dec-09	40.75954	-98.51631	1930	9:34
596	6-Dec-08	40.75056	-98.55127	1950	11:31
596	7-Dec-08	40.75058	-98.55119	1958	14:58
596	16-Dec-08	40.74995	-98.54427	1968	15:09
626	2-Feb-09	40.73748	-98.63503	1991	13:29
626	18-Feb-09	40.77401	-98.48779	1927	15:59
626	23-Feb-09	40.75162	-98.55669	1957	10:55
626	27-Feb-09	40.75158	-98.55674	1979	10:34
626	4-Mar-09	40.75068	-98.55354	1984	15:32

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
626	10-Mar-09	40.74963	-98.54168	1926	14:30
626	31-Mar-09	40.74778	-98.55714	1951	16:47
626	7-Apr-09	40.75159	-98.55672	1980	11:22
626	16-Apr-09	40.78502	-98.45892	1901	11:28
626	20-Apr-09	40.75161	-98.55666	1950	10:24
626	21-Apr-09	40.75004	-98.54423	1927	10:57
626	24-Apr-09	40.78502	-98.45888	1919	13:43
626	27-Apr-09	40.78502	-98.45895	1894	12:04
626	29-Apr-09	40.74189	-98.56876	1955	14:03
626	4-May-09	40.78497	-98.45892	1935	11:35
626	13-May-09	40.78503	-98.45891	1909	15:50
626	27-May-09	40.78698	-98.45525	1924	11:13
626	29-May-09	40.78699	-98.45528	1931	11:01
626	5-Jun-09	40.78696	-98.45525	1905	11:32
626	15-Jul-09	40.74569	-98.60916	1955	12:16
626	21-Jul-09	40.79512	-98.48816	1909	11:33
626	23-Jul-09	40.74726	-98.59611	1983	13:26
626	10-Aug-09	40.78724	-98.45460	1927	12:37
626	11-Aug-09	40.78802	-98.43697	1909	10:32
626	12-Aug-09	40.78241	-98.45886	1904	10:03
626	24-Aug-09	40.80014	-98.43723	1898	11:30
626	5-Nov-09	40.78808	-98.43631	1927	9:30
626	18-Nov-09	40.75666	-98.52204	1932	14:24
626	4-Dec-09	40.79790	-98.47063	1946	11:30
626	16-Dec-09	40.78525	-98.45821	1908	10:56
626	29-Oct-08	40.74215	-98.56542	1973	14:31
626	7-Nov-08	40.74949	-98.54568	1932	13:00
626	10-Nov-08	40.74949	-98.54521	1967	10:54
626	12-Nov-08	40.75529	-98.58994	2084	12:00
626	14-Nov-08	40.73991	-98.57801	1985	10:43
626	24-Nov-08	40.74949	-98.54521	1967	10:00
626	16-Dec-08	40.75499	-98.59019	1971	14:38
626	14-Sep-09	40.80002	-98.43645	1904	14:52
626	25-Nov-08	40.74949	-98.54521	1967	10:22
684	17-Dec-08	40.77398	-98.48788	1947	11:09
684	26-Nov-08	40.78108	-98.47706	1934	11:08
684	27-Jan-09	40.73891	-98.62788	1952	12:11
684	10-Mar-09	40.78054	-98.49118	1940	13:25
684	23-Mar-09	40.77895	-98.49049	1982	12:20
684	2-Apr-09	40.77896	-98.49045	1918	15:30

Appendix A. Continued.

Otter Number	Date	Northing (°)	Westing (°)	Elevation (ft)	Time
684	9-Apr-09	40.78064	-98.49123	1930	11:28
684	24-Apr-09	40.77661	-98.48055	1923	16:14
684	27-Apr-09	40.77897	-98.49035	1889	15:34
684	4-May-09	40.78048	-98.49132	1940	16:01
684	9-Jul-09	40.78818	-98.43659	1925	16:06
684	3-Aug-09	40.78818	-98.43659	1925	10:17
684	5-Aug-09	40.78104	-98.46179	1918	10:46
684	6-Aug-09	40.78818	-98.43659	1925	10:41
684	7-Aug-09	40.78818	-98.43659	1925	9:43
684	12-Aug-09	40.78104	-98.46179	1918	10:10
684	24-Aug-09	40.80014	-98.43723	1898	11:30
684	22-Oct-09	40.78053	-98.49272	1918	8:45
684	12-Nov-09	40.78072	-98.49530	1919	15:50
684	21-Oct-08	40.73915	-98.62739	1991	9:43
684	7-Nov-08	40.74701	-98.55534	1979	12:25
684	14-Nov-08	40.73732	-98.63389	2005	12:03
684	24-Nov-08	40.77216	-98.49457	1961	13:11
684	30-Nov-08	40.77400	-98.48792	1935	11:59
684	2-Dec-08	40.78107	-98.47711	1923	11:21
684	3-Dec-08	40.78110	-98.47713	1921	10:50

Appendix B. Figures of locations of unique den/resting sites for each of the 16 otters tracked.

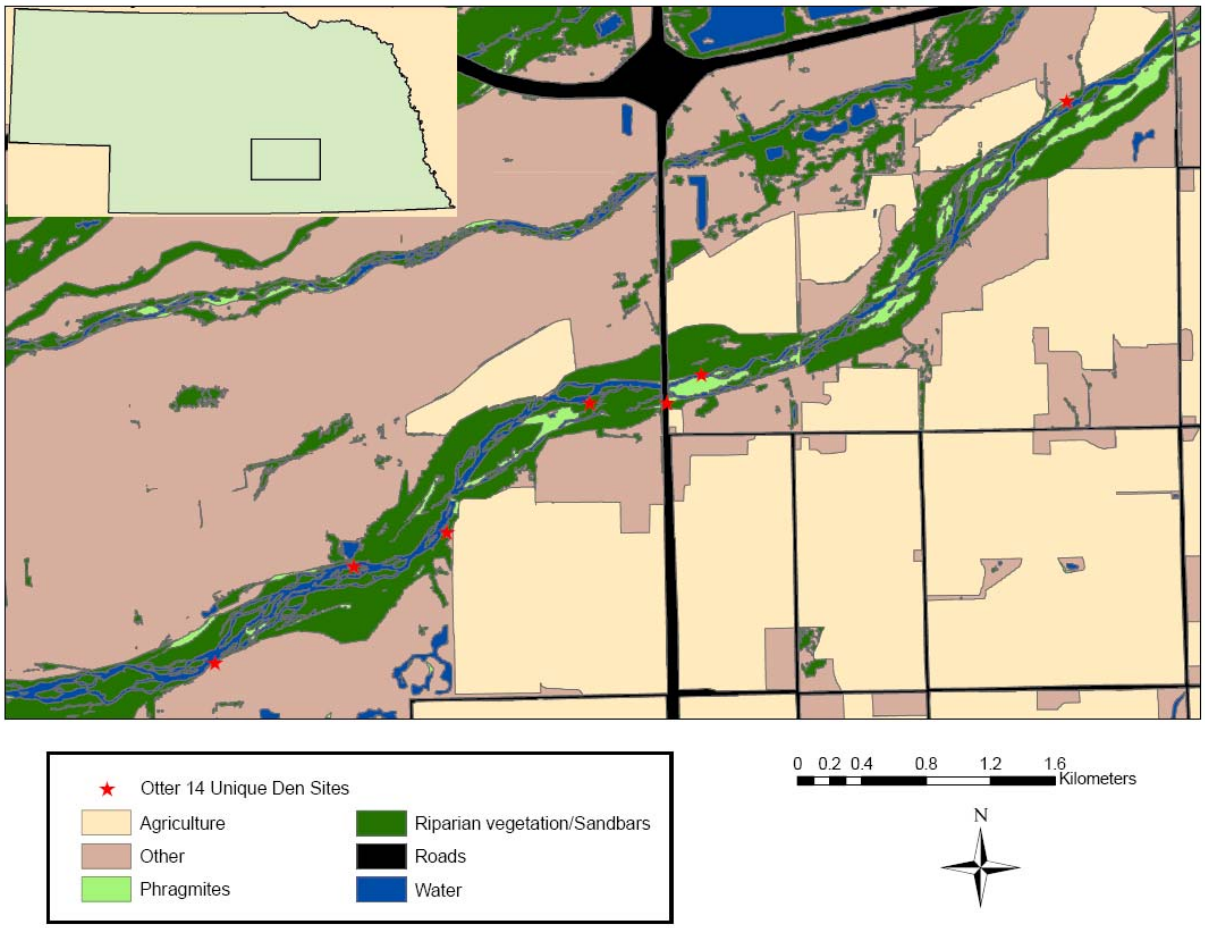

Figure B.1. Unique den/resting sites for female otter 14 along the Big Bend reach of the central Platte River, Nebraska, USA from 2008 to 2009.

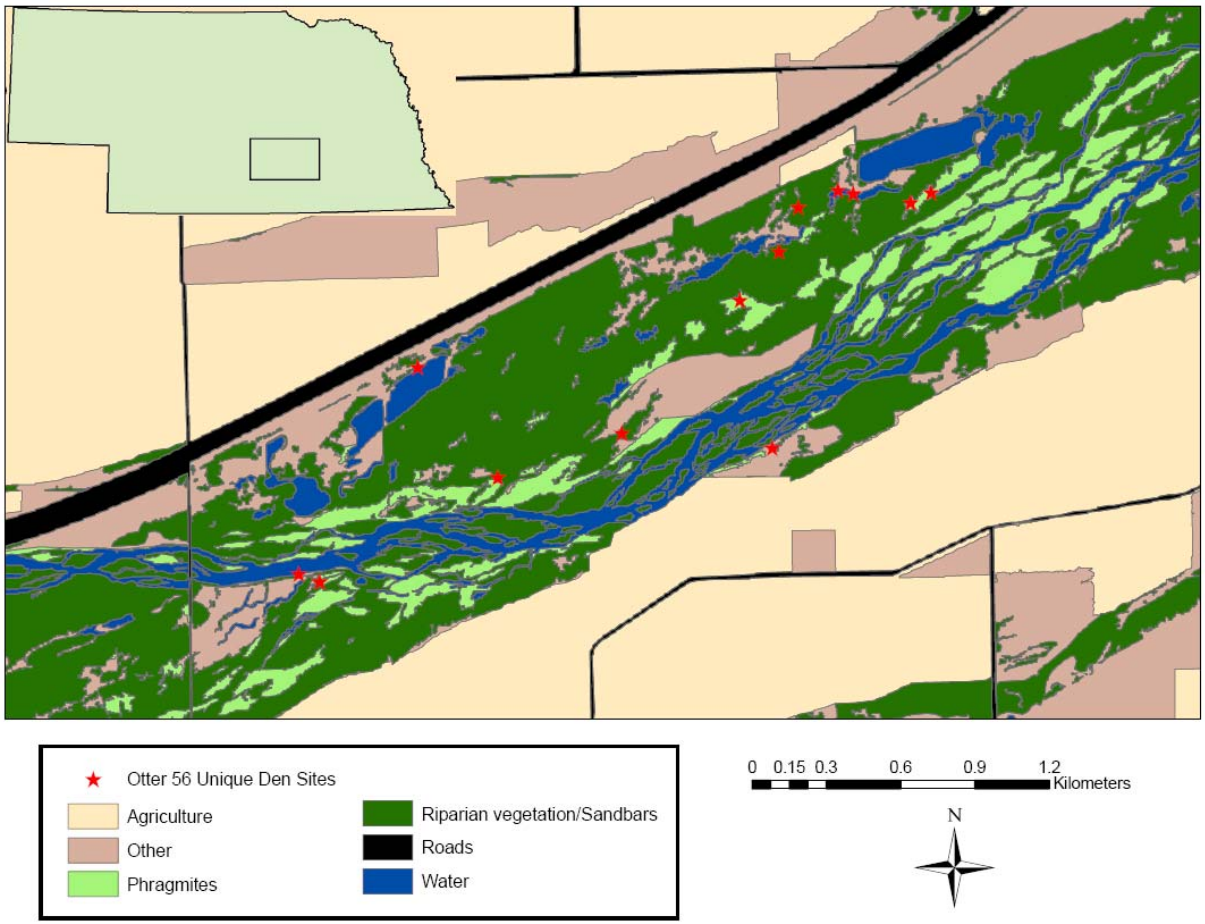

Figure B.2. Unique den/resting sites for female otter 56 along the Big Bend reach of the central Platte River, Nebraska, USA from 2008 to 2009.

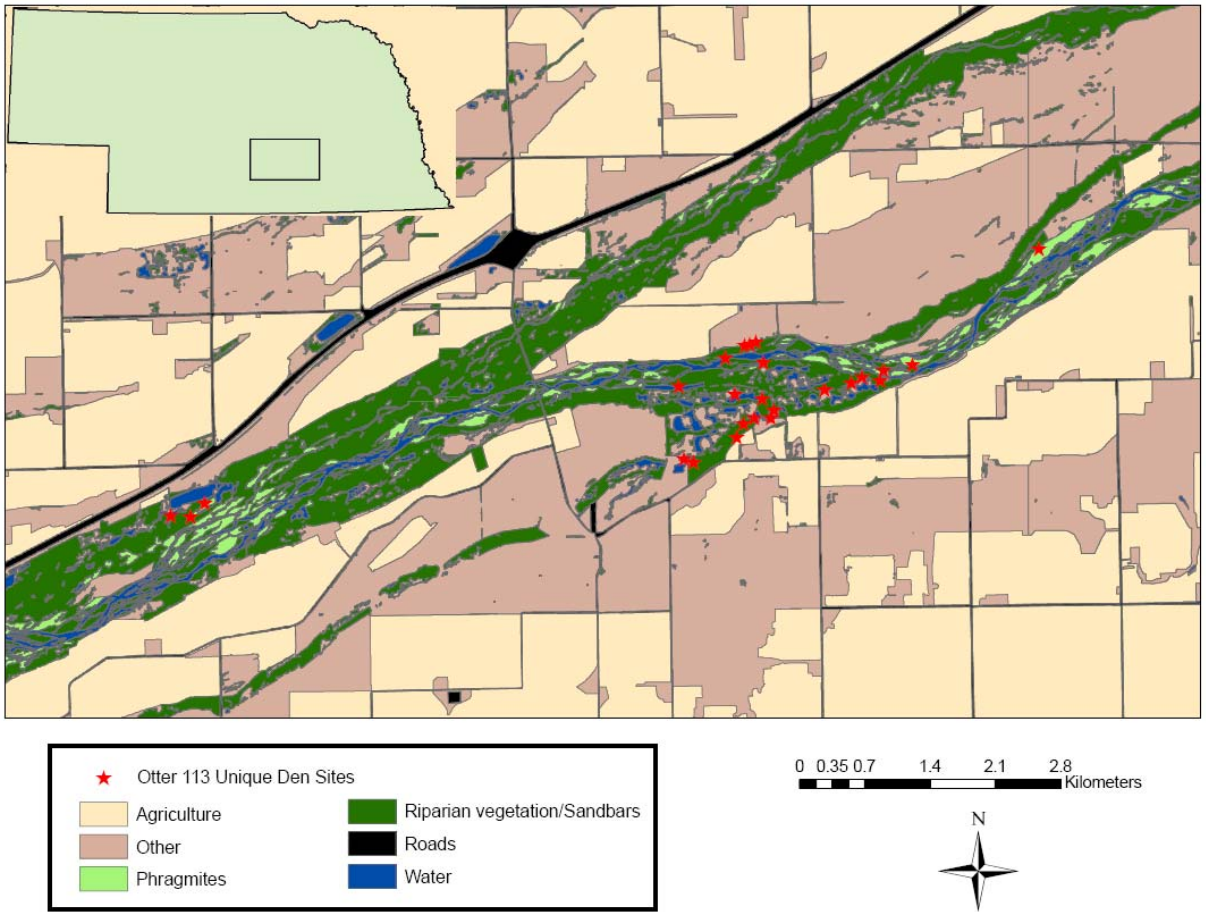

Figure B.3. Unique den/resting sites for female otter 113 along the Big Bend reach of the central Platte River, Nebraska, USA from 2006 to 2008.

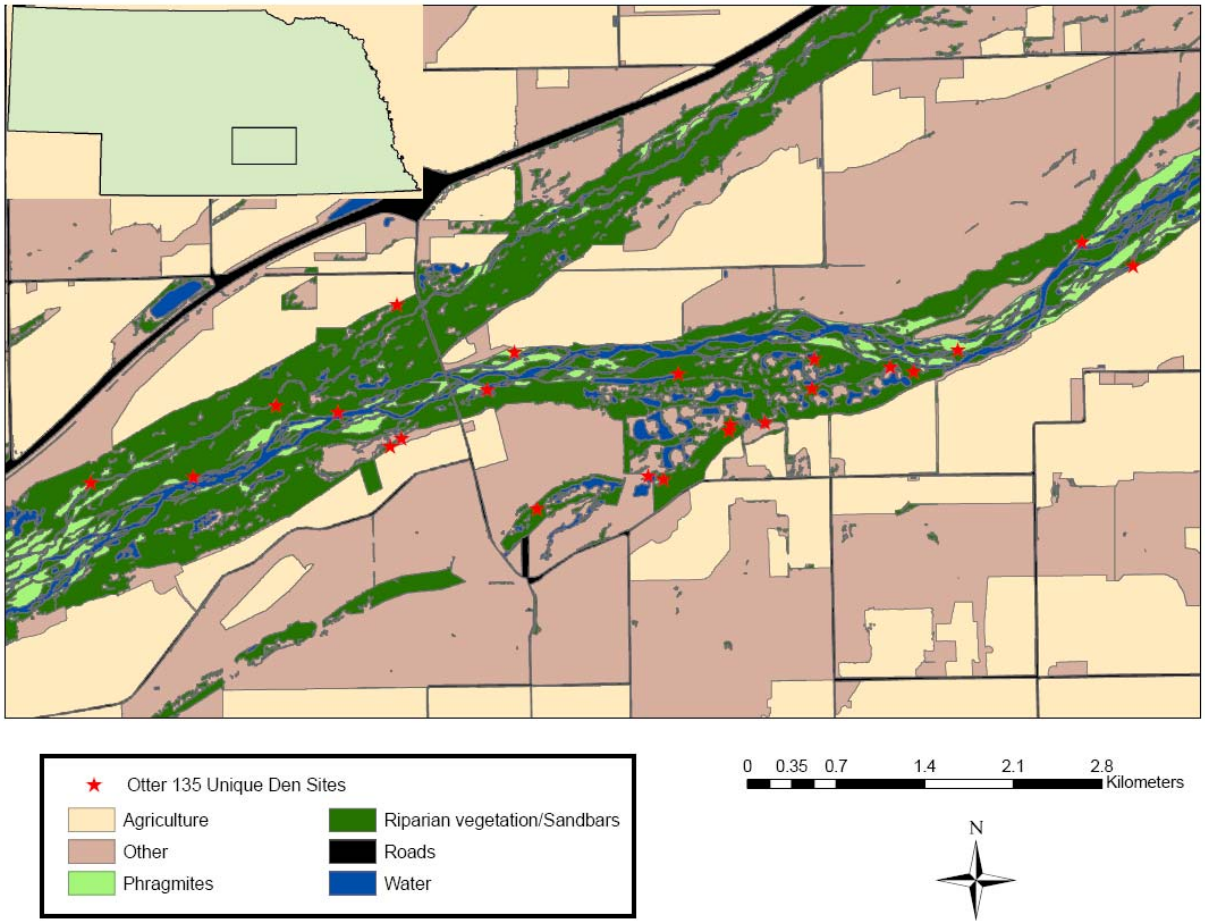

Figure B.4. Unique den/resting sites for female otter 135 along the Big Bend reach of the central Platte River, Nebraska, USA from 2007 to 2008.

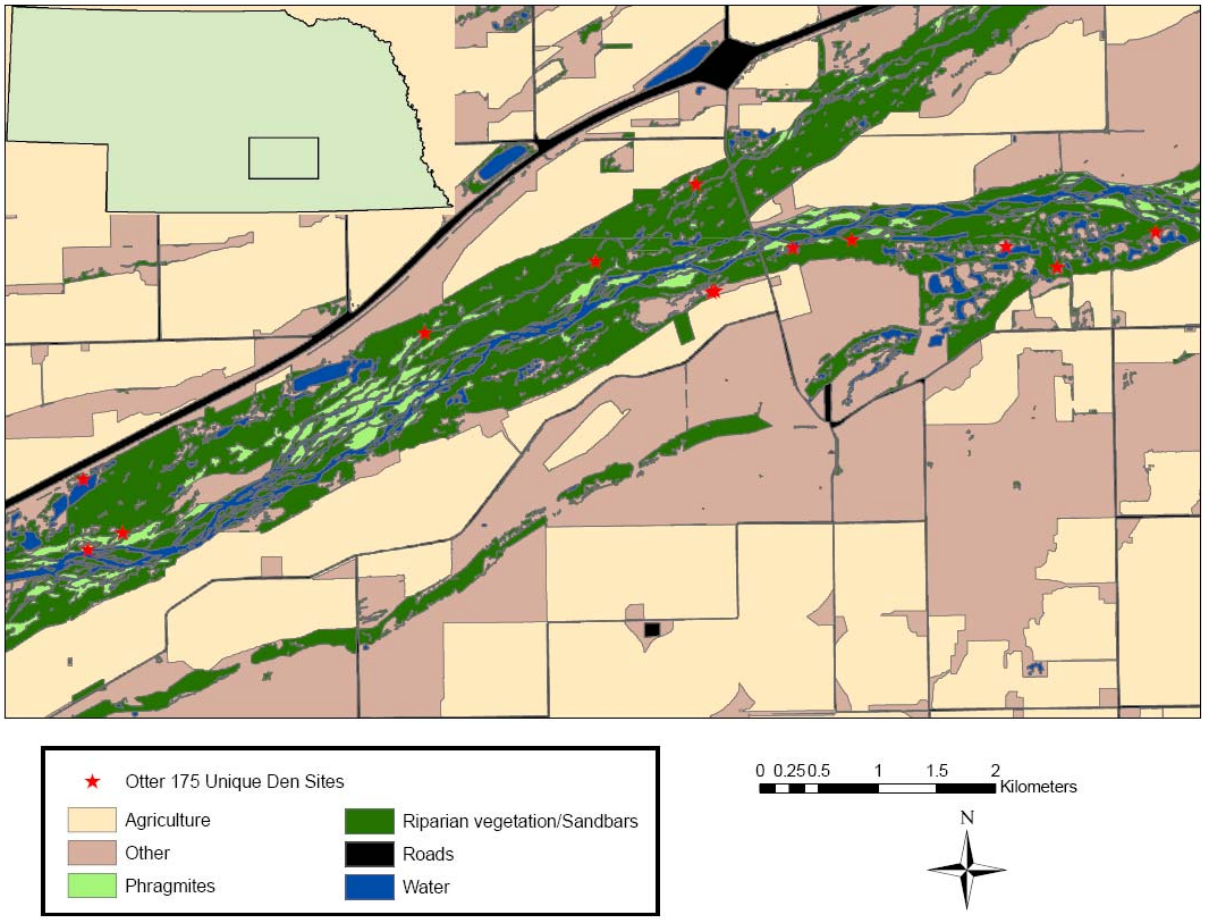

Figure B.5. Unique den/resting sites for male otter 175 along the Big Bend reach of the central Platte River, Nebraska, USA in 2008.

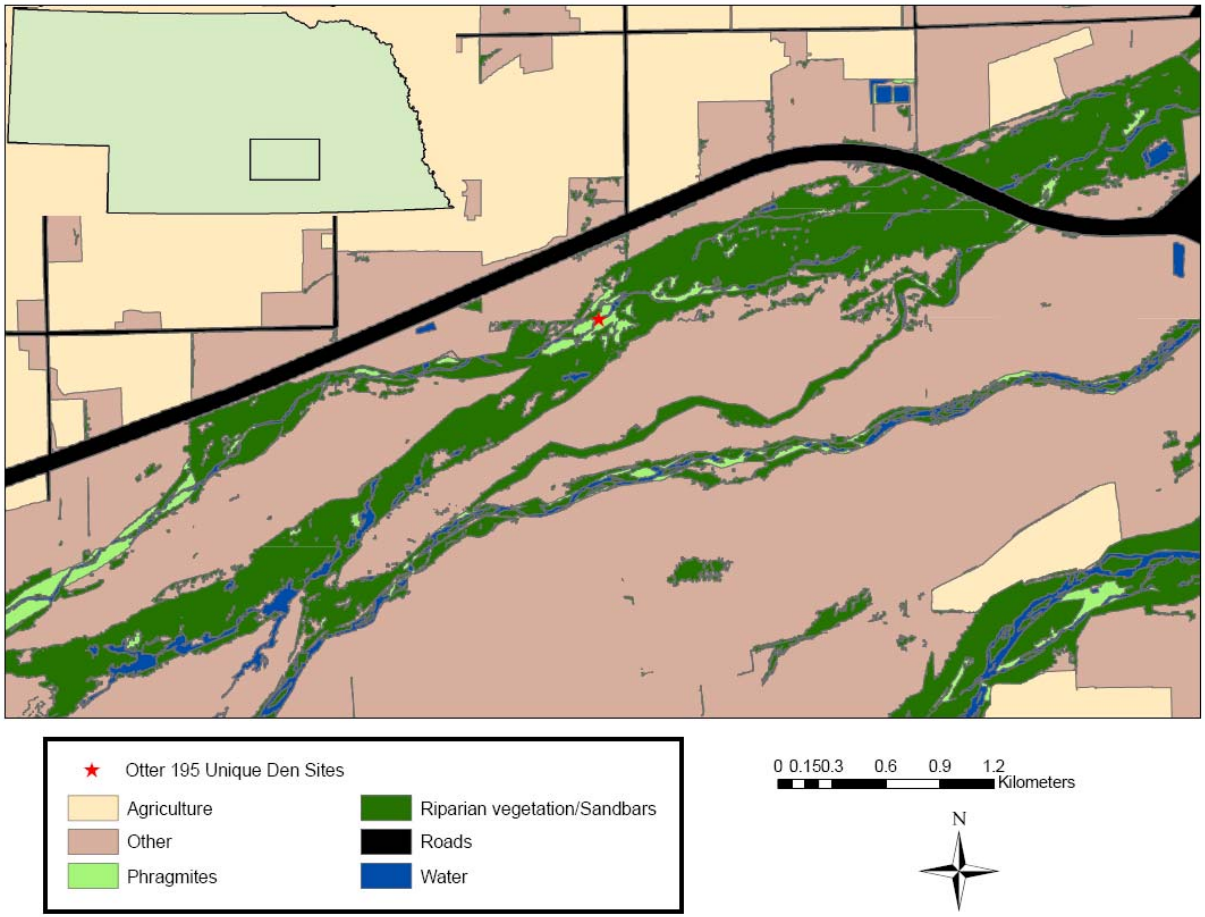

Figure B.6. Unique den/resting sites for male otter 195 along the Big Bend reach of the central Platte River, Nebraska, USA in 2006.

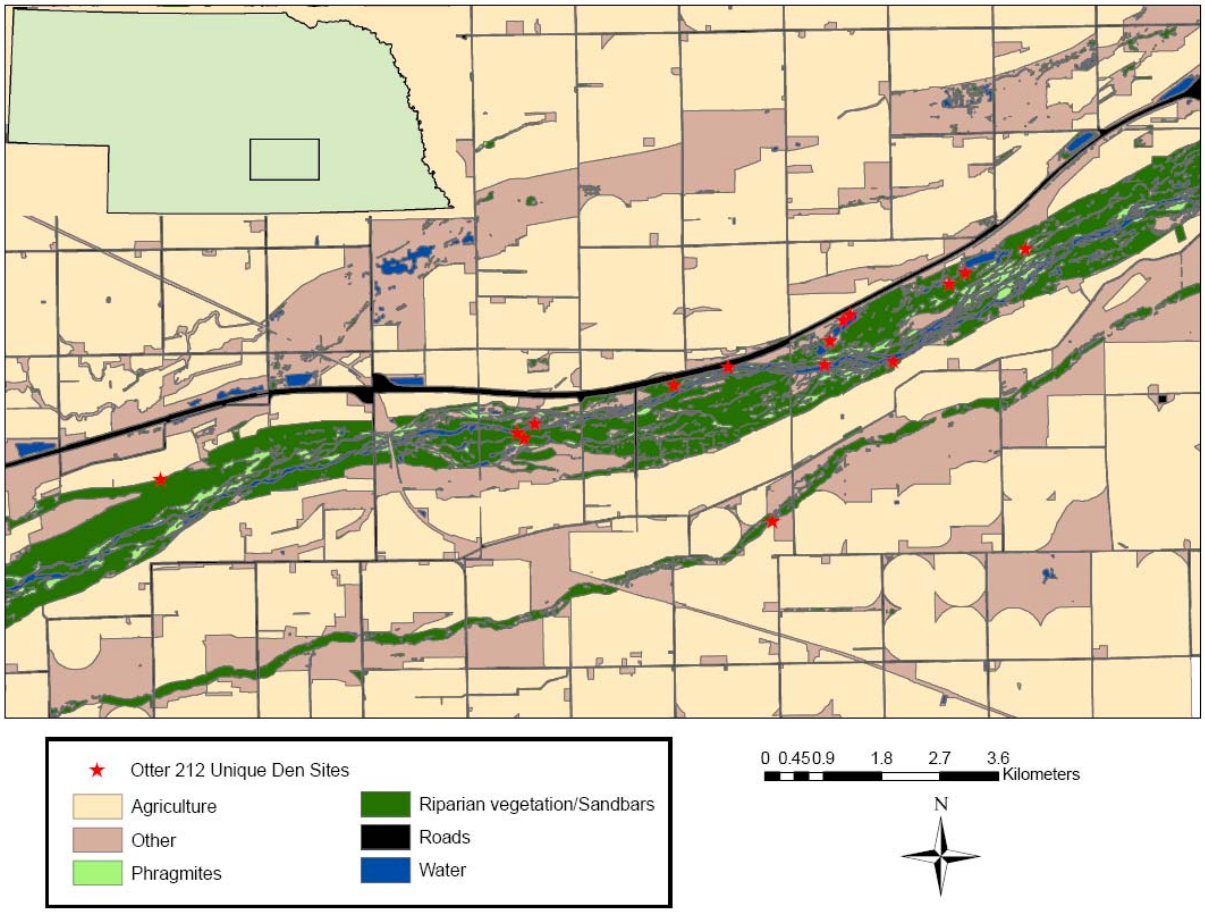

Figure B.7. Unique den/resting sites for male otter 212 along the Big Bend reach of the central Platte River, Nebraska, USA from 2006 to 2009.

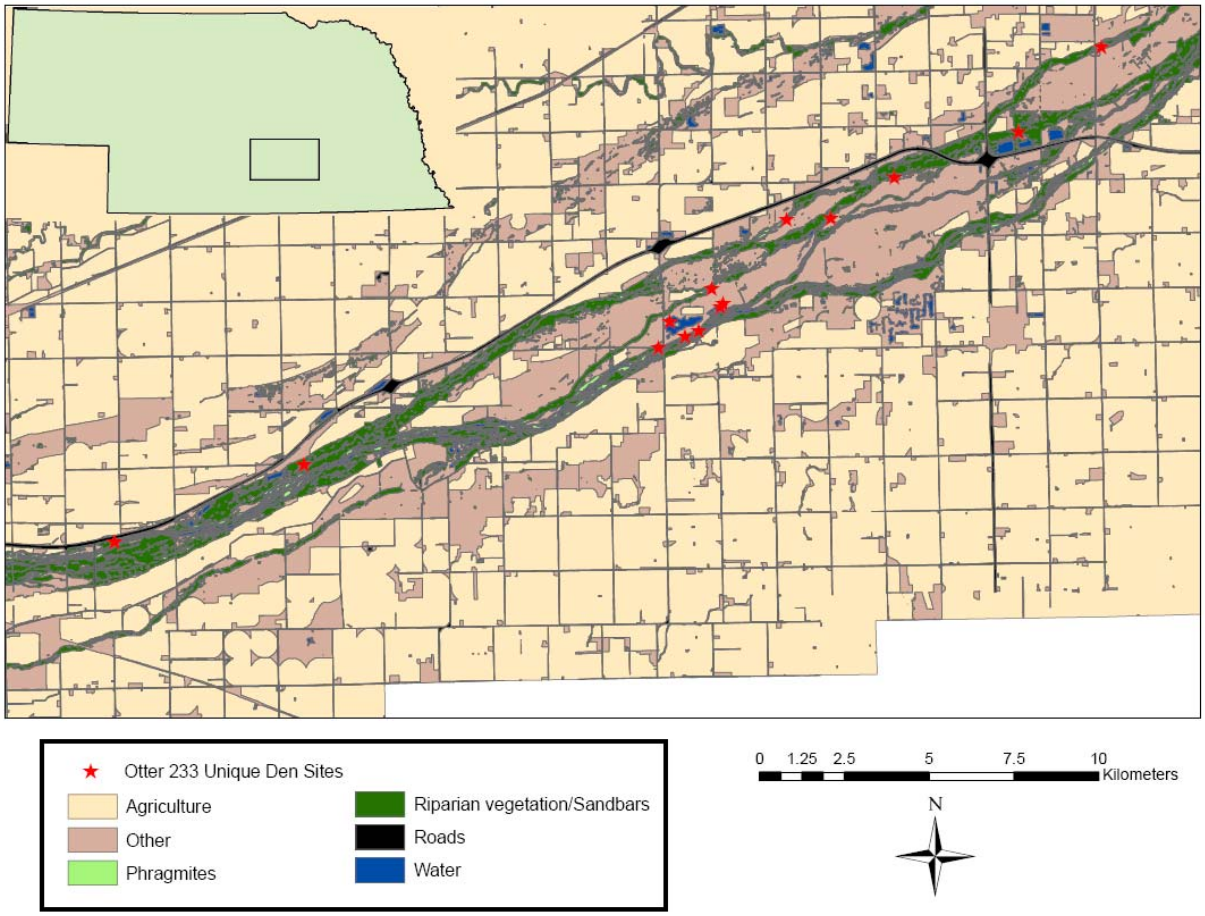

Figure B.8. Unique den/resting sites for male otter 233 along the Big Bend reach of the central Platte River, Nebraska, USA from 2008 to 2009.

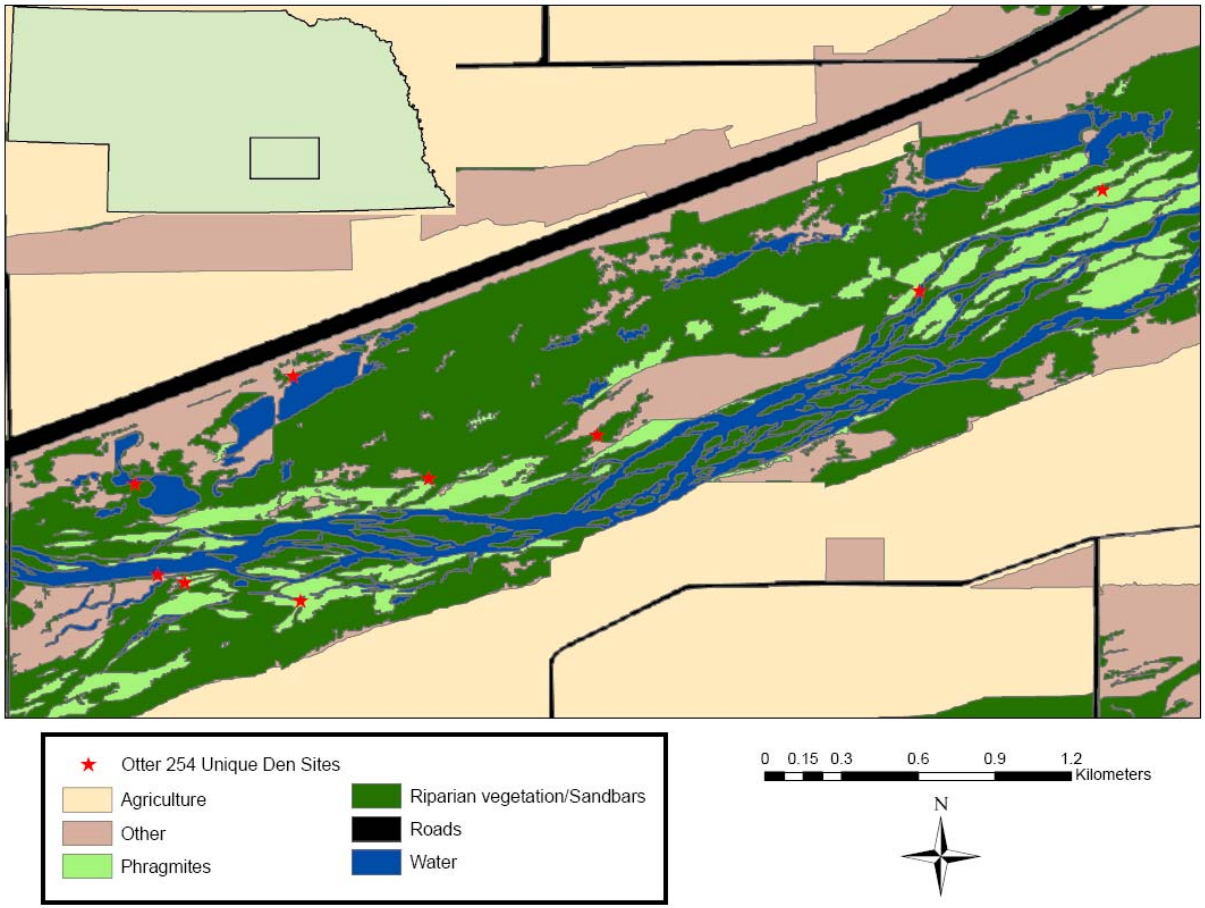

Figure B.9. Unique den/resting sites for female otter 254 along the Big Bend reach of the central Platte River, Nebraska, USA from 2007 to 2008.

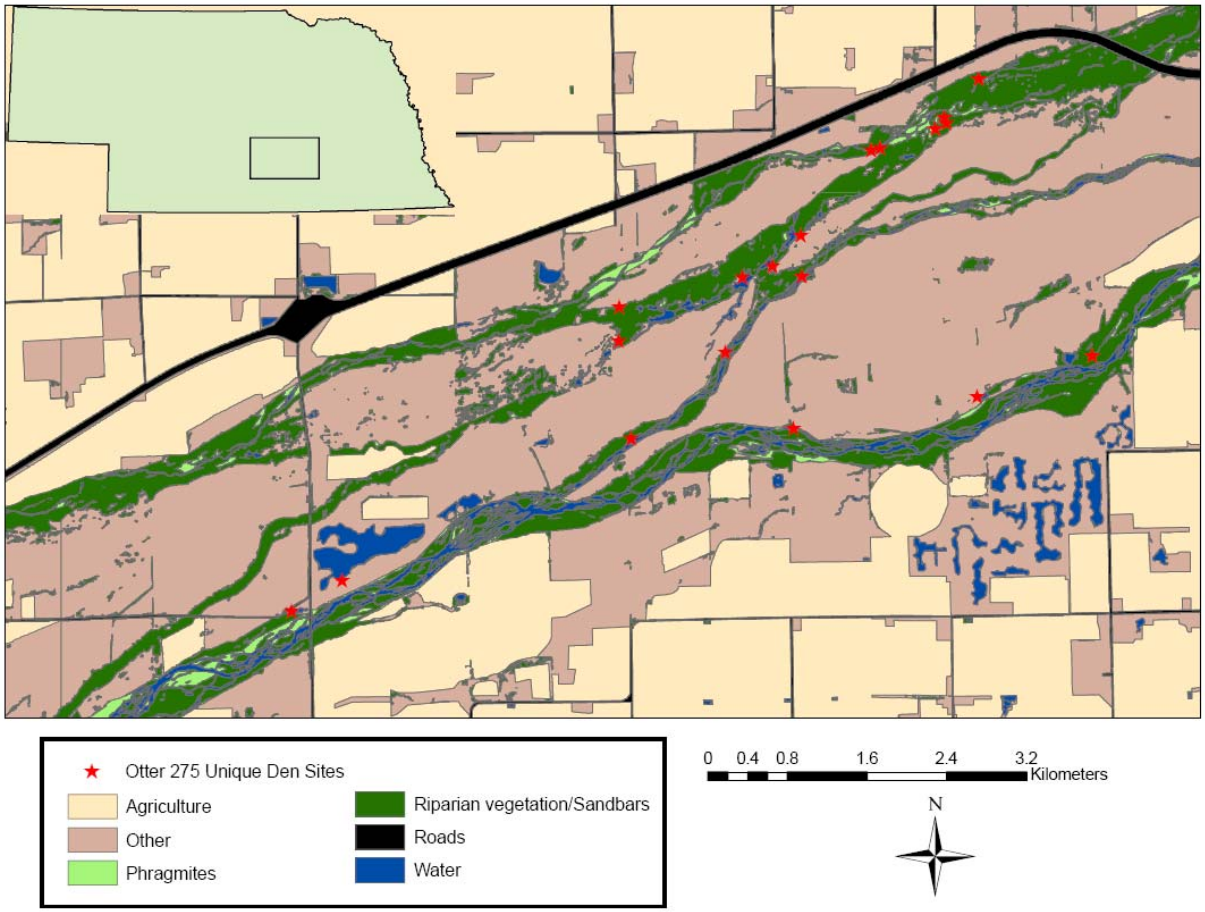

Figure B.10. Unique den/resting sites for female otter 275 along the Big Bend reach of the central Platte River, Nebraska, USA from 2006 to 2007.

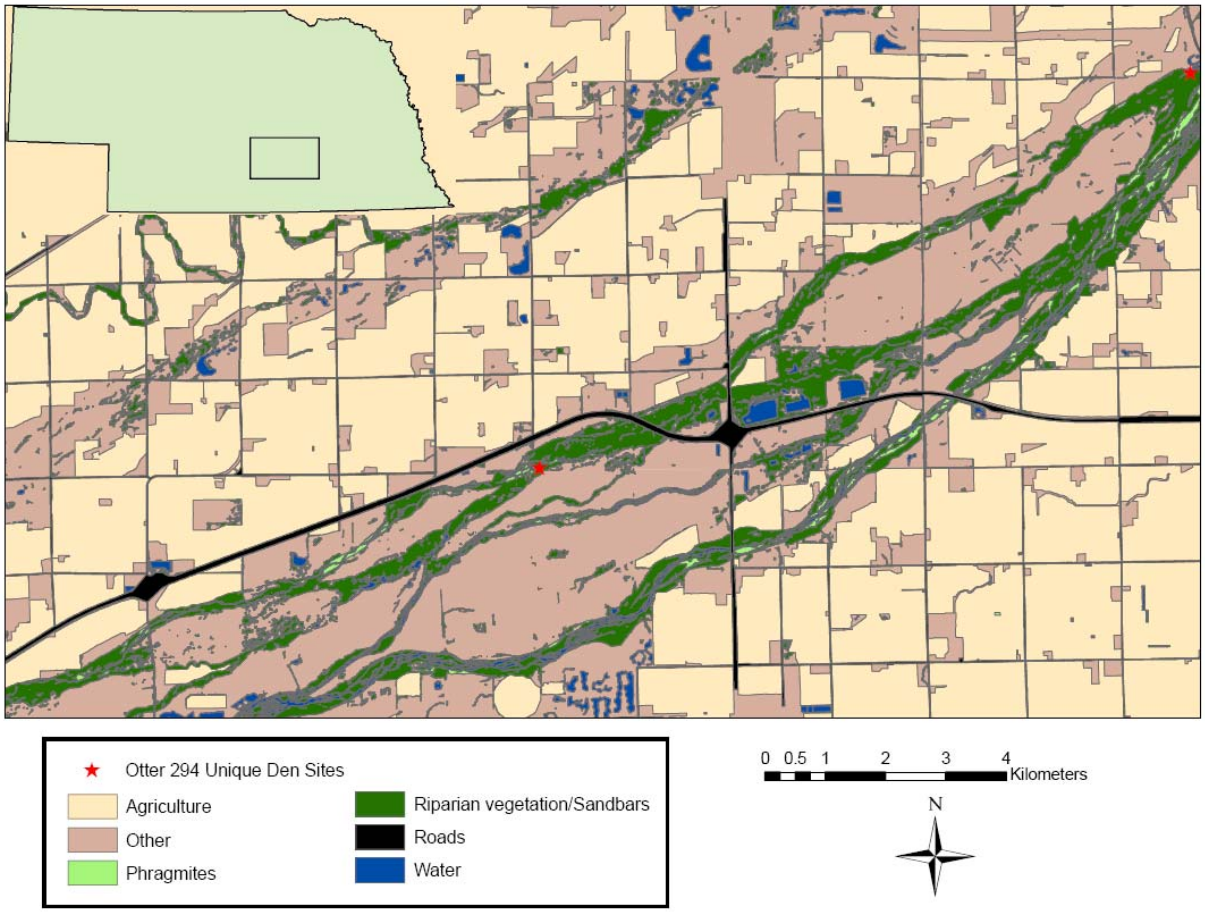

Figure B.11. Unique den/resting sites for male otter 294 along the Big Bend reach of the central Platte River, Nebraska, USA from 2008 to 2009.

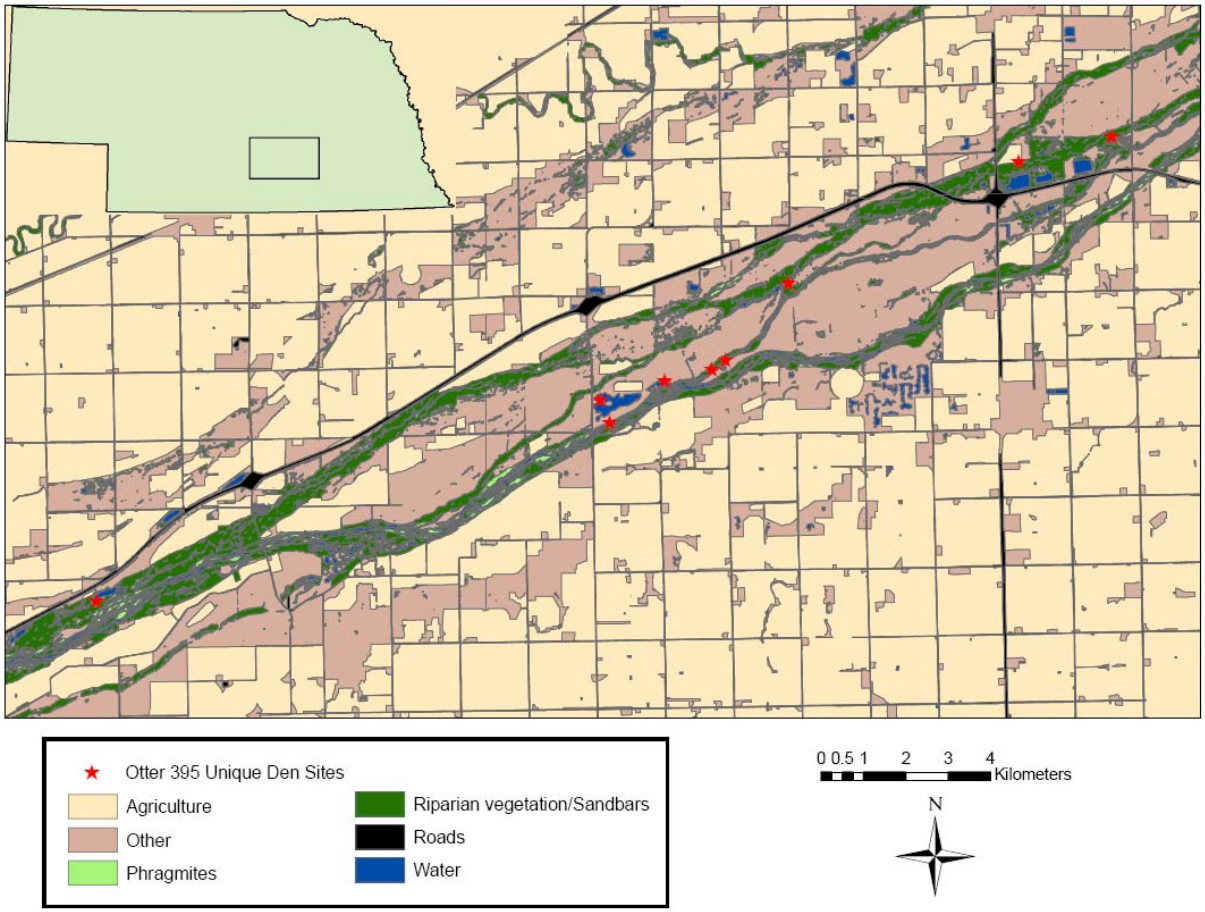

Figure B.12. Unique den/resting sites for male otter 395 along the Big Bend reach of the central Platte River, Nebraska, USA from 2008 to 2009.

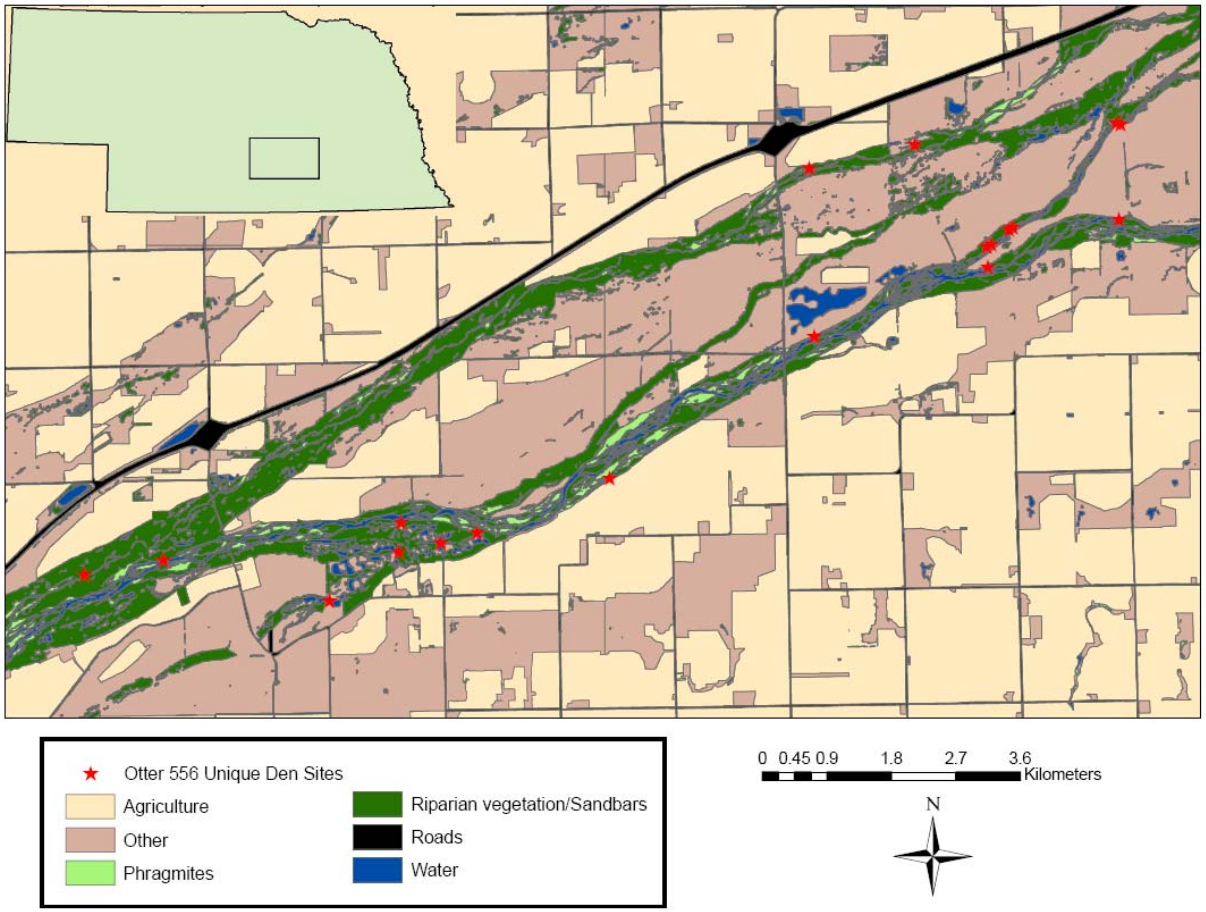

Figure B.13. Unique den/resting sites for male otter 556 along the Big Bend reach of the central Platte River, Nebraska, USA in 2009.

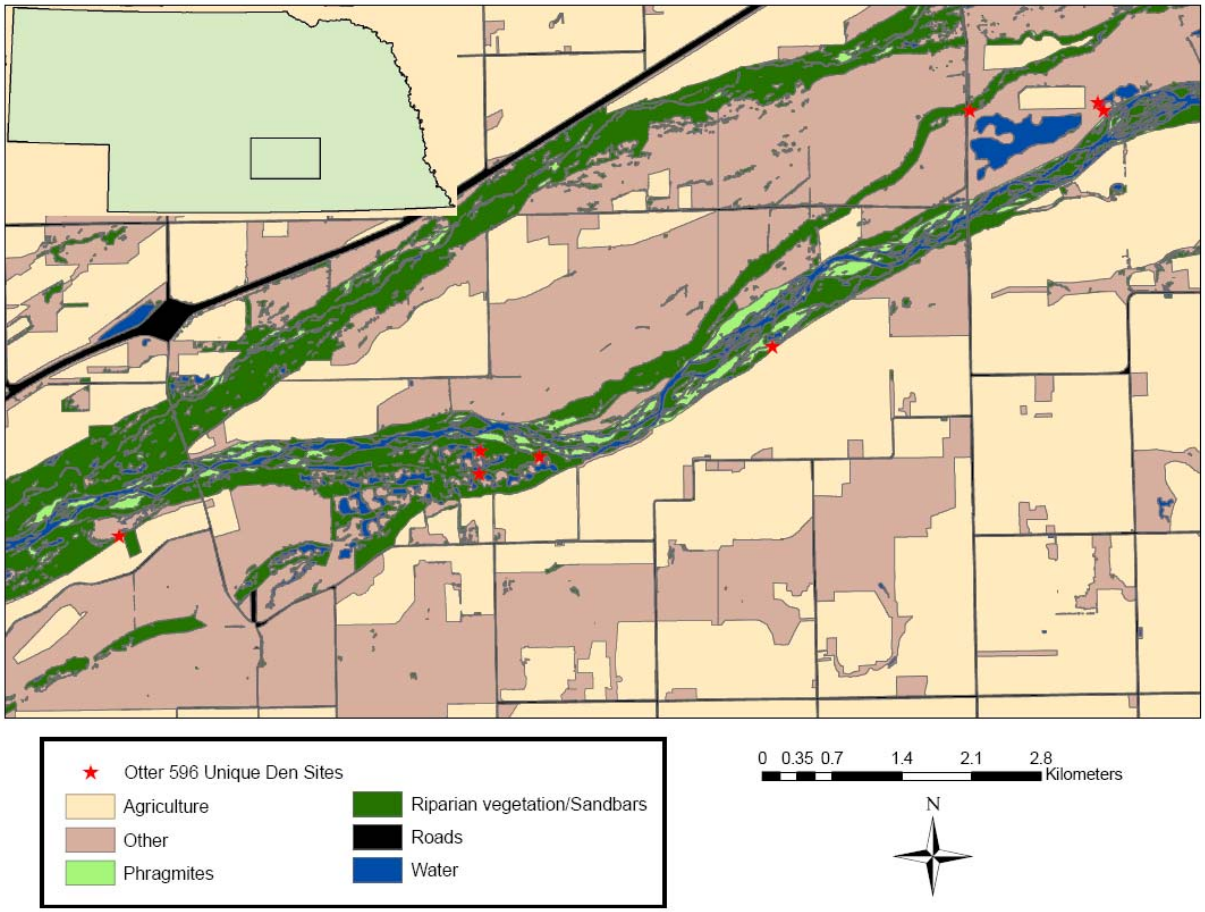

Figure B.14. Unique den/resting sites for female otter 596 along the Big Bend reach of the central Platte River, Nebraska, USA from 2008 to 2009.

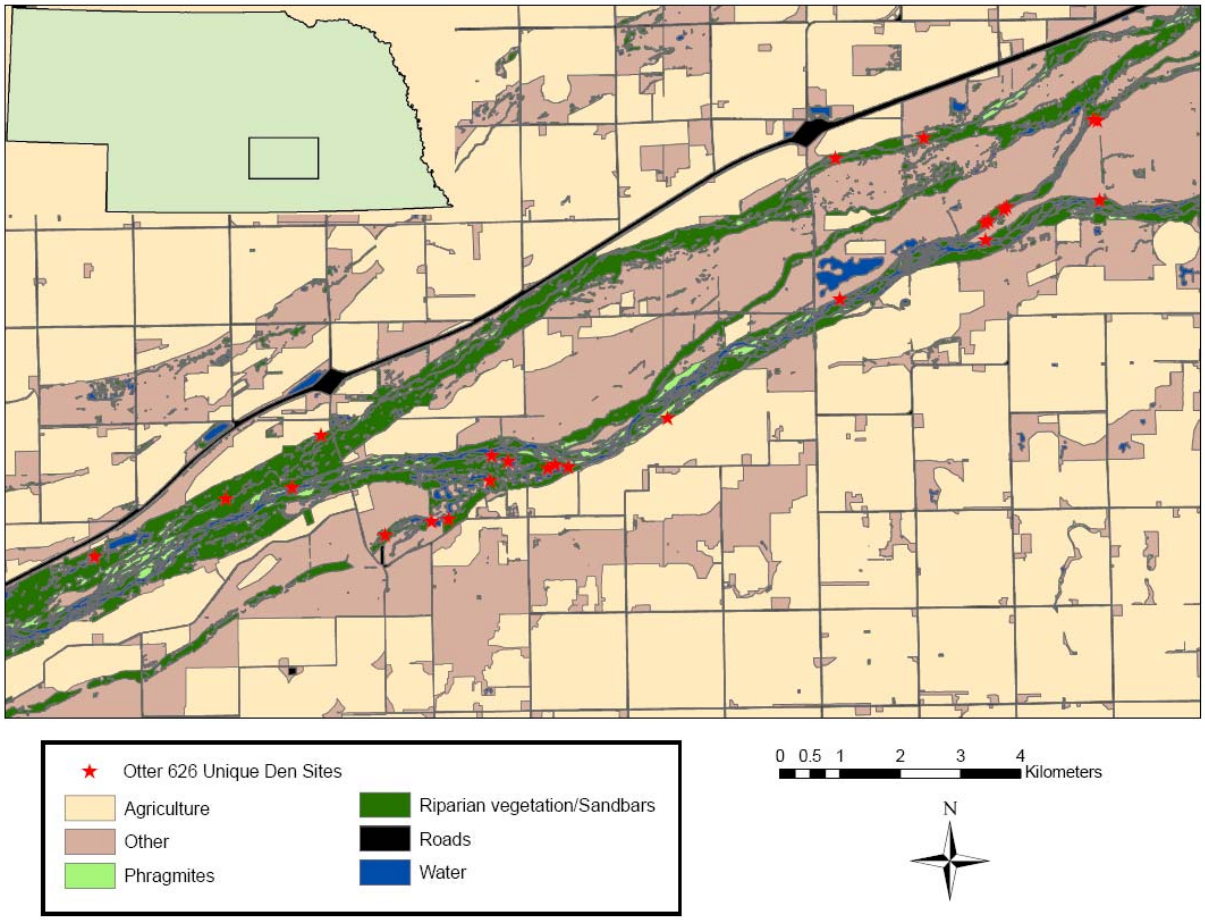

Figure B.15. Unique den/resting sites for male otter 626 along the Big Bend reach of the central Platte River, Nebraska, USA from 2008 to 2009.

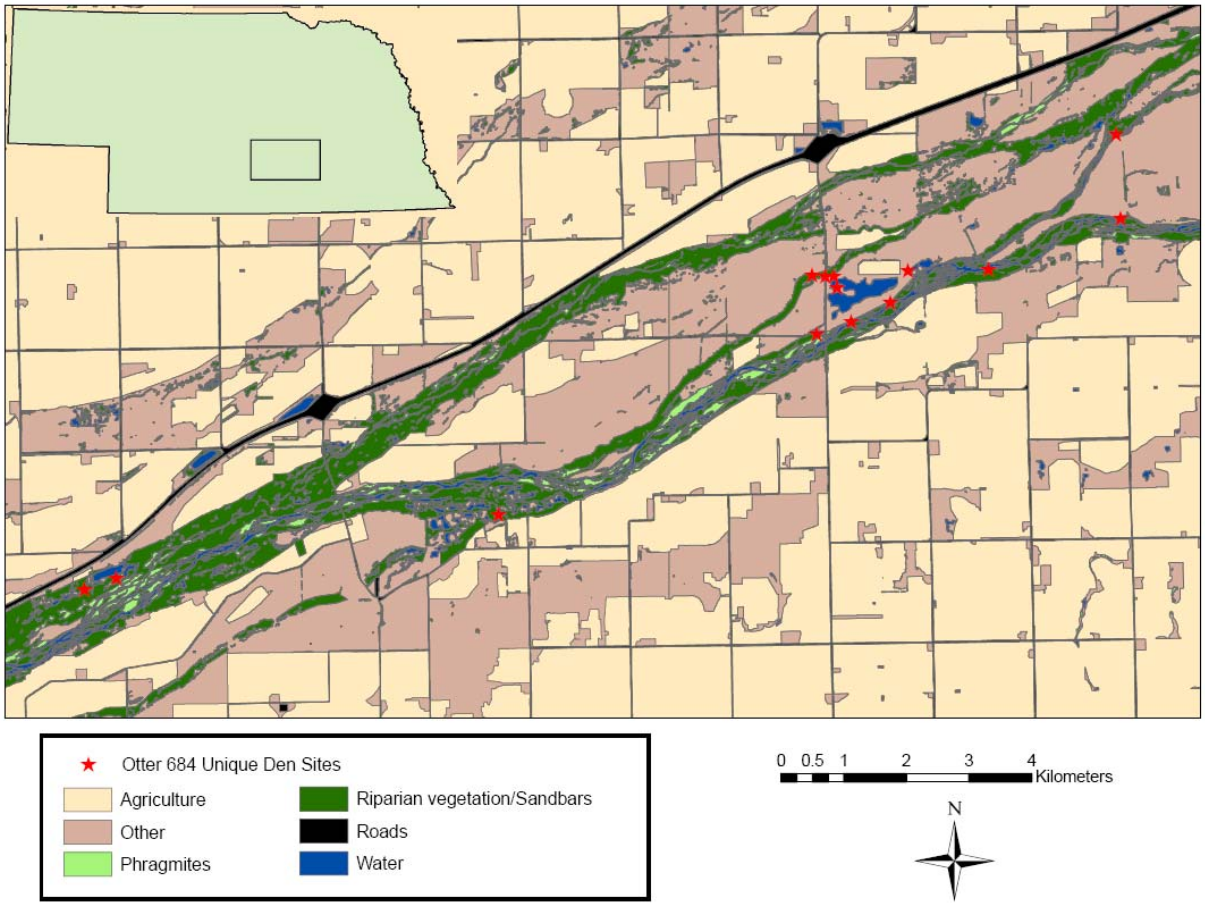

Figure B.16. Unique den/resting sites for male otter 684 along the Big Bend reach of the central Platte River, Nebraska, USA from 2008 to 2009.

Appendix C. Equations used to estimate population size of river otters on the Platte River between Gibbon and Alda, Nebraska.

Equation 1. Lincoln–Petersen model for estimating population size (N), adjusting for small sample size, where n_1 is the number of individuals captured during the first sampling session, n_2 is the number of individuals captured during the second sampling session, and m_2 is the number of recaptured individuals.

$$N_c = \frac{(n_1 + 1)\,(n_2 + 1)}{(m_2 + 1)} - 1$$

Equation 2. Model for estimating variance of population size estimate (N) where n_1 is the number of individuals captured during the first sampling session, n_2 is the number of individuals captured during the second sampling session, and m_2 is the number of recaptured individuals.

$$var(N_c) = \frac{(n_1 + 1)(n_2 + 1)(n_1 - m_2)(n_2 - m_2)}{(m_2 + 1)^2(m_2 + 2)}$$

Equation 3. Lincoln–Petersen model to estimate population size (N, without adjusting for sample size) and detection probability (β), where n_1 is the number of individuals captured during the first sampling session, n_2 is the number of individuals captured during the second sampling session, and m_2 is the number of recaptured individuals.

$$N = \frac{n_1 n_2}{m_2} = \frac{n_2}{\beta}$$

Appendix D. Genotypes of otter scat samples, collected during the first sampling session on September 23, 2009 between Gibbon and Alda, Nebraska, with genotypes identified at seven or more of the ten microsatellite loci. Samples 106–124 were collected on the Gibbon to Shelton river stretch, samples 206-247 were collected on the Shelton to Wood River stretch, and samples 305–362 were collected on the Wood River to Alda stretch.

	Microsatellite locus									
Sample Number	RIO01R2	RIO02R	RIO04R	RIO06R	RIO07R	RIO08R	RIO11	RIO13R	RIO15R	RIO16R
106	146146	127129	110118	126126	096100	105105	153153	157159	138138	
108	146146	127131	110110	126126	100102	105107		147147	138138	155157
114		127135	110110		096096	103103		145161	138138	157157
118	146154	129131	110110	126126	096100	105107	157159	147159	138138	
122	146154	129131	110110	126126	098100			157159	138138	
123	146154	129131	110118	126126	100102			159163	138138	
124	146154	129131	110118	126126	100100	103107		157159	138138	155155
206	146154	127131	110118	126134	094102	105107	153153		138138	
215	146154	129131	110110	126126	100100	105107	153157	147159	138138	155157
217	146154	129131	110110	126126	098100	103105	157157	159159	138138	157157
224		119131	110114	134134	092102	105107	153155	145159	138138	155157
229	142146	129131	110110	126132	096100	105109	153155	147159	136138	155157
230	146154		110118	126134		105107	153153	147159	138138	155157
241		129131	112114	126134	088096	107109	153157	145159	138138	155157
242	146154	129131	110110		100102	103105	157157		138138	155157
243	146146	127131	110110	126126	100102	105107		147159	138138	
246	146150	119131	110114	126134	096102	105109		159159	138138	

Appendix D. Continued.

	Microsatellite locus									
Sample Number	RIO01R2	RIO02R	RIO04R	RIO06R	RIO07R	RIO08R	RIO11	RIO13R	RIO15R	RIO16R
247	146154	129131	110110		096100	103105		159163	138138	
305		131131	110110	126126	096096	105105	153155		138138	
306		131131	110114	126134	088092	107107	145145	138138		
307		127131	112112	126134	102102	103107		145145	138138	
319		129131	110114	134134	088092	107109		145157	138138	155157
322	154154	127131		126134	096102	103105	155155	145145	138138	
327	150158	129131	110114	134134	088092	107109	153155		138138	155155
329	146158		110114	126142	088096	103109		145145	136138	
338			110112	126134	102102	109109		157159	138138	155157
348		131131	114114	134134	088092	107109		145159	138138	
350	154154		110112	126134	096102	105105		145145	136138	155155
353		131131	110114	134134	088092	107109		145145	138138	
354	146154	127131	112114	126134	102102	103107	153153	145145	138138	155157
356	154154	127129	110110	126134	100102	103109		145159	138138	157157
362	154154		110110	126134	102102	103109		145145	138138	

Appendix E. Genotypes of otter scat samples, collected during the second sampling session on October 7, 2009 between Gibbon and Alda, Nebraska, with genotypes identified at seven or more of the ten microsatellite loci. Samples 409–421 were collected on the Gibbon to Shelton river stretch, samples 501-552 were collected on the Shelton to Wood River stretch, and samples 602–656 were collected on the Wood River to Alda stretch.

	Microsatellite locus									
Sample Number	RIO01R2	RIO02R	RIO04R	RIO06R	RIO07R	RIO08R	RIO11	RIO13R	RIO15R	RIO16R
409	146146	127129	110118	126126	096100	105105	153155	159159	138138	155157
421	146146	127129	110118	126126	096100	105105	153155	159159	138138	155157
501		119129	110114	126142	088096	103109		145165	136138	155155
507	146154	131131	110110	126134	096100	103105		159159		
519	146146				096096	105109	153159	159163	138140	155155
531	146146	127131	110110	126126	100102	105107	153157	147159	138138	155157
539	146154	131131	110110	126132		103107	153153	145159	138138	155157
543	154146	131131	110110	126134		103105		159163	138138	155155
544	146146	127129	110118	126126	096100	105107		159159	138138	
551	146146	127131	110110	126126	100102	105107	157157	147159	138138	
552	146146	129131	110110	126126	100100	105107		147159	138138	155155
602	154154	127131	110112	126134	096102		153155		138138	155157
603	146154	127131	110112	126134	088096	103105	153155	143145	138138	155155
614	154154	129129	110112		096102	105109	157159	159163	138138	155157
619	150158	129131	110114	134134	088092	107109	153155	145159	138138	155157
622	146154	127131	112114	126134	092102	103107		145145	138138	155157
624	146154			126126	102102	103107		145145	138138	155157

Appendix E. Continued.

	Microsatellite locus									
Sample Number	RIO01R2	RIO02R	RIO04R	RIO06R	RIO07R	RIO08R	RIO11	RIO13R	RIO15R	RIO16R
645	146154	127131	112114	126134	102102	103107		145145		155157
648	154154	127129		126134	102102	103109	153153	145157	138138	
653		129131	110114	134134	088092	107109	153155	145157	136138	155157
656	146154		112114	126134	102102	103107		143145	138138	155157

Appendix F. Unique Nebraska river otters (identified by the liberal genotyping method, in which two samples where there was uncertainty regarding identification were considered different individuals) and the sample numbers that were collected for each of those individuals (and genotyped at seven or more of the ten microsatellite loci).

Individual ID	Session 1 Samples	Session 2 Samples
River Otter 1	243	531, 551, 552
River Otter 2	354, 307	622, 645, 656, 624
River Otter 3	319, 327, 353, 348	619, 653
River Otter 4	247, 305	543, 507
River Otter 5	322, 350	602
River Otter 6	329	501
River Otter 7	338	
River Otter 8	215	
River Otter 9	224	
River Otter 10	242, 362, 123	
River Otter 11	356	648
River Otter 12	230, 206	
River Otter 13	106	544, 409, 421
River Otter 14		539
River Otter 15		614
River Otter 16	217	
River Otter 17	229	
River Otter 18	241	
River Otter 19	246	
River Otter 20	306	
River Otter 21		519
River Otter 22		603
River Otter 23	108	
River Otter 24	114	
River Otter 25	118	
River Otter 26	122	
River Otter 27	124	

Appendix G. Unique Nebraska river otters (identified by the conservative genotyping method, in which two samples where there was uncertainty regarding identification were considered the same individual) and the sample numbers that were collected for each of those individuals (and genotyped at seven or more of the ten microsatellite loci).

Individual ID	Session 1 Samples	Session 2 Samples
River Otter 1	106	409, 421, 544
River Otter 2	114	
River Otter 3	215, 243, 108, 305, 118	531, 551, 552
River Otter 4	217, 242, 123, 247, 122, 124	
River Otter 5	224	
River Otter 6	229	
River Otter 7	230, 206	
River Otter 8	241	
River Otter 9	246	
River Otter 10	306	
River Otter 11	322, 350	
River Otter 12	327, 319, 353, 348	619, 653
River Otter 13	329	501
River Otter 14	338	
River Otter 15	354, 307	622, 645, 656, 624
River Otter 16	356, 362	648, 602
River Otter 17		519
River Otter 18		539
River Otter 19		543, 507
River Otter 20		603
River Otter 21		614