Summaries of Wildlife Research Findings
2014

Minnesota Department of Natural Resources
Division of Fish and Wildlife
Wildlife Populations and Research Unit

MNDNR

SUMMARIES OF WILDLIFE RESEARCH FINDINGS 2014

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MNDNR

EVALUATION OF LOCALIZED DEER MANAGEMENT FOR REDUCING AGRICULTURAL DAMAGE CAUSED BY WHITE-TAILED DEER IN MINNESOTA

Gino D'Angelo

SUMMARY OF FINDINGS

Minimizing damage caused by white-tailed deer (*Odocoileus virginianus*) is an important consideration for managing deer densities in Minnesota. I am conducting an ongoing study, which began in April 2014 in southeast Minnesota to assess the effectiveness of localized management of deer (i.e., targeted removal of deer in a limited area) to reduce damage to agricultural crops. The objective of this study is to evaluate the effectiveness of localized management for reducing fine-scale deer abundance and to examine whether damage caused by deer to agricultural crops is reduced on properties where deer densities are lowered. One field season of the study was completed during 2014 in southeast Minnesota. Baited infrared camera surveys were used to estimate deer abundance on focal properties, and spotlight surveys were used to estimate deer abundance in the local area surrounding focal properties. Yields of corn in fenced and unfenced plots were evaluated to estimate the impacts of browsing by deer. Corn yield loss was seemingly low on most properties, and there was no difference in corn damage between properties where localized management was utilized versus normal sport-hunting. Corn damage could not be explained solely by deer abundance at the property level or deer abundance in the area surrounding focal properties. However, extra deer harvest opportunities were utilized when requested. Deer management was >2 times as intensive on properties where integrated management was used versus normal sport-hunting. A second field season is being conducted in 2015. The results of this study will provide a basis for improving the framework for future application of localized management in agricultural regions.

INTRODUCTION

Damage caused by white-tailed deer can be severe in the United States with ≥$100 million lost annually by agricultural producers (Conover 1997). Results from previous studies have demonstrated only through anecdotal evidence that population reduction of deer can reduce damage to agriculture (McShea et al. 1993, Frost et al. 1997, Conover 2001). In some situations, localized management has effectively reduced the abundance of deer to maintain lowered deer densities over time (McNulty et al. 1997). As a result, damage to resources targeted for protection should be reduced because fewer deer are available to cause damage. However, conditions including high deer densities in surrounding areas (Miller et al. 2010), seasonal migratory behavior of deer (Vercauteren and Hygnstrom 1998), and colonization by deer from adjacent populations (Comer et al. 2007) may inhibit the creation of sufficient temporal periods of low deer densities to provide resource protection. Studies of the effectiveness of localized management to reduce damage to specific properties in agricultural settings are lacking.

Minimizing damage caused by deer is an important consideration in managing their populations in Minnesota. In many deer permit areas in Minnesota, deer are managed at or near population goals annually. However, complaints of deer damage from agricultural producers are common. During years 2003-2012, wildlife managers fielded an average of 130

complaints annually about damage caused by deer. Complaints of depredation by deer in Minnesota include consumption of forage stored for livestock, damage to specialty crops (e.g., produce, Christmas trees, nursery stock), row crops (corn [*Zea mays*] and soybeans [*Glycine max*]), alfalfa (*Medicago sativa*), and forest stands. Deer damage is reported throughout Minnesota, but a distinct cluster of complaints occurs in the Southeast region of the state (Nelson and Engel 2013).

In Southeast Minnesota the majority of complaints involve standing row crops and alfalfa in the field. Farmers who enter into a Cooperative Damage Management Agreement with MNDNR are eligible for cost-sharing to install exclusion fencing. However, funds for deer damage assistance are limited and fencing is only practical for protecting areas that are relatively small (i.e., stored forage and specialty crops). Sound and visual deterrents and taste and smell repellents have proven ineffective for reducing deer damage in agricultural fields (Belant et al. 1996, Belant et al. 1998, Gilsdorf et al. 2004). Therefore, most attempts to reduce damage to standing crops in Southeast Minnesota involve the use of localized deer damage management techniques such as shooting permits and depredation permits (herein, localized management).

MNDNR Regional Offices have issued shooting permits to agricultural producers experiencing extreme damage caused by deer for use outside of hunting seasons. Shooting permits allow landowners to shoot deer at any time of day or night and with a high-powered rifle. For years 2004 through 2012, an average of 95 shooting permits for nuisance deer were issued annually for use during summer and winter (Nelson and Engel 2013). In Southeast Minnesota, landowners with support from local legislators requested shooting permits to be issued during the regular hunting seasons to reduce depredation to standing row crops. As an alternative to their request, a pilot program using depredation permits allocated to specific properties was instituted in 2012 in Southeast Minnesota (Luedtke 2013). Depredation permits were to be used by private sport-hunters during regular hunting seasons. Additionally, a temporary DNR position, the Landowner Assistance Specialist, was created to administer the program in Fillmore, Goodhue, Houston, Olmsted, Wabasha and Winona counties.

Depredation permits allowed up to 15 hunters per property to harvest up to five antlerless deer in addition to established bag limits during regular hunting seasons–75 deer could be harvested on an individual property using depredation permits. To be eligible, applicants had to demonstrate: 1) a history of deer damage documented through complaints to the DNR Area Wildlife Office, 2) crop losses, 3) enrollment in a Cooperative Damage Management Agreement with MNDNR including a plan for deer hunting management, and 4) hunting was allowed on the property during the previous hunting season.

Localized management in Southeast Minnesota increased deer harvest on individual properties from previous years and anecdotally landowners and hunters involved in the program were satisfied (Luedtke 2013). However, the effect of localized management on agricultural damage caused by deer is unknown. Also, logistical limitations and eligibility guidelines restrict the number of properties where depredation permits may be issued annually. Given the onerous nature of administering localized management from an agency perspective, it is important to establish whether such management aids in reducing agricultural damage as intended.

The purpose of this study is to evaluate whether localized management of deer reduces agricultural damage and to provide a basis for improving the framework for future application of localized management in Minnesota. No previous studies have examined the effectiveness of localized management for reducing damage to agricultural crops. Other research has suggested that using recreational hunting to institute localized management of overabundant deer and effectively reduce damage may be difficult (Simard et al. 2013). If localized management can be used to minimize damage, these techniques should be utilized wherever feasible in Minnesota. Otherwise, alternative strategies for balancing local deer populations with social carrying capacity should be explored.

OBJECTIVES

1. To evaluate the effects of localized white-tailed deer management techniques– Including shooting permits, and depredation permits–on localized deer densities in Southeast Minnesota.

2. To quantify the amount of damage caused by white-tailed deer to agricultural crops relative to localized management in Southeast Minnesota.

STUDY AREA

This study was conducted in the Minnesota counties of Fillmore, Houston, and Winona. Southeast Minnesota is characterized by a mosaic of rolling limestone uplands dominated by agriculture (Mossler 1999). Typical crops include corn, soybeans, alfalfa, and small grains. Steep ravines cut by narrow streams are interspersed throughout the uplands. Ravines are rocky and primarily forested by mature hardwoods (Omernik and Gallant 1988).

Pre-fawn deer densities in these Southeast Minnesota averaged 5 deer per km^2 (Grund 2013), which represents the highest deer densities found in the farmland zone of Minnesota. An average of 1.5 deer per km^2 was harvested in these Southeast Minnesota during 2012, which was nearly twice the statewide average (McInenly 2013).

METHODS

Experimental Design

My objective was to evaluate the effectiveness of localized management for reducing fine-scale deer abundance and to examine whether damage caused by deer to agricultural crops is reduced on properties with higher management intensity. Therefore, I examined deer depredation to crops and deer abundance on individual focal properties in Southeast Minnesota. On properties used as treatments, localized management strategies were utilized in addition to regular sport-hunting. On control properties, normal sport-hunting was allowed by the landowner. I included 7 focal properties in the study, including 4 treatments and 3 controls.

Data Collection

Corn Evaluations–Within each field, I delineated 8 plots, which were stratified into interior (>10 m from the field edge) and edge (0-5 m from the field edge). Each plot included two paired 5-m X 5-m subplots (~$6/1000^{th}$ acre) separated by 5 m and within the same rows of corn. One subplot of each pair was fenced to exclude deer and the other subplot was an unfenced control. Within each pair, the treatment and control were assigned randomly. Square exclosures were constructed with 2-m high heavy-duty plastic mesh attached to four 2.4-m u-posts. Exclosures surrounding subplots were approximately 6 m X 6 m to reduce the effect of fencing on plants within the subplot. Exclosures were installed immediately following planting and herbicide treatment or initial cultivation. When necessary, exclosures were removed for $\leq$24 hours to allow farmers to conduct additional field treatments. I evaluated corn crops near the estimated date of plant maturity before senescence. Within each subplot I recorded the number of rows, number of plants, and for 30 randomly selected plants, I measured plant height, level of herbivory per plant, and classified the quality of each ear of corn relative to damage caused by deer. I estimated grain yield (total seeds produced per 30 plants) for fenced and unfenced subplots, and calculated the proportional loss of corn for each fenced and unfenced plot as: ((total seeds in fenced plot minus total seeds in unfenced plot) divided by total seeds in the fenced plot. I consulted with the agricultural producer to determine the variety of corn planted in each field.

Deer Abundance Estimates on Focal Properties–To aid in estimating deer abundance and management intensity (i.e., deer harvested per deer available for harvest) on focal properties, I used baited infrared camera surveys to obtain estimates of the abundance of deer at a fine scale in the area of crop fields designated for evaluation. This method of survey was conducted according to previous research by Jacobson et al. (1997) and a pilot study I conducted in Southeast Minnesota during 2013 (G. D'Angelo, unpublished data). The abundance of deer in an area can be determined using baited surveys, where bucks can be uniquely identified by antler characteristics and their number used to infer the number of does and fawns visiting repeatedly a bait site. Cameras were placed at a density of one camera per 65 hectares in wooded or brushy habitat immediately adjacent to crop fields. This relatively high density of cameras was intended to reduce bias associated with capturing adult bucks at a higher rate at lower camera densities because males have larger home ranges (Jacobson et al. 1997). A bait site was established at each camera location during a 7-day pre-baiting period. During pre-baiting, whole kernel corn and trace mineral salts were placed at each bait site in a quantity sufficient to maintain consistent access by deer 24 hours per day. Following this acclimatization period, an infrared camera was set to record still photographs of deer 24 hours a day at 10-minute intervals during a 14-day survey period. As in the pre-baiting period, bait was provided ad libitum. I generated deer abundance estimates using data pooled from all cameras on a property. Deer abundance estimates were conducted during August. This timing increased the likelihood that: 1) fawns were mobile with their dams and available for survey, 2) antler growth of bucks was sufficient to uniquely identify individuals, 3) deer photographed near crop fields were those that caused damage during the growing season and were available for harvest in the same area, and 4) harvest mortality and disturbance of deer by hunting activities was minimized since the survey preceded deer hunting seasons.

Deer Abundance Estimates in 5-km Area including Focal Properties–I bounded focal properties with a 5-km square quadrat and established transects totaling 5.5 km in length along roads to conduct spotlight surveys. Surveys were conducted in early November after leaf senescence of most deciduous trees, after most corn was harvested, and before firearms deer season. Surveys were conducted between 1 hour after sunset and 1 hour before sunrise. Two replicate surveys were conducted for each focal property. Two observers in the cab of an MNDNR vehicle scanned for deer in the landscape along survey routes using handheld 12-volt spotlight (1000 m at 1 Lux of illumination, Lightforce, Orofino, ID). A real-time, moving-map software program (DNRSurvey; Wright et al. 2011), coupled to a global positioning system receiver and a convertible tablet computer, was used to guide transect navigation and record deer locations directly to ArcGIS (Environmental Systems Research Institute, Redlands, CA) shapefiles. Observers recorded the number of deer per group and estimated the distance of deer groups from the transect using a laser rangefinder (Leupold and Stevens, Beaverton, OR). I estimated the deer density for individual surveys using Distance software (Thomas et al. 2010) and averaged the two estimates for each focal property.

Management Intensity–I asked agricultural producers to report deer harvested on their properties by season. I quantified management intensity as: number of deer harvested divided by the total number of deer estimated to be on the property via infrared camera surveys. I also classified properties under two management strategies: hunting (herein HUNT, i.e., hunting conducted by sport-hunters during the regular season framework, or integrated management (herein INT, i.e., in addition to hunting, deer were harvested using depredation and shooting permits outside of the regular season framework).

RESULTS AND DISCUSSION

The portion of the study described in this summary occurred during April 2014-December 2014, and field work is ongoing during 2015. HUNT was used to manage deer on three properties and INT was used on four properties. I sampled 112 subplots in corn fields including 56 unfenced subplots and 56 fenced subplots. I excluded 2 pairs of fenced and

unfenced subplots (i.e., 4 subplots total) on one property from analysis because the growth of corn plants was severely affected by soil erosion.

Deer abundance via infrared camera surveys was similar on HUNT and INT properties (Table 1, $t = 0.139$, $df = 5$, $P = 0.896$). Likewise, deer abundance was similar in the area surrounding HUNT and INT properties as determined via spotlight surveys ($t = 0.120$, $df = 5$, $P = 0.910$). Agricultural producers on INT properties utilized extra deer harvest opportunities, and management intensity on INT properties was more than double the management intensity on HUNT properties (HUNT = 0.19, INT = 0.44, $t = -2.393$, $df = 5$, $P = 0.097$). Despite increased harvest pressure on INT properties, deer damage to corn was similar on all properties regardless of the deer management strategy employed (HUNT = 7% mean proportional corn loss, INT = 8% mean proportional corn loss, $t = -0.121$, $df = 5$, $P = 0.908$). There was no difference in proportional loss of corn between edge and interior plots ($t = 0.529$, $df = 12$, $P = 0.606$).

The primary objective of this study was to evaluate the effectiveness of localized management for reducing fine-scale deer abundance and to examine whether damage caused by deer to agricultural crops is reduced on properties where deer densities are lowered. The true effects of integrated deer management conducted during 2014 and 2015 on deer abundance will not be evident until the field season is completed in 2015. During 2014, corn yield loss was seemingly low on most properties. There was no difference in corn damage between properties where localized management was utilized versus normal sport-hunting, and the level of corn damage could not be explained by deer abundance at the property level or in the surrounding area. However, extra deer harvest opportunities were utilized by landowners when requested. Management was more intensive on INT properties versus HUNT properties. Also, deer were harvested earlier and more continuously throughout the growing season, corn drydown period, and crop harvest seasons on INT properties. Increased deer harvest pressure on INT properties may have prevented corn damage from being worse had additional deer not been harvested. Therefore, extra opportunities to harvest deer should be afforded on properties where landowners consult with MNDNR staff about their concerns for potential deer damage. These concerns are likely legitimate and landowners are basing their concerns on prior experiences and current conditions.

A second field season is being conducted in 2015. I will also examine landscape characteristics associated with levels of deer damage to corn, deer damage to alfalfa over winter and during the growing season, and the human dimensions associated with application of localized deer management strategies. The results of this study will provide a basis for improving the framework for future application of localized management in agricultural regions.

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Table 1. Estimates of the abundance of white-tailed deer, management intensity of deer, and corn damage caused by deer on 7 privately owned properties in Southeast Minnesota, 2014.

Property	Deer management strategy[1]	Estimated deer abundance (deer per camera)[2]	Local deer density (deer per km^2)[3]	Management intensity[4]	Mean proportional corn loss[5]
A	HUNT	26	17	0.16	0.07
B	HUNT	22	10	0.21	-0.01
C	HUNT	13	14	0.21	0.14
D	INT	26	28	0.35	0.24
E	INT	21	13	0.39	-0.06
F	INT	22	2	0.28	0.00
G	INT	11	9	0.74	0.12

[1]On properties with HUNT management deer harvest was conducted by sport-hunters during the regular season framework. On properties with INT management deer harvest was through integrated methods including by sport-hunters during the regular season framework and using depredation and shooting permits outside of the regular season framework.
[2]Deer abundance estimated from infrared camera surveys indexed as deer per camera with camera densities of 1 camera per 65 ha on each focal property.
[3]Deer density estimated from spotlight surveys in 5-km quadrat encompassing each focal property.
[4]Proportion of the number of deer estimated to be using a property that were harvested.
[5]Negative values indicate higher average yield estimates in unfenced subplots versus subplots fenced to exclude deer.

MNDNR

PILOT STUDY TO ASSESS HARVEST MORTALITY RATES OF GRAY AND FOX SQUIRRELS ON PUBLIC LAND IN MINNESOTA

Rachel Curtis and Nicole Davros

SUMMARY OF FINDINGS

Small game hunting is a popular recreational activity in Minnesota but the number of squirrel hunters and the squirrel harvest has declined since 1985. In addition, metropolitan hunters have indicated that they have limited access to private land and heavy hunting pressure exists on publicly owned land. We intend to study the contribution of harvest mortality to overall gray and fox squirrel mortality rates on public hunting lands; but first we conducted a small pilot study to evaluate trapping, handling, and tracking methods. During September-October 2014 we set traps using different trap placement arrays and baits in Minneopa State Park. We captured 20 squirrels and tested 2 styles of radio-transmitting collars. We tracked the collared squirrels from October 2014 through May 2015. In the expanded study we will use baits and trap placement grids that were effective in this study. We will use the collar style that contained a mortality sensor and took the longest time for the squirrels to remove. We will also only assess survival status and sources of mortality instead of determining locations for collared squirrels.

INTRODUCTION

Small game hunting is a popular recreational activity in Minnesota, but since 1985 the number of squirrel hunters has declined by almost 25% and the squirrel harvest has declined by about 40% (Dexter 1997, Dexter 2013). The DNR conducted a survey of squirrel hunters to assess squirrel hunter perceptions and opinions (Dunbar 2009). More hunters in the Twin Cities metropolitan area (hereafter, metro) responded that they believed squirrel populations were declining (51%) than their statewide counterparts (19%) and more metro hunters hunt exclusively on public land. Metro hunters indicated that there was limited access to private land and heavy hunting pressure existed on publicly-owned land (Dunbar 2009). Previous research has shown that hunting pressure can be considerably higher on forests open to the public than on privately-owned property (Nixon et al. 1974) so this perception of hunters could be a real management issue for squirrel populations around the metro area.

The DNR Section of Wildlife has considered changes to the squirrel season structure in the metro area based on these survey results. However, many factors cause squirrel populations to fluctuate naturally (see Barkalow et al. 1970, Nixon et al. 1975, Healy and Welsh 1992, Descamps et al. 2009, Vander Haegen et al. 2013) and limited population-level data exists for Minnesota's squirrel populations. Therefore, it is unclear whether the squirrel harvest is declining due to overexploitation on high-use wildlife management areas or if the decline is due to reduced hunter participation. We have proposed a study to assess the contribution of

harvest mortality to overall mortality rates of gray and fox squirrels (*Sciurus carolinensis* and *S. niger*, respectively) on public hunting lands. Prior to initiating this large research project, we initiated a pilot study to evaluate squirrel trapping, handling, and tracking methods.

OBJECTIVES

This project was a pilot study to evaluate trapping, handling, and tracking methods for gray and fox squirrels as part of a larger study that will evaluate squirrel mortality rates on public lands (beginning July 2015). Our pilot study objectives included the following:

1) Determine the number of traps that can be monitored each day.
2) Determine the spacing of traps for effective capture rates.
3) Assess handling methods to determine if improvements could be made to further reduce stress and handling time of squirrels while maintaining safety for handlers.
4) Deploy 15-20 collars on gray and fox squirrels (combined total).
5) Track collared squirrels once per week to evaluate logistics such as collar battery life, signal strength, and staff time required for monitoring efforts.

METHODS

Study Area

We conducted our study at Minneopa State Park because the park had abundant gray and fox squirrels to meet our target sample size and good forest habitat to test and refine radio-tracking methods for squirrels. Additionally, the park was within easy commuting distance from our research station and from the locations of our student volunteers [Mankato (Minnesota State University) and St. Peter (Gustavus-Adolphus College)]. We conducted our trapping efforts in the deciduous forest in and around the campground (Figure 1). This area had a large number of squirrels and road access in all seasons.

Trapping

During September-October 2014, we trapped gray and fox squirrels using wire box traps (48 x 15 x 15 cm; 2.5 x 1 cm mesh) baited with dried corn, sunflower seeds, peanut butter, pecans, and/or hickory nuts. We used two different trap placement methods (Figure 2). The first method involved placing ≤25 traps at known squirrel-use sites within the campground. We set and removed traps each day to reduce interference with park visitors. We set traps when the last camping group left in the morning, typically from 1000 to 1100 h, and removed them when guests began arriving in the evening, typically from 1500 to 1600 h. The campground continued to have visitors during the day; therefore, we checked these traps and released trapped animals every 1.5 h to reduce stress. Our second trapping method involved setting 30 traps in the forest interior. Using GIS, we placed a 30 m buffer around all trails and roads. We created a grid of points 30 m apart in the area outside the buffer and placed baited traps at these points. Peak squirrel activity is in the morning and evening; therefore, we checked traps three times per day (late morning, afternoon, and night) to reduce the amount of time squirrels remained in the traps. We closed the traps each night to prevent animals from staying in the traps overnight, and

opened them again the following morning. We closed the traps during inclement weather and removed them over the weekend.

We weighed squirrels in the trap using a digital hanging scale. We restrained captured squirrels using a modified handling cone which allowed us to handle and radio-collar without sedation (Koprowski 2002). Handling cones were constructed of denim with Velcro© straps to secure the squirrel and a zipper to allow access to the head and neck during collar attachment (Figures 3 & 4). We placed a removable plastic funnel around the squirrel's neck to protect handlers from bites during collaring (McCleery et al. 2007; Figure 5). We released a squirrel back into the trap if it was not oriented correctly or became twisted in the cone. We intended to release un-collared any squirrel that could not be collared after being placed in the cone twice to avoid stress mortality. However, all squirrels were properly aligned in the cone by the first or second try and none needed to be released un-collared. We deployed two different VHF necklace-style radio-collars: 13.0 g collars with integrated mortality sensors and 5.0 g collars without integrated mortality sensors. Mortality sensors change the pulse rate of the signal if an animal has not moved for 8 h. All squirrels were immediately released after handling was complete (Figure 6). We counted handling time as the time from when we approached the trap to when we released the collared squirrel. We immediately released any non-target animals.

Tracking

We tracked the collared squirrels biweekly from October 2014 through January 2015 and monthly from February through May 2015. For squirrels carrying a 13.0 g collar, we determined the location of squirrels using triangulation techniques, and determined survival status by listening for the mortality signal. This technique provided the least amount of disturbance to the animal. When a 13.0 g collar transmitted a mortality signal, we attempted to retrieve the transmitter. For squirrels carrying a 5.0 g collar without mortality sensor, we used a homing radio-tracking technique in an attempt to locate the animal to determine survival status and location. Survival status could not always be determined when squirrels with a 5.0 g collar were inside of a tree cavity that prevented visual inspection.

RESULTS AND DISCUSSION

We trapped and collared 20 squirrels (Table 1). Total handling time per squirrel ranged from 5-11 min ($\bar{x}$ = 7 min). Our handling times were faster with ≥2 handlers. Handling times were slower when squirrels were collared by one person or when a squirrel had to be re-aligned in the cone. Overall, our handling cone and funnel method provided a safe, effective way to handle squirrels for researchers and animals alike.

We collared 8 squirrels in the campground and 12 squirrels from the forested grid (Figure 7). In the campground, the percentage of traps containing a gray or fox squirrel heavy enough to collar was 9.4% per day (85 trap days), and 2.4% per trap check (333 trap checks). In the forested grid, the percentage of traps containing a gray or fox squirrel heavy enough to collar was 7.7% per day (155 trap days), and 3.2% per trap check (372 trap checks). The slightly higher trapping success at the campground may be due to higher squirrel densities, trap placement in known-use locations, preferred baits, habituated animals, and/or shorter times between checks. Although the targeted trapping method had a higher per day success rate, it is likely unreliable for the contiguous forest habitats on wildlife management areas, and we will use the grid system to trap squirrels in our expanded study. We determined that 40-50 traps will be

a reasonable number for each handler to monitor each day, and that 25-30 m spacing between traps efficiently covers an area of forest.

We collared 19 gray squirrels and 1 fox squirrel. Our trapping sites, particularly in the forest grid, were primarily gray squirrel habitat and contained little of the open, savannah-type habitat preferred by fox squirrels. We collared 6 female and 14 male squirrels. Squirrel weights ranged from 440-660 g ($\bar{x}$ = 550g). We were unable to definitively determine age because most juveniles had reached adult size and reproductive status was difficult to determine in October.

Two study animals are still collared. Of the 18 losses, 2 were possible mortalities, 3 were likely transmitter failures, 2 batteries have failed, and 11 squirrels were able to remove their collars either by slipping them over their heads or chewing through the plastic collar. We will not use the 5.0 g collars for our expanded study. All 3 transmitter failures were this type, the thin zip-tie collar attachment style is easy for the squirrels to chew through, and without the mortality sensor it is very time consuming to determine survival status. Of the 11 slipped collars, 6 were 13 g collars, and 5 were 5 g collars; however, the squirrels removed the 5 g collars soon after collaring while the 13 g collars began to slip after several months. The large number of slipped collars is likely due to attaching collars too loosely. Additionally, we collared animals in fall during the peak in their body weight. As winter progressed and squirrels became slimmer, squirrels began to lose their collars.

In the expanded study, we will only use radio-collars with integrated mortality sensors as this will allow us to more easily determine survival status of collared squirrels. We will not triangulate squirrel locations because it is too time intensive. However, we will use the homing technique to locate collars emitting a mortality signal and determine cause of death, if possible. Survival status can be determined remotely ≤0.5 km from the collared squirrel, depending on transmitter battery strength and topography. Scanning collar frequencies to listen for mortality signals takes only a few minutes but it will take 30-60 min to recover a collar and determine cause of death. Additionally, it will take more time to assess survival status in the expanded study because squirrels will be dispersed throughout the site and some transmitter signals may not be audible from the nearest road.

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Table 1. Squirrels collared at Minneopa State Park, September and October 2014.

Collar Frequency	Date Trapped	Handling Time	Location	Species	Sex	Weight (g)	Collar Size	Current Status
164.715	9/30/2014	6:00	Forest	Gray	Female	660	13 g	Collar off, unrecovered
164.443	10/7/2014	9:00	Forest	Gray	Female	550	5 g	Missing, likely transmitter failure
164.304	10/7/2014	9:00	Forest	Gray	Male	550	5 g	Slipped collar
164.633	10/8/2014	5:00	Forest	Fox	Male	610	13 g	Collar off, unrecovered, likely mortality
164.614	10/8/2014	6:00	Forest	Gray	Female	610	13 g	Slipped collar
164.043	10/8/2014	9:00	Campground	Gray	Male	440	5 g	Missing, likely transmitter failure
164.091	10/8/2014	10:00	Campground	Gray	Female	620	5 g	Collar off, unrecovered
164.061	10/9/2014	6:00	Campground	Gray	Male	520	5 g	Collar off, unrecovered
164.073	10/9/2014	6:00	Campground	Gray	Male	520	5 g	Alive
164.512	10/9/2014	7:00	Campground	Gray	Male	480	5 g	Collar off, unrecovered
164.625	10/9/2014	8:00	Campground	Gray	Male	550	13 g	Collar off, unrecovered, likely mortality
164.323	10/9/2014	6:00	Forest	Gray	Male	490	5 g	Alive
164.654	10/9/2014	7:00	Forest	Gray	Female	560	13 g	Chewed through collar
164.546	10/9/2014	6:00	Campground	Gray	Male	660	13 g	Slipped collar
164.432	10/9/2014	11:00	Forest	Gray	Male	510	5 g	Missing, likely transmitter failure
164.733	10/10/2014	5:00	Forest	Gray	Male	620	13 g	Slipped collar
164.053	10/10/2014	5:00	Forest	Gray	Male	490	5 g	Chewed through collar
164.585	10/10/2014	6:00	Forest	Gray	Female	500	13 g	Missing, possible battery failure
164.703	10/10/2014	7:00	Forest	Gray	Male	630	13 g	Missing, possible battery failure
165.105	10/14/2014	9:00	Campground	Gray	Male	500	13 g	Slipped collar

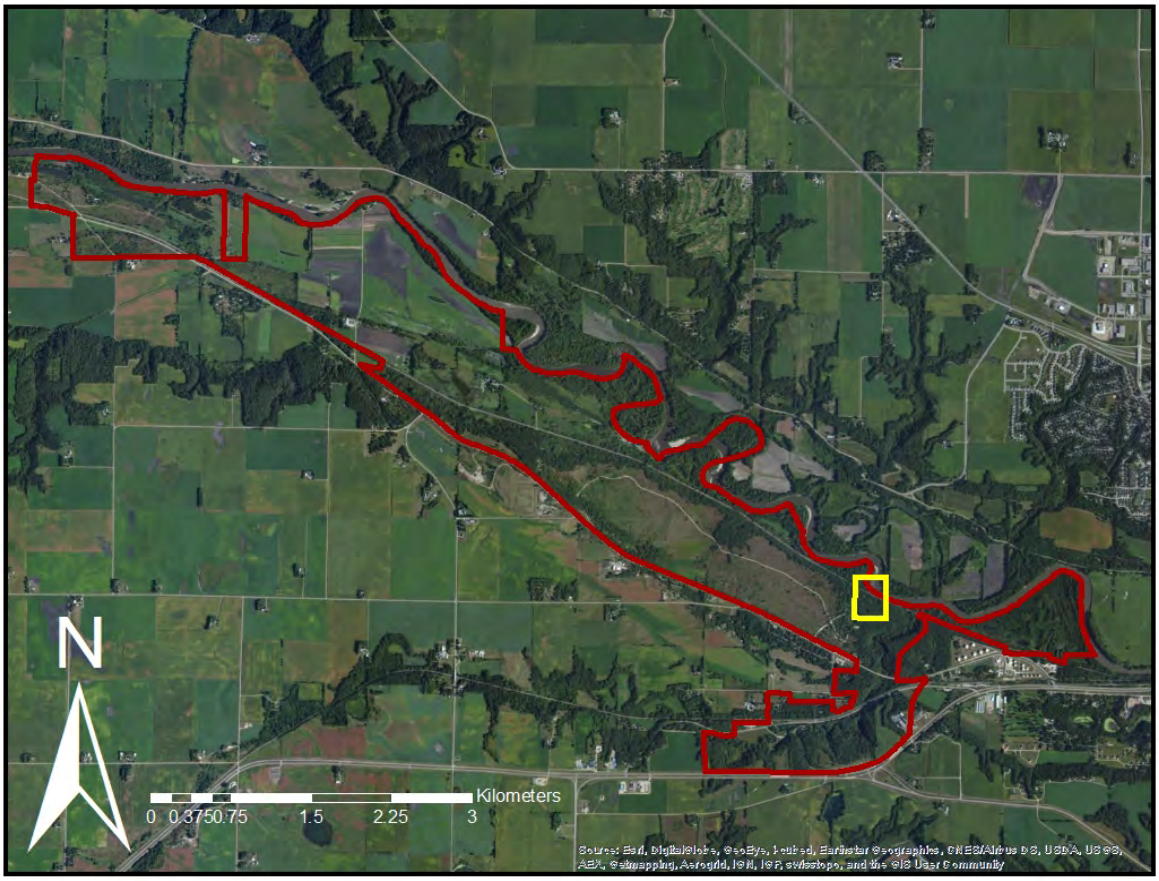

Figure 1. Our pilot study was conducted in the area surrounding Minneopa State Park's campground (trapping area shown in yellow). The red border defines the park boundary.

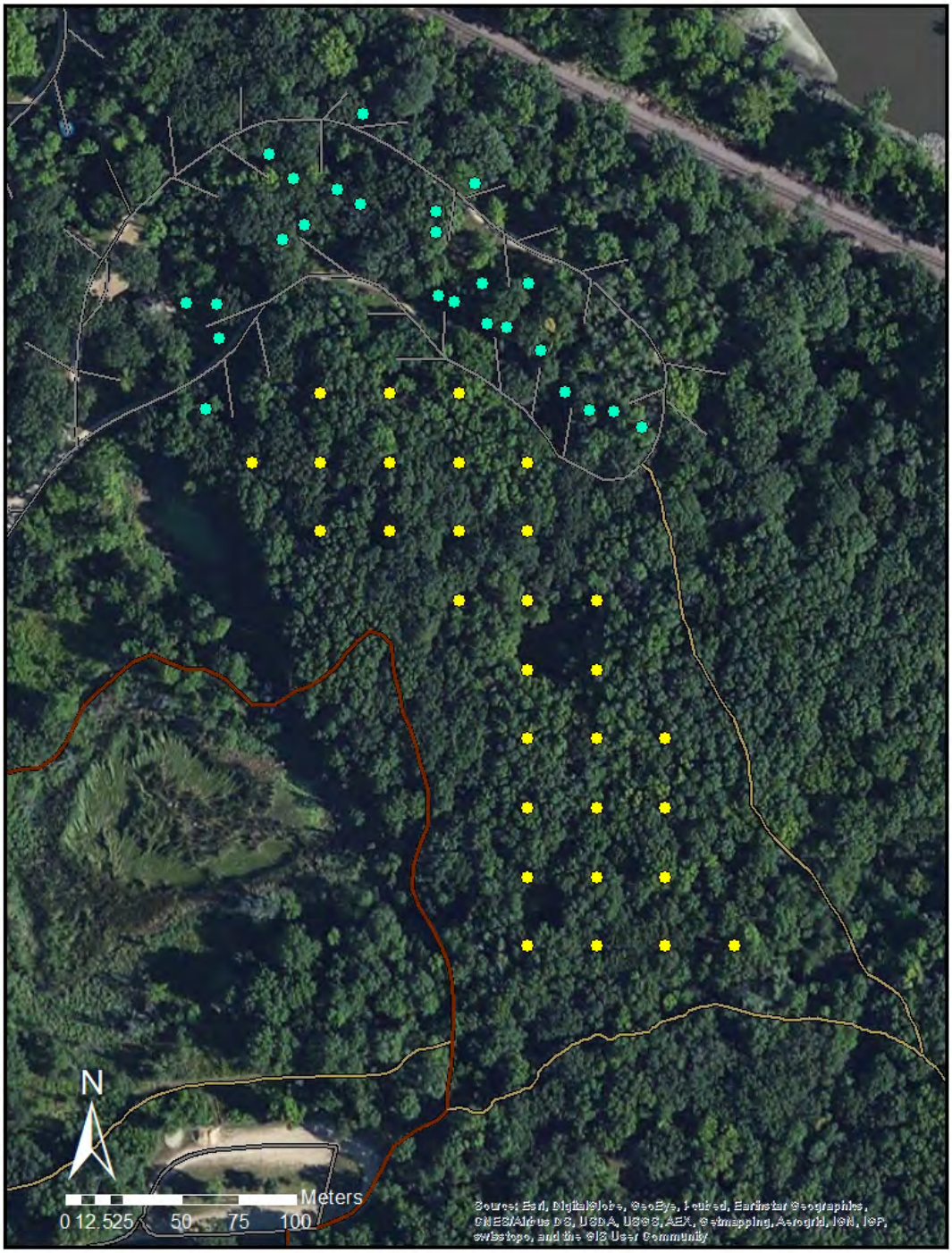

Figure 2. Trap distribution near the campground. Green dots represent known squirrel-use campground sites. Yellow dots mark the forested grid trap sites.

Figure 3. Placing the wide end of the handling cone over the trap door allowed us to release the squirrel directly into the cone. The squirrel exited the trap and became caught in the constricted end of the cone. Velcro© straps secured the trapped squirrel.

Figure 4. By partially unzipping the handling cone, we were able to expose the squirrel's head and then secure a plastic funnel around its neck.

Figure 5. With the squirrel secured, we were able to safely attach radio-transmitters.

Figure 6. We removed the plastic funnel, loosened the straps, and unzipped the cone to release the squirrel.

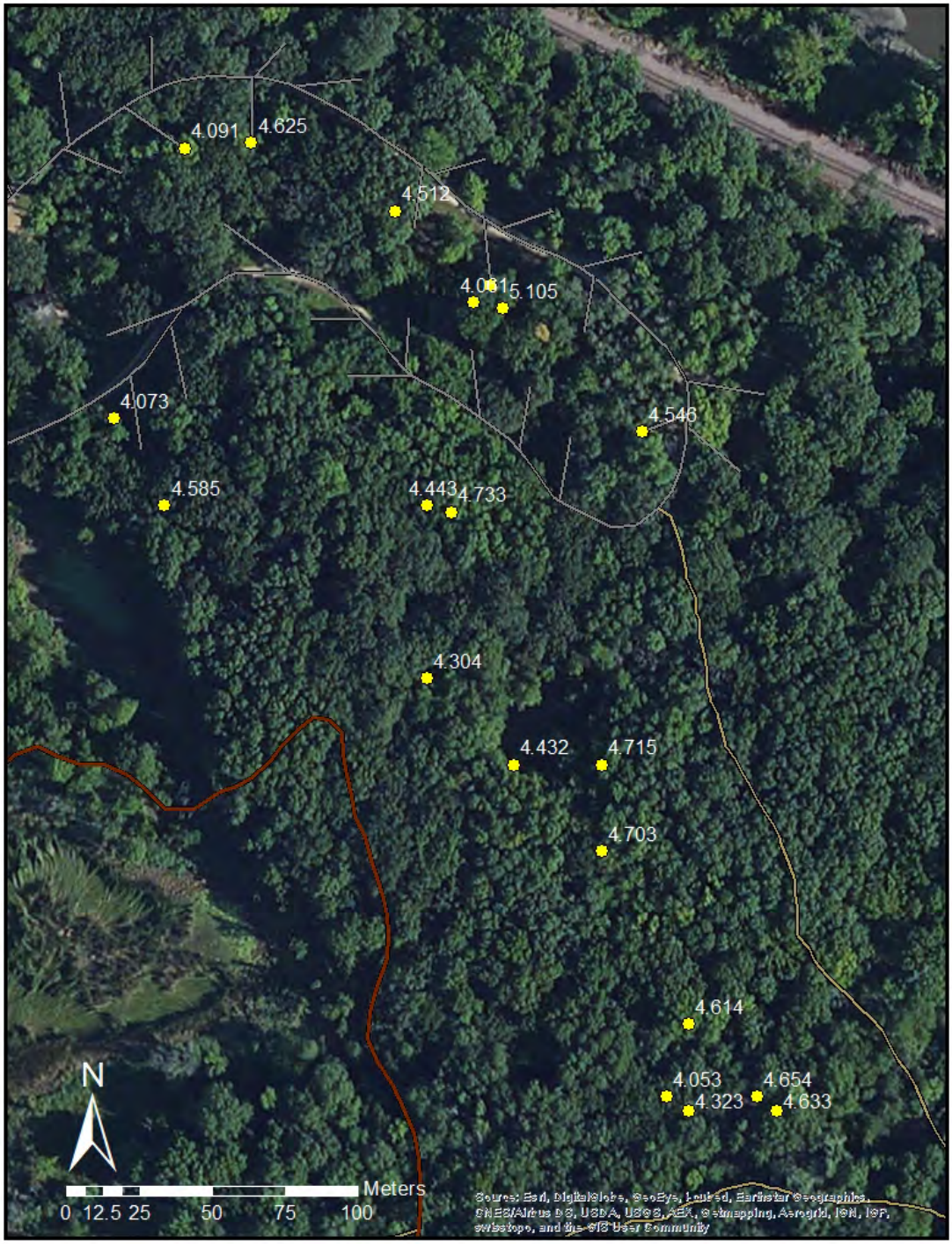

Figure 7. Location of initial squirrel captures. A total of 20 squirrels were collared.

MNDNR

AN EVALUATION OF NESTING AND BROOD-REARING HABITAT SELECTION AND SURVIVAL RATES OF RING-NECKED PHEASANTS IN RELATION TO VEGETATION STRUCTURE AND COMPOSITION

Nicole Davros and Rachel Curtis

SUMMARY OF FINDINGS

Ring-necked pheasant (*Phasianus colchicus*) responses to the amount of grassland acres in the landscape have been well documented but we lack current information on the individual components of reproductive success (e.g., nest success, brood success, chick survival) that are driving pheasant population dynamics in Minnesota. Better understanding the factors that limit reproductive success can help natural resource agencies prioritize their management and acquisition strategies. We radiocollared 20 hen pheasants across two study areas in southwestern Minnesota during spring 2015 to monitor them during nesting and brood-rearing. We are currently capturing and radiotagging chicks to estimate juvenile survival rates and collecting vegetation data to evaluate nest-site and brood habitat selection. The results from our 2015 field season will provide the basis for a broader study aimed at assessing the influence of vegetation structure and composition on pheasant hen nest-site selection, nest success, brood success, brood habitat selection, and chick survival.

INTRODUCTION

Ring-necked pheasant population dynamics are largely driven by variation in survival rates, and predation is the primary cause of mortality for hens and their young (Peterson et al. 1988, Riley et al. 1998). Predator control efforts can help improve reproductive output over short time periods, but such efforts are economically and ecologically inappropriate at the landscape scale (Chesness et al. 1968, Riley and Schulz 2001). Management of pheasant populations has instead focused mainly on providing abundant nesting cover to minimize the effects of predation and maximize reproductive success to increase populations. As acres enrolled in CRP and similar cropland retirement programs decline in Minnesota, providing suitable habitat on public lands to sustain populations will become more critical for mediating the effects of predation on pheasant population dynamics. However, the interaction between habitat and predation will no doubt remain, and gaining new insights into old problems will be important for improving management strategies on publicly-owned lands.

Predation during the nesting season is a major factor affecting pheasant population dynamics. Nest predation is the leading cause of nest failure for many grassland-nesting birds, including pheasants (Chesness et al. 1968, Clark et al. 1999), and can limit productivity.

Additionally, hens take only short recesses from incubating which puts them at greater risk to predation during nesting (Giudice and Ratti 2001, Riley and Schulz 2001). Management efforts aimed at increasing patch size and reducing edge effects are assumed to alleviate rates of predation on birds and their nests (e.g., Johnson and Temple 1990, Sample and Mossman 1997, Winter et al. 2000); however, the composition of the landscape surrounding a patch (Clark et al. 1999, Heske et al. 2001) and the vegetation within a patch (Klug et al. 2009, Lyons 2013) also play important roles in determining susceptibility to nest predation.

Recent advances in video camera technology have allowed better monitoring of bird nests and provided evidence that nest predator communities are more complex than previously thought (Pietz et al. 2012). In particular, the predators associated with nest depredation events can vary with the structure and diversity of nesting cover (e.g., percent cover of litter, forbs, or cool-season grasses; Klug et al. 2009, Lyons 2013) and landscape context (Benson et al. 2010). Thus, management actions attempting to mitigate the impact of predators may not necessarily reduce rates of nest predation but rather create a spatial or temporal shift in the nest predator community and susceptibility to nest predation (Benson et al. 2010, Thompson and Ribic 2012). Nest predator communities also vary across regions and habitats and results from studies of other species or in other states may not be entirely applicable to Minnesota's pheasant population (Thompson and Ribic 2012). Understanding how management at both the site level (e.g., vegetation structure, composition, and diversity) and the landscape level (e.g., tree removal, wetland restoration) impacts the dynamics of nest predation is an important but as of yet unintegrated step in our ability to manage habitat for increased productivity of pheasants and other grassland birds (Jiménez and Conover 2001).

Chick survival is also a vital component of pheasant population dynamics but it remains poorly understood (Riley et al. 1998, Giudice and Ratti 2001). Assessing the causes of pheasant chick mortality has been difficult because many previous studies have relied on estimates of brood survival (e.g., the proportion of broods in which ≥1 chick survived to a certain age) rather than survival of individual chicks within a brood (e.g., Meyers et al. 1988, Matthews et al. 2012; but see Riley et al. 1998). Using brood survival estimates is likely unreliable because brood mixing can occur (Meyers et al. 1988). Further, lack of data on individual chicks (e.g., body condition, cause of death) prevents us from understanding the role of different factors (e.g., exposure, food limitation, predation) that lead to variation in recruitment. Evidence that predation is the leading cause of chick mortality for grassland gamebirds in North America is well-established (e.g., Riley et al. 1998, Schole et al. 2011). Food availability has been implicated as an important factor explaining chick survival for many gamebird species in Europe (Green 1984, Hill 1985, Potts 2012); however, strong evidence that food is a major limiting factor for survival of chicks in North America is still lacking. Moreover, food availability and rates of predation likely interact in relation to vegetation structure and composition and confound conclusions from chick survival and food resource studies (Hill 1985). Finally, death from exposure has been shown to decrease chick survival rates, especially after periods with increased precipitation when chicks are still very young and unable to fully thermoregulate (Riley et al. 1998, Schole et al. 2011). Risk of exposure and starvation may interact to decrease chick survival, but few studies have been able to directly address this question (but see Riley et al. 1998). Therefore, better data are needed to understand the interplay between these potential limiting factors on brood habitat selection and chick survival in different habitats and landscapes within Minnesota's pheasant range.

Minnesota DNR wildlife managers in the farmland region have indicated a need for more information on pheasant nesting, brood habitat suitability, and chick survival in relation to management activities and agricultural land use practices. Indeed, better understanding the factors that limit brood production and chick survival will help natural resource agencies prioritize their management strategies at both the local level (e.g., forb interseeding) and landscape level (e.g., acquisition priorities) in this new era of reduced CRP acreages. Additionally, obtaining data on individual components of pheasant population dynamics will aid in future assessment of DNR management activities [e.g., Prairie Plan implementation (Minnesota Prairie Plan Working Group 2011), conservation grazing] and agricultural land use practices (e.g., pesticide use) on Minnesota's pheasant population.

OBJECTIVES

Our long-term research objective is to evaluate the relative importance of potential limiting factors (e.g., vegetation cover type, food, predation, weather) on pheasant productivity. We will evaluate hen nest site selection, nesting and brood-rearing success, brood habitat selection, and hen and chick survival in Wildlife Management Area (WMA) project areas with varying amounts of site-level diversity [e.g., sites dominated by smooth brome (*Bromus inermis*), warm-season grasses, and high diversity grass-forb mixtures]. Specific objectives include:

1) Evaluate nest site selection, nesting success, and survival of ring-necked pheasant hens in relation vegetation cover and composition.
2) Evaluate pheasant brood-rearing habitat selection, brood success, and chick survival rates in relation to vegetation cover and composition.
3) Evaluate the relative importance of different factors (e.g., predation, food limitation, weather) on pheasant nesting success, brood success, and hen and chick survival to help guide management priorities.

STUDY AREA

Our study is being conducted in the southwest region of Minnesota (Figure 1). Topography ranges from flat to gently rolling. This region is intensively farmed, and corn and soybeans combined account for approximately 75% of the landscape (U.S. Department of Agriculture 2013a, U.S. Department of Agriculture 2013b). Grassland habitats, including those on private land [Conservation Reserve Program (CRP), Reinvest in Minnesota (RIM), Conservation Reserve Enhancement Program (CREP), and Wetlands Reserve Program (WRP)] and public land [MN DNR Wildlife Management Areas (WMA) and U.S. Fish and Wildlife Service Waterfowl Production Areas (WPA)] account for 5.7% of the landscape in this region (Davros and Curtis 2014). The southwest region lies within the core of Minnesota's pheasant range, and MN DNR's 2014 August roadside counts indicated 50.7 pheasants/100 mi (Davros and Curtis 2014).

We focused our efforts at two project areas for the 2015 field season. Each project area is about 9 m^2 in size and has extensive amounts of permanently protected habitat. The Lamberton WMA project area (Redwood County) is a large, nearly contiguous WMA complex

with >1,100 acres of permanently protected upland and wetland habitats. The Worthington Wells project area (Nobles County) has >1,500 acres of permanently protected habitat that spans multiple WMAs, the Okabena-Ocheda Watershed District, and U.S. Fish & Wildlife Service (USFWS) lands.

METHODS

We captured hen pheasants from 2 February – 15 April 2015 using baited walk-in traps and nighttime spotlighting via 6-wheel utility-task vehicle (UTV). We also opportunistically captured roosters during our efforts. We weighed each hen to the nearest 5.0 g, measured the right tarsus to the nearest 0.5 mm, banded her with a unique combination of leg bands (1 numbered aluminum band and 3 colored plastic bands), and fitted her with a 16.0 g necklace-style VHF radiotransmitter with integrated mortality switch before release. Roosters were weighed, measured, and banded with a unique leg band combination before being released.

We began radiotracking hens 3-5 times per week in late April to determine the onset of incubation. We assume that incubation has begun when the radio signal is projected from the same location for several consecutive days. Once incubation is initiated, we flush hens from their nest to determine clutch size and float a subset of eggs to estimate hatch dates (Westerskov 1950, Carroll 1988). We mark the location of nests using a global positioning system (GPS) receiver. We also place flagging within 5-8 m of nests to aid relocation efforts. If a hen begins making large daily movements prior to us flushing her to locate a nest, we assume her nest has failed and we wait for her to re-localize and begin incubating her next nest before we flush her. We use the homing technique on radiocollars emitting a mortality signal to retrieve the collar and determine a cause of death when possible.

We place miniature color video cameras at a random subset of nests to document nesting behavior, hatching, and nest predation events (Cox et al. 2012). Cameras have infrared light-emitting diodes (LEDs) to allow recording at night and are connected to digital video recorders (DVRs) with SD cards and deep-cycle marine batteries housed in waterproof containers >20 m away from nests. We use a portable monitor to adjust camera settings and check video feeds and we switch batteries and SD cards every 4-7 days. Video footage is reviewed in the office weekly and relevant video clips are archived.

Near the estimated hatch date, we monitor hen activity 2-3 times daily to determine if hatching is occurring. We assume hatching is occurring when the hen's signal fluctuates in intensity (Riley et al. 1998). We also occasionally flush hens on the estimated hatch date to determine if hatching is occurring. We capture chicks on day 0 (hatch day) or day 1 (i.e., 1-day post-hatching) while they are still on the nest by flushing the hen off early in the morning. If the hen and her brood have already moved from the nest, we flush the hen from the brood and immediately play a recording of a hen's brood-gathering call or a hen turkey call until 1-5 chicks are captured by hand. We never capture more than 50% of the brood at one time. If the chicks do not respond to either playback within 30 min, we leave the area to allow the hen to gather her brood and try to capture them again the next day. We discontinue chick capture attempts for a particular brood if we are unsuccessful at capturing any chicks by the end of day 2.

We transport captured chicks in a small box cooler heated with hand-warmers to a nearby field truck for processing. We determine the mass of each chick to the nearest 0.1 g and

we measure tarsus length to the nearest 0.5 mm. We surgically suture a 0.65 g backpack-style VHF radio-transmitter to the backs of 1-3 chicks/brood (Burkepile et al. 2002, Dahlgren et al. 2010). If more than 3 chicks are captured, we subcutaneously implant a passive integrated transponder (PIT) tag on the back between the scapula and neck (Nicolaus et al. 2008) on each of the additional captured chicks to allow for identification of individuals during future recapture efforts. Handling time lasts <5 min per chick and chicks are returned to the hen within 30-60 min of capture. We follow the methods of Riley et al (1998) to return chicks to the hen.

We monitor hens and their broods 2-3 times daily at least 3 times per week to determine their locations and estimate brood and chick survival. First, we triangulate a hen to estimate her location and we take each bearing from approximately 100-200 m away. We then flush the hen and note the presence of any chicks by sight or sound. We make conservative estimates of the number of chicks detected each time. We also make note of the hen's behavior after flushing (e.g., approximate distance flown when flushed, returned immediately to the area or stayed away, gave a brood-gathering call) to aid in determining whether she has any surviving chicks if the chicks themselves are not detected. We triangulate the location of chicks that are radiotagged from that brood during this same sampling period. If a chick is detected >50 m from a hen or doesn't appear to be moving (as determined by signal fluctuation), we use the homing technique to locate the chick and determine survival status. When a chick is found dead, we examine the carcass and surrounding area to assign a cause of death, if possible. Following Riley et al (1998), we classify mortality as due to "predation" if we find puncture marks from teeth, hemorrhaging, or parts of the body consumed and when there are predator tracks, fur/feathers, scat, or a den present. We classify mortality as due to "exposure" when evidence of predation is lacking and death was associated with recent rain and/or cold temperatures. We classify mortality as "other" when other circumstances are obvious (e.g., killed by machinery in a mowed patch of grass). We classify mortality as "unknown" when the transmitter has fallen off the back with no obvious signs of tampering or suture failure.

We collect vegetation data at the nest site within 7 days after a nest has hatched, failed, or been abandoned. We estimate litter depth and the percent canopy cover (Daubenmire 1959) of grasses, forbs, litter, bare ground, woody vegetation, and other (e.g., logs, rocks) using a 0.5 m^2 sampling quadrat. We estimate percent cover on an overlapping basis using 7 classes: 0%, 0.1-5%, 5-25%, 25-50%, 50-75%, 75-95%, and 95-100%. We count the number of grass and forb species to determine species richness within the quadrat. We also record visual obstruction readings (VOR; Robel et al. 1970) in the 4 cardinal directions to determine vegetation vertical density around the nest and we record the maximum height of live and standing dead vegetation within 0.5 m of the Robel pole. Finally, we repeat these sampling efforts at two random points within 15 m of the nest site.

RESULTS AND DISCUSSION

Data collection is ongoing at the time of this report; therefore, we provide only a summary overview of the data collected and note adjustments to field methods that we have made thus far.

We captured and collared 10 hens at Lamberton and 10 hens at Worthington Wells. Two roosters were opportunistically captured and banded at Lamberton. The baited walk-in traps were not a productive capture technique. Tracks in the intermittent snow cover showed that

birds walked near the traps but were unwilling to enter them. We speculate that this result is due to a mild winter with above-average food availability for pheasants. Only 2 hens were captured using the walk-in traps (10%) whereas 18 hens (90%) and 2 roosters (100%) were captured by spotlighting/UTV. The onset of the breeding season limited our spotlighting/UTV capture efforts in the spring. In the future, we plan on also conducting spotlighting/UTV capture efforts in the fall and early winter to help increase our sample sizes. We will also use the baited walk-in traps if winter conditions are conducive to this capture method.

To date, 3 collar crimps have failed (15%) which led to the hens' collars falling off. Extreme cold temperatures during the collaring process likely led us to incorrectly install the crimps, resulting in the failures. Two hens (10%) died due to predation during the early nesting season (late April – early May). Prior to losing their collars or being depredated, two hens made short movements (<800 m) to initiate nesting on privately-owned grasslands enrolled in CRP. Fifteen hens (75%) are currently being monitored via radiotelemetry. Neither marked rooster has been re-sighted yet.

Fourteen of the 15 remaining hens (93%) have stayed within 400-800 m of their initial capture location for their nesting attempts whereas one hen (7%) has made a >4 mi movement to nest in a roadside outside of the Worthington Wells project area. To date, 6 hens (40%) have successfully hatched a nest. One additional nest has been located during our field efforts and has since hatched. Therefore, 7 out of 16 monitored nests have been successful (43.7% apparent nest success) to date. At least 2 nests failed due to cold, wet weather in mid-May and 2 nests have failed due to predation thus far. Three nests were abandoned in the laying stage in early May due to our monitoring efforts. In all three cases, the hen was sitting on her nest extensively while laying and our telemetry efforts therefore seemed to indicate that each hen had begun incubating. We have modified our protocol for flushing hens to locate nests and determine clutch size to allow hens to incubate for longer (>5 days) before we disturb them. Two hens began moving again after telemetry indicated that they had begun incubation but before we could locate their nests; therefore we do not know their clutch sizes or the cause of nest failure for these nesting attempts. We have begun to mark the general nest location by placing flagging 10-15 m to the north and south of the nest during laying and early incubation so that we can avoid losing these data in the future if nests fail before we flush the hen.

Cameras have been deployed on 7 nests to date. No predation events have been captured on video yet. Notable observations include a rooster visiting a hen at her nest (Figure 2) and a chick appearing on video (Figure 3) within 2 h of the hen leading the brood away from the nest site. We initially set cameras to record video continuously at 6 frames per second (fps) but later changed to 12 fps to increase the quality of video. Hens have been extremely tolerant of the cameras and we have been able to place the cameras within 1 m of the nest bowl.

We are currently monitoring 6 broods. We believe 3 of these broods hatched between 27 May and 1 June and the other broods hatched between 11 June and 16 June. We have captured and radio-tagged 4 chicks from 2 broods. In all cases thus far, hens have flown <200 m when flushed from their broods and have returned to within 5-50 m of the brood almost immediately, regardless of our presence. One transmitter has fallen off of a chick for unknown reasons; the remaining 3 chicks are currently alive and being monitored. Future efforts will be aimed at capturing and radiotagging chicks between 3-4 weeks old to allow tracking beyond the first 30 days post-hatch. We expect these efforts will be difficult because chicks will be capable of flying at this age. We will suture 3.4 g backpack-style VHF radiotransmitters onto the backs of

these older chicks. These heavier transmitters are expected to last approximately 90 days and will allow us to estimate juvenile survival to the beginning of October. The PIT tags implanted into hatchlings will allow us to identify older individuals during this second round of chick captures and help refine our survival analyses.

Vegetation data is currently being collected around nest sites. Vegetation data related to brood habitat selection will be collected over the remainder of the field season.

This first field season has aided in the refinement of field techniques necessary for assessing survival and habitat selection of pheasants. Furthermore, the data that is being collected will be used to plan the expansion of the study in 2016.

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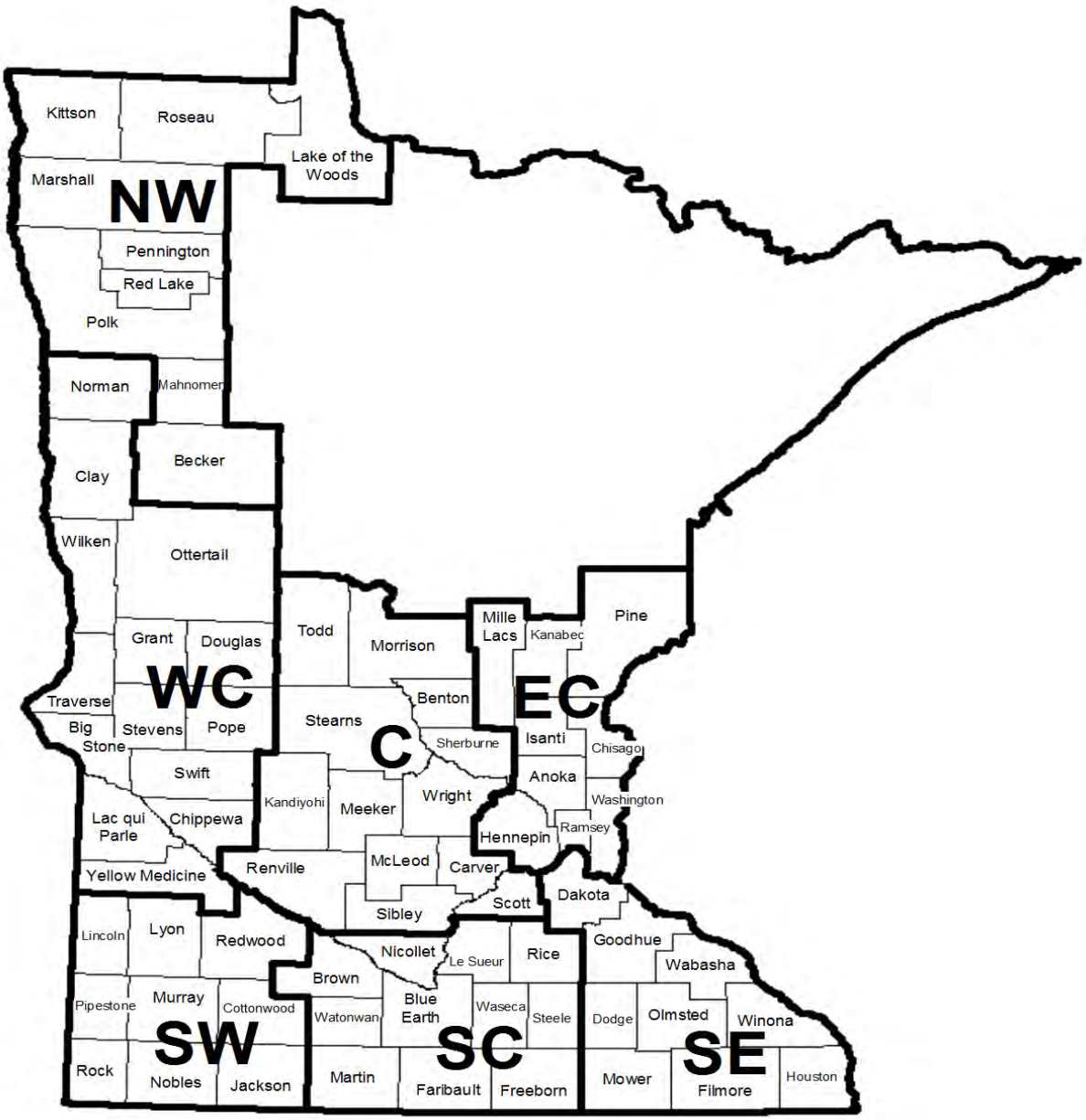

Figure 1. This study is being conducted in Redwood and Nobles Counties in southwestern Minnesota, which lies within the core of Minnesota's pheasant range.

Figure 2. A rooster visits a hen at her nest during incubation.

Figure 3. A chick appears <1 m from a nest within hours of hatching. About 2 h later, the video showed the hen leaving the nest with her brood.

QUANTITATIVE ASSESSMENT OF BULLET FRAGMENTS IN VISCERA OF SHEEP CARCASSES AS SURROGATES FOR WHITE-TAILED DEER[1]

Luis Cruz-Martinez, Marrett D. Grund, and Patrick T. Redig

ABSTRACT

Research indicates that avian scavengers, such as bald eagles (*Haliaeetus leucocephalus*), can be exposed to lead through the consumption of spent lead from ammunition in carcasses of animals shot with lead-based projectiles. Few studies have examined the degree of bullet fragmentation in viscera (offal) of game mammals. Our objective was to quantify the number of bullet fragments deposited in sheep carcasses shot with different types of lead and nonlead, high-velocity centerfire rifle bullets and with lead projectiles fired from shotguns and muzzleloader rifles marketed for hunting white-tailed deer (*Odocoileus virginianus*). We hypothesized that after controlling for velocity, angle of entry, distance from target, and shot placement (thoracic region), most of the bullet fragments would be deposited in the impact zone (heart and lungs). After examining all viscera from each carcass, we detected metal fragments in 96% of the viscera and found that metal fragments were deposited in greater quantities in the abdominal viscera (organs caudal to the diaphragm) compared to the thoracic viscera (heart and lungs). Additionally, bullets fired from the centerfire rifle fragmented more than the projectiles fired from the shotgun and muzzleloader rifle. Rapid-expansion lead bullets fragmented more than controlled-expansion lead bullets and lead-free bullets. However, 1 type of controlled-expansion bullet that is comprised almost entirely of lead and advertised to retain >90% of its weight, fragmented similarly to the rapid expansion lead bullets. We observed lead fragments produced by centerfire rifle bullets and shotgun and muzzleloader projectiles present in sheep carcasses and conclude that lead is made available to scavengers from the distribution of lead fragments lodged in the carcasses of game through viscera left in the field by hunters.To eliminate this type of lead exposure, shooters must employ the use of nonlead projectiles or completely remove the remains of shot animals from the field.

VALUATING COMPETING PREFERENCES OF HUNTERS AND LANDOWNERS FOR MANAGEMENT OF DEER POPULATIONS[1]

Gino J. D'Angelo and Marrett D. Grund

ABSTRACT

Most state wildlife agencies consider public input in the management of white-tailed deer (*Odocoileus virginianus*) populations. In 2013, we surveyed deer hunters (*n* = 3,600) and landowners (*n* = 4,604) in southwest Minnesota to gauge their preferences for managing deer. We sought to identify whether a priori assumptions about these main stakeholder groups in a primarily rural, agricultural region of the Midwest U.S. aligned with their perceptions of the impacts of deer. We hypothesized that irrespective of their perceived impacts of deer, hunters would prefer deer populations to be increased and landowners would prefer deer populations to be decreased. Our findings suggest that defining stakeholder groups according to primary associations with deer (i.e., farming and/or hunting) accurately categorized differences in tolerance levels for deer populations in our study area. Deer damage was considered relatively minor by landowners, yet 51% of landowners wanted deer densities reduced. Although 59% of hunters were satisfied with the number of deer, 62% of hunters still wanted deer densities increased in the future. Almost two-thirds of hunters were not satisfied with the number or quality of bucks where they hunted, and an antler point restriction was the only potential regulation supported by hunters to reduce harvest mortality rates of bucks. To enable managers to monitor trends in public satisfaction relative to the fundamental objectives of deer management in an area, we recommend conducting frequent surveys of primary stakeholders.

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IDENTIFYING BARRIERS TO MOVEMENT AND THE EFFECTIVENESS OF CORRIDORS FOR CONNECTING CORE AREAS: LANDSCAPE GENETICS OF PRAIRIE GROUSE IN FRAGMENTED LANDSCAPES

Charlotte Roy, Eric Nelson[1], and Andrew Gregory[2]

SUMMARY OF FINDINGS

Landscape genetics is an emerging field that examines landscape connectivity by combining a GIS with information about genetic variation in a population. This study aims to identify landscape features that pose barriers to prairie grouse movement and those that enable movements among areas of suitable habitat by using genetic information from feather samples in a landscape genetic approach. During the spring of 2014, cooperators and staff collected 174 sharp-tailed grouse (*Tympanuchus phasianellus*) and 162 greater prairie-chicken (*Tympanuchus cupido*) feather samples from leks. Hunters also submitted wings from 30 sharp-tailed grouse and 22 greater prairie-chickens during fall 2014. In spring 2015, 657 feather samples were collected from sharp-tailed grouse leks and 347 samples were collected from greater prairie-chicken leks. Wings will also be submitted by hunters in the fall of 2015. Genotyping is expected to be completed during summer 2016 with subsequent landscape genetic analysis during fall 2016.

INTRODUCTION

The grassland habitats that prairie grouse require have become increasingly fragmented as a result of competing pressures on the land (Berg 1997). Core habitat areas are isolated from each other by unsuitable areas that may prevent successful movement and the colonization of newly created habitat. The Minnesota Prairie Conservation Plan recognizes the importance of providing dispersal corridors to connect isolated core areas and identifies the greater prairie-chicken as an indicator species for upland prairie and grassland habitat (Minnesota Prairie Plan Working Group 2011). Similarly, sharp-tailed grouse must be able to move among isolated grassland, brushland, savanna, and peatland habitat patches (Berg 1997), through areas that may pose difficulty for successful movement. If the resistances of various landscapes to movement are understood, then more effective corridors can be identified, and management efforts can be prioritized using this information (Epps et al. 2007, Braunisch et al. 2010, Spear et al. 2010).

Landscape genetics is an emerging field that provides methods to examine connectivity on the landscape by combining a GIS with information about genetic variation in a population (Braunisch et al. 2010, Lowe and Allendorf 2010, Sork and Waits 2010, Haig et al. 2011). This tool can be used to examine effective dispersal (gene flow) on the landscape, without having to rely on telemetry techniques, which can be expensive and may require large numbers of marked animals if successful dispersal events are infrequent (Coulon et al. 2004, Spear et al. 2010). Landscape genetic methods have been used in recent years to identify barriers to dispersal, including human development, non-habitat land cover types, and distance in species

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like capercaillie (*Tetrao urogallus*, Braunisch et al. 2010), northern bobwhite (*Colinus virginianus*, Berkman et al. 2013a,b), and prairie-chickens (Gregory 2011). Thus, landscape genetics can be used to examine the movements of birds in a spatially explicit manner.

OBJECTIVES

1- To identify barriers to movement for sharp-tailed grouse and greater prairie-chickens in Minnesota (e.g., distance, urban development, treed areas) as measured by genetic connectivity
2- To identify landscape features and types that enable movements of prairie grouse among areas of suitable habitat in Minnesota (e.g., agriculture) as measured by genetic connectivity
3- To improve corridor planning and provide guidance to keep connected populations connected

METHODS

Wildlife managers, cooperators, and seasonal technicians surveyed prairie-chickens and sharp-tailed grouse at leks throughout Minnesota in the springs of 2014 and 2015. Feathers lost during male contests, copulations, and as a result of other activities were collected from discrete locations on leks to maximize the probability of sampling different individuals. Each cluster of feathers, or single feather when necessary to ensure that only one individual was represented, was placed in an envelope and labeled with the lek location (coordinates or Township, Range, Section, and quarter-section information), date, collector name, contents, and species. Information from each envelope was recorded in a database and assigned a unique sample number. Areas underrepresented in 2014 were given greater effort in the spring of 2015.

Feather samples from leks were supplemented with samples from hunter-harvested birds in 2014. Wings from harvested birds were aged based on plumage characteristics (Bihrle 1993). Collection of hunter-harvested samples will continue in fall 2015. After the close of hunting season, samples will be sent to a commercial lab (Wildlife Genetics International, British Columbia).

At the lab, DNA will be extracted and amplified at 15 microsatellite loci. Microsatellites are highly variable, neutral (non-coding) genetic loci. Recent studies of prairie-chickens and sharp-tailed grouse identified polymorphic microsatellite loci in these species (and populations, see citations in Gregory 2011 and Malone 2012). The sex of birds will be determined molecularly using techniques such as those in Fridolfsson and Ellegren (1999).

Genetic information will be linked to spatial information in a GIS to examine the connectivity of the landscape. Areas that share greater connectivity will be similar genetically, whereas areas with restricted connectivity will be more dissimilar genetically. Analytical methods will be revisited for the most recent advances prior to initiating data analysis.

RESULTS/DISCUSSION

We collected 174 sharp-tailed grouse and 162 greater prairie-chicken feather samples from leks during the spring of 2014 (Figures 1 and 2). Thirty sharp-tailed grouse and 22 greater prairie-chicken wings were submitted by hunters during fall 2014 (Figures 1 and 2). In spring 2015, 657 sharp-tailed grouse samples and 347 greater prairie-chicken samples were collected (Figure 3). We plan to collect wings from hunters again in the fall of 2015. Genotyping is expected to be completed during summer 2016 with subsequent landscape genetic analysis during fall 2016.

This study is expected to provide information about landscape features that isolate habitat fragments and those that promote connectivity. We can also use landscape genetic

analyses to understand the relative influence of different landscape elements to promote or inhibit dispersal (Gregory 2011, Barton et al. 2010). This information will be useful to target management efforts in ways that can more effectively accomplish the goal of connecting core areas, enhancing local habitat conditions, and providing new habitat sufficiently close to existing leks to promote colonization.

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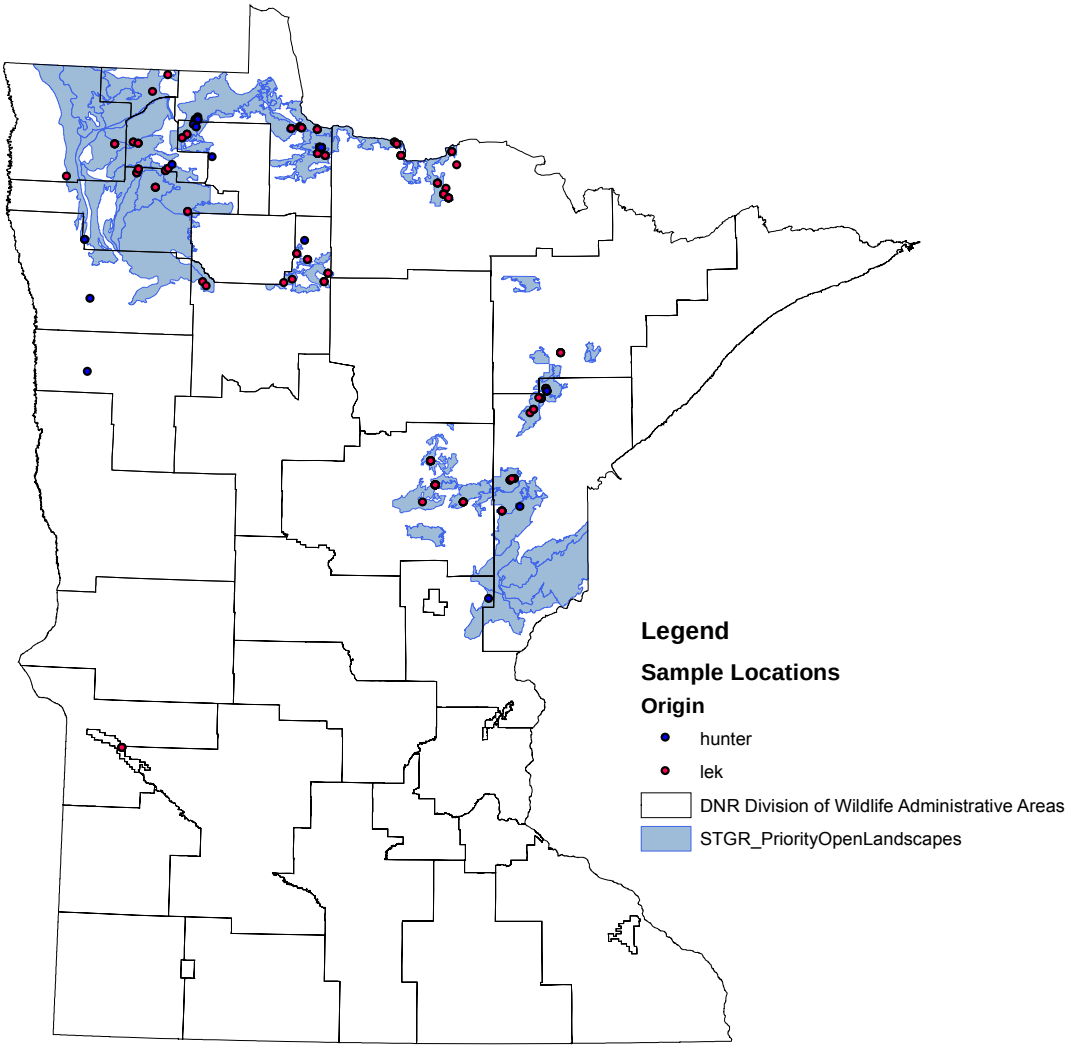

Figure 1. Locations where sharp-tailed grouse feather samples (*n* = 174) were collected at leks or by hunters (*n* = 30) in Minnesota during 2014.

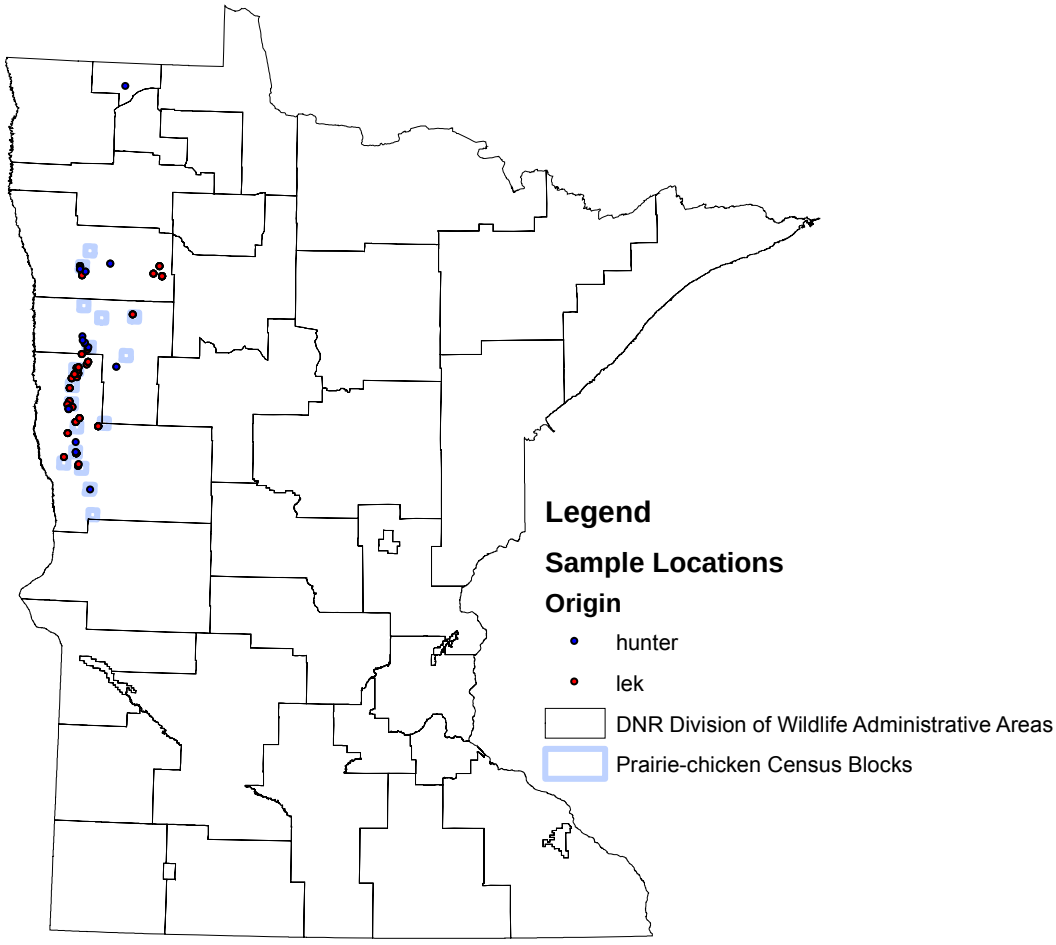

Figure 2. Locations where greater prairie-chicken feather samples (*n* = 162) were collected from leks and hunter-harvested sample collection sites (*n* = 22) during 2014.

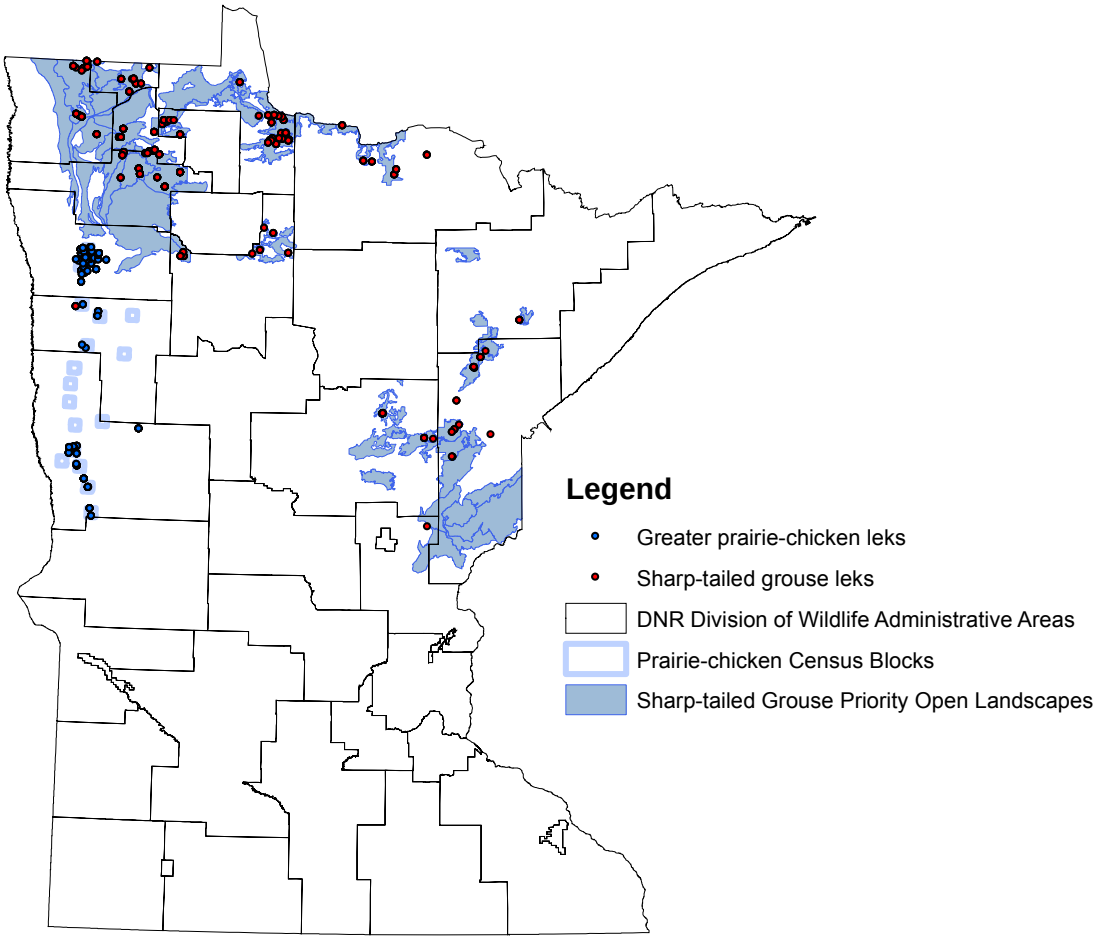

Figure 3. Collection sites for sharp-tailed grouse samples (*n* = 657) and greater prairie-chicken samples (*n* = 347) in spring 2015.

MONITORING SPRUCE GROUSE IN MINNESOTA: A PILOT STUDY (2014–2015)

Charlotte Roy, John Giudice, and Chris Scharenbroich

SUMMARY OF FINDINGS

Data collection began in 2014 for a 2-year pilot study to develop survey methodology for spruce grouse in Minnesota. We examined 2 primary methods of spruce grouse detection, broadcast of a cantus call and a pellet survey. We also used dogs to detect spruce grouse in survey areas as an additional method in 2014. Based on field work conducted in April and May 2014, we determined that spruce grouse responses to the cantus call were more frequently detected earlier in the day, earlier in the 15-min broadcast period, earlier in the season (April > May), and when habitat occurred on both sides of the road. Pellet surveys along circular transects centered on call survey points (i.e., radii of 75 m and 100 m) had 5 times the apparent detection rate of call surveys (20% and 4%, respectively), which were more similar to detection rates with dogs (4%). When all 3 methods were conducted at the same points, the methods had 66% concordance, but no spruce grouse were detected by any method in 64% of surveyed points. GIS forest data layers correctly predicted habitat cover types for spruce grouse with 85% accuracy. However, the western portion of the study area at Red Lake Wildlife Management Area (RLWMA) contained areas of forest habitat with no spruce grouse detections, which is consistent with this study area being on the southwestern periphery of spruce grouse range in Minnesota. Based on findings in 2014, we modified our methodology in 2015 to restrict call surveys to before 0930 hours, in areas where habitat occurred on both sides of the road, and in portions of the study area where spruce grouse had been detected in 2014. We also reduced the call survey period to 9 min and added a second study area near Isabella in 2015, which was more central to spruce grouse range in Minnesota, to contrast with the more peripherally located site at RLWMA. During 2015, we conducted pellet and call surveys at paired points on and off roads to allow examination of the effects of roads on survey counts with both methods. Data collection for 2015 ended in May. We anticipate design of a road-based pellet survey to be piloted at a large scale in 2016, pending analysis of findings from 2015.

INTRODUCTION

The spruce grouse (*Falcipennis canadensis;* SPGR) is considered a Species of Special Concern in Michigan (Michigan DNR 2005) and was listed as threatened in Wisconsin in 1997 (Wisconsin DNR 2004). Minnesota is unique among the Lake States in having a sizeable spruce grouse population that still permits spruce grouse hunting. Yet, the only data the Minnesota Department of Natural Resources (MNDNR) collects on spruce grouse is estimated total harvest as part of the annual MNDNR small game mail survey (Dexter 2013). Estimated total harvest has been 9,000–27,000 birds/year over the last 10 years (Dexter 2013). However, spruce grouse harvest may be more reflective of ruffed grouse hunter numbers than spruce grouse numbers; thus these data cannot be used as a population index (Gregg et al. 2004). The MNDNR mail survey also provides some information on geographic distribution via a "county hunted most" question, but it is probably insufficient for monitoring anything less than large scale range changes. Hence, the MNDNR has limited data on spruce grouse distribution, abundance, and population trends in Minnesota despite a responsibility to manage spruce

grouse during a period of expected habitat loss due to climate change (see Roy et al. 2013). Thus, there is a need for better population-monitoring data for spruce grouse in Minnesota.

Developing large-scale monitoring programs that are both reliable and cost effective is a challenging exercise, especially when the species is relatively rare and occupies habitats that are not easily accessible. New York (Fritz 1979) and Wisconsin (Worland et al. 2009) have conducted statewide surveys of spruce grouse. Wisconsin used a spatially balanced stratified sampling design with 4 stand size classes (range: 8.1–1,242 ha), in which they surveyed multiple points in 81 swamps during 3 visits. In New York, 67 habitat patches were surveyed during 220 visits. However, these surveys were only conducted a few years, were labor intensive, and were not designed to be long-term monitoring projects. Any long-term, large-scale monitoring effort of spruce grouse in Minnesota would need to be easy to execute, repeatable, and representative of spruce grouse populations. Logistical, financial, and resource constraints often limit survey-design options for large-scale monitoring efforts. In this case, spruce grouse occupy habitats that are very difficult to access away from roads. A roadside survey would possess the logistical ease desirable for a statewide effort, but several potential biases would need to be addressed.

As part of a pilot study (Roy et al. 2013), we evaluated survey methods that might be useful for monitoring spruce grouse populations in Minnesota or investigating questions related to habitat use and metapopulation dynamics. More specifically, we evaluated a time-of-detection auditory survey using playback of female cantus calls (Fritz 1979, Boag and McKinnon 1982, Whitcomb et al. 1996, Lycke et al. 2011). We also conducted pellet surveys and used pointing dogs to locate birds on survey plots (e.g., following completion of a cantus-call survey).

Surveys for spruce grouse are usually conducted using playback of female cantus calls (Fritz 1979, Boag and McKinnon 1982, Whitcomb et al. 1996, Lycke et al. 2011, among others). The duration of responsiveness to playback varies among years depending on spring phenology (i.e., shorter period of responsiveness in early springs, Anich, pers. comm.), which is consistent with findings in our study area by M. Larson (DNR, unpublished data) in 2011 and 2012. The duration of responsiveness may also vary throughout the day, although Lycke et al. (2011) determined that birds responded to cantus calls between sunrise and noon.

OBJECTIVES

The primary objectives of the pilot study were to

1. Assess the feasibility of using a roadside survey to determine distribution and population trends of spruce grouse in Minnesota; and
2. Estimate capture success and identify constraints to radiotracking (for a subsequent, more intensive study of habitat use and survival).

STUDY AREAS

In 2014 we focused on the Red Lake Wildlife Management Area (RLWMA) and Beltrami Island State Forest (BISF; Figure 1). Gretchen Mehmel and Scott Laudenslager, managers at these locations, have an interest in managing for spruce grouse and have resources they can commit to the project (e.g., financial support, equipment, lodging for seasonal workers). Their interests include development of survey methods and studying population-level responses to timber harvest. In addition to logistical and financial support, this study area was attractive because it is on the southwestern edge of the surmised range of spruce grouse in Minnesota, where changes (range contraction or negative trends in abundance, density, or patch occupancy) might occur earlier than in more central portions of the species range.

In 2015, we reduced our focal area at RLWMA and BISF to include only portions where spruce grouse detections occurred in 2014. This was done so survey methods would be evaluated in areas where birds were known to occur (Figure 2), which was of importance given this study area location on the edge of their distribution in Minnesota.

The long term goal is to expand the survey throughout spruce grouse range in northern Minnesota. Therefore, in 2015 we expanded the pilot study to include a second study site near Isabella (Figure 3). The second study site is more central in SPGR range within Minnesota, and thus this study site offered insights into application of survey methods where populations might be more robust to initial habitat changes.

METHODS

Identifying SPGR Habitat

The literature is conflicting with respect to forest ages of importance for spruce grouse; earlier successional stages have been reported to be important in the western U.S. (Boag and Schroeder 1992), but mature forest was important in Wisconsin (Anich et al. 2013). In 2014, we included forest types reported to be preferred by spruce grouse in our region including jack pine (*Pinus backsiana*), black spruce (*Picea mariana*), and tamarack (*Larix lariana;* Robinson 1969, Pietz and Tester 1982, Anich et al. 2013). We included all stand ages because of the lack of clarity in the literature, but focused on preferred habitat types rather than all used habitat types. We also included white cedar (*Thuja occidentalis*) which was reported to be used but not a preferred habitat type (Anich et al. 2013), because managers at the study area were specifically interested in surveying this forest type.

In 2015, we added balsam fir (*Abies balsamea*) and red pine (*Pinus resinosa*) forest types to our survey points. This decision was based on 2014 detections in stands with these species components that exceeded our expectation of use based on their representation in the sample. We also added white spruce (*Picea glauca*) because it was reported as used but not preferred in the literature, and inclusion of these other used but not preferred stand types seemed to warrant its inclusion for consistency. We used Forest Stand Inventory (FIM) data layers to identify survey points in these forest stand types and age classes at both sites. We excluded stand ages listed as “under development” (i.e., 0–5 years) in the FIM data to exclude areas that might not have established as forest. Timber harvest data (US Forest Service 2015a), Motor Vehicle Use Maps (U.S. Forest Service 2015b), and fire records (National Interagency Fire Center 2013) were also used for the Isabella site to exclude stands that were recently harvested or burned and to identify roads suitable for survey routes.

Survey Routes and Listening Points

2014.– We used GIS road layers (MNDOT and MNDNR) to identify roadways that were within 40 m of potential habitat polygons (jack pine, black spruce, tamarack and white cedar; see above). We then classified roadways as primary or secondary based on their accessibility during the April-May survey period (e.g., plowed vs. not plowed). Minimum maintenance forest roads and other system forest roads that served as snowmobile trails were excluded from consideration. We then established listening points on road segments that bisected or were within 40 m of habitat polygons. Points were spaced $\geq$300 m apart to ensure independence among points based on estimates that playback calls can be heard 100–150 m from the speaker (Schroeder and Boag 1989; Lycke et al. 2011; Anich unpubl. data). Road segments and associated listening points were then grouped into survey routes based on logistical considerations.

2015.– We used the same GIS layers to select survey points in 2015, but also used current data for U.S. Forest Service roads, harvest, and fire data for the Isabella study site (U.S. Forest Service 2013, National Interagency Fire Center 2013, U.S. Forest Service 2015a,b). However, our focus in the second season was a comparison of off-road and on-road survey points to examine the impact of roads on survey detections. We selected paired points that had at least 30% SPGR habitat (based on selected forest types) within 150 m of each point, but limited our selection to areas where habitat occurred on both sides of the road. Off- and on-

road points were separated by 300 m, and we alternated the side of the road where off-road points were selected, except when creeks limited access to points on foot.

Cantus Call Surveys

2014.– We used a playback of female cantus calls to conduct point-count surveys of SPGR (Fritz 1979, Boag and McKinnon 1982, Schroeder and Boag 1989, Whitcomb et al. 1996, Lycke et al. 2011). We surveyed as many points as possible to provide information on survey duration (1–15 min), time needed to complete multiple surveys, habitat associations, and the responsiveness of SPGR to cantus calls (in terms of time of day and duration of season). Surveys were conducted during April-May, beginning at sunrise, when winds were <10 mph and precipitation was absent or light. Each point count lasted 15 min (Lycke et al. 2011, Anich et al. unpubl. data) and was divided into five consecutive 3-min listening intervals. The 8-second cantus call was broadcast once per minute for the duration of the 15-min listening period (i.e., 3 times per listening interval). Observers recorded initial and subsequent detections of each SPGR by listening interval, which allowed us to construct individual detection histories for a potential time-of-detection analysis (Alldredge et al. 2007). We also recorded the estimated distance (<50, 50–100, 101–150, >150 m) to each initial detection, type of initial detection (flutter flight, approach, etc.), survey date, arrival time, wind speed, temperature, dominant tree species (as classified from the roadway: jack pine, black spruce, tamarack, white cedar, red and white pine, balsam fir, deciduous, other), and background noise (none, low, medium, high).

In addition to computing summary statistics, we used the function 'occup' in R package 'unmarked' (Fiske and Chandler 2011; R Core Team 2014) to fit some exploratory hierarchical occupancy models to the cantus call survey data. We included detection covariates for survey date and arrival time, and occupancy covariates for relative amount of SPGR habitat around each listening point (habitat sides = 0, 1, 2) and survey date. Continuous covariates (survey date and arrival time) were standardized prior to analysis. We used AIC to select a best approximating model for the detection process and then used that structure to examine occupancy covariates. We also included a time covariate that allowed probability of detection to vary by listening interval. If a model was within 2 AIC units of the 'best' model (lowest AIC), we selected the most parsimonious model for inference. For simplicity, we restricted our exploratory analysis to initial visits (revisits were excluded) and we excluded surveys with background noise = 3 (high). We also excluded surveys with missing data for any of the covariates. The final analysis dataset consisted of 459 cantus-call surveys, which were all unique listening stops.

2015.–Call survey methods were modified to incorporate our findings from 2014. Specifically, we reduced the survey length from 15 minutes to 9 minutes and ended call surveys before 0930 hours. We also initiated surveys 30 minutes prior to sunrise to provide for more survey time before 0930 hours. In 2015, we discontinued estimating the distance from the observer to initial detections because it was difficult to assess distance accurately for auditory detections in varying densities of forest vegetation.

Dog and Pellet Surveys

2014.– After completion of playback surveys, we surveyed a subset of listening points with trained dogs and their handlers (Robinson 1969, Keppie 1987, Ratti et al. 1984) to locate grouse within a 150-m radius of the listening point later the same day. We attempted to quantify the variability in skill among dogs by surveying the same points with multiple dogs, but dogs surveyed these points on different days. We also counted grouse-pellet piles within 1 m of circular survey paths located at 75-m and 100-m radii from the listening point. We distinguished ruffed grouse pellets from spruce grouse pellets on the basis of length, thickness, uric acid wash, and color (N. Anich and A. Ross, personal communication). Ruffed grouse pellets tend to be shorter, thicker, and usually have a uric acid wash, whereas spruce grouse pellets are

longer, thinner, and infrequently have a uric acid wash. Spruce grouse pellets are also darker green in color when spruce grouse are consuming conifer needles (during winter), but color changes depending on diet (pers. observ.); spruce grouse pellets can have a similar color to ruffed grouse pellets later in the spring. Finally, we recorded dominant and subdominant tree species along each circular path to compare forest-type classification based on GIS, roadside observations, and pellet surveys.

2015.– We surveyed all paired points on and off roads for pellets along circular transects of 100-m radius. Other pellet survey methods remained the same. We did not use dogs in 2015 because of the limited success of dogs at survey points in 2014 and no intent to incorporate dogs into a range-wide survey method.

Radio-marking

We radiomarked 10 spruce grouse (4 males, 6 females) at the RLWMA in 2015 to gather pilot data on movements and tracking constraints in study areas with limited road access. Tracking will continue while transmitters are active (i.e., approximately 1 year).

RESULTS

2014

Cantus-call surveys.– We surveyed 56 roadside routes with 2-12 stops (listening points)/route for a total of 530 unique listening points. Surveys were conducted 6 April–28 May (median = 7 May). Three hundred ninety-six listening points (75%) were visited once, 120 points (23%) were visited twice, and 14 points (3%) were visited three times for a total of 678 cantus-call surveys. We detected SPGR at 26 points (4%), but only 1 survey had >1 bird detection (max = 2 birds). Ten of the 27 SPGR detected were females, 7 were males, and 10 were unknown. Twenty-six percent of detections involved flutter flights and 74% of detections involved SPGR vocalizations. Birds were detected in all 5 listening intervals, although 78% of birds were detected in the first 3 intervals (9 min, Figure 4). Most birds (89%) were first detected within 100 m of the listening point.

Pellet surveys.– We conducted pellet surveys at 230 listening points and detected pellets at 45 (20%) of these points. Pellet surveys and cantus-call surveys had 82% concordance in terms of the presence-absence of SPGR. However, we detected pellets at 36 points (16%) where we failed to detect a bird during cantus-call surveys, and we failed to detect pellets at 5 (2%) points where we detected SPGR during cantus-call surveys. If we condition on sites where SPGR pellets were detected, the cantus-call survey resulted in SPGR detections 20% of the time (an index of sensitivity; but the probability of detecting pellets given a bird was present was <1). The proportion of points where pellets were detected was also influenced by survey effort (i.e., radius from the listening point; $\chi^2 = 23.6$, df = 1, $P < 0.01$). More specifically, the 100-m radius survey path resulted in 28 detections (39%) compared to 18 (11%) detections with a 75-m radius path.

Dog surveys.– We conducted dog surveys at 123 listening points (118 points had a concurrent pellet search). The dogs found SPGR at 5 (4%) points and we found SPGR pellets at 37 (31%) points. If we restricted the analysis to 118 listening points where all 3 surveys were conducted (cantus call, pellet, and dog), then concordance was 66%. However, 64% of these sites had no detections (from any method) and dogs found SPGR on 10% of sites where pellet or cantus-call surveys indicated SPGR presence or use (n = 41 points). If we condition on surveys where both cantus-call and dog surveys were conducted on the same day and the cantus-call survey detected at least 1 bird (n = 23 surveys), then dogs found spruce grouse on 4 (17%) occasions.

Forest-stand characteristics.– We had 217 listening points with GIS, roadside, and ground-truth (based on walking surveys) data on forest-stand characteristics. There was a 94% agreement between roadside and ground-truth assessments of forest type, at least in terms of classifying functional SPGR habitat (forest stands with ≥30% mature black spruce, jack pine, red pine, white cedar, tamarack, or balsam fir). Likewise, we had an 85% agreement between GIS and roadside classifications (again, in terms of identifying potential SPGR habitat). Overall, 83% of the survey areas had ≥30% SPGR habitat. However, 14% of the survey areas had <30% SPGR habitat based on ground-truth assessments. In most cases there was still some component of the forest stand that was comprised of potential SPGR habitat. A complicating factor was that SPGR habitat was not always uniformly distributed around listening points. Seventy-eight percent of listening points had potential SPGR habitat on both sides of the road, but 21% of points had habitat on only 1 side.

Time-of-detection analysis.– Our best approximating hierarchical occupancy model included detection covariates for survey date, arrival time (hours), whether the bird was detected in a previous listening interval, and an occupancy covariate describing the relative amount of SPGR habitat surrounding the listening point (habitat sides = 0, 1, 2). Mean probability of detection (for any given listening interval) was negatively associated with survey date and arrival time; however, sample sizes were too small to precisely describe the magnitude of the effects (Figures 5 and 6). Not surprisingly, probability of detection increased dramatically if a bird was detected in a previous listening interval [i.e., p(recapture) > p(capture); Figure 7]. The mean probability of occupancy for a listening stop with good SPGR habitat on both sides of the road was 0.23 (95% CI = 0.02–0.78; Figure 8), and the overall probability of detection for the entire 15-min survey given mean covariate values for survey date and arrival time was 0.25 (95% CI = 0.02–0.93).

2015

The field season ended in late-May and data have yet to be analyzed for 2015.

DISCUSSION

We intend to design a pilot survey that can be conducted at a large scale beginning in 2016, based on our findings in 2014 and 2015. We anticipate this survey will be pellet-based. Pellets are numerous, more easily detected, and can be easily learned by field biologists with limited instruction.

ACKNOWLEDGMENTS

We thank the volunteer dog handlers including Earl Johnson, Donna Dustin, Meadow Kouffeld-Hansen, David Andersen, Chris Petro, Frank Spaeth, Gary Huschle, Greg Kvale, Jerry Forgit, and Charlie Tucker. We also thank Gretchen Mehmel, her staff, and family for supporting this project financially, logistically, and personally. We also send a sincere thanks to Luke Nolby, Clara Olson, Kyle Kuechle, Tyler Garwood, and Michael Schleif, our seasonal technicians. Charlie Tucker also radio-tracked spruce grouse outside the spring season and facilitated photography efforts.

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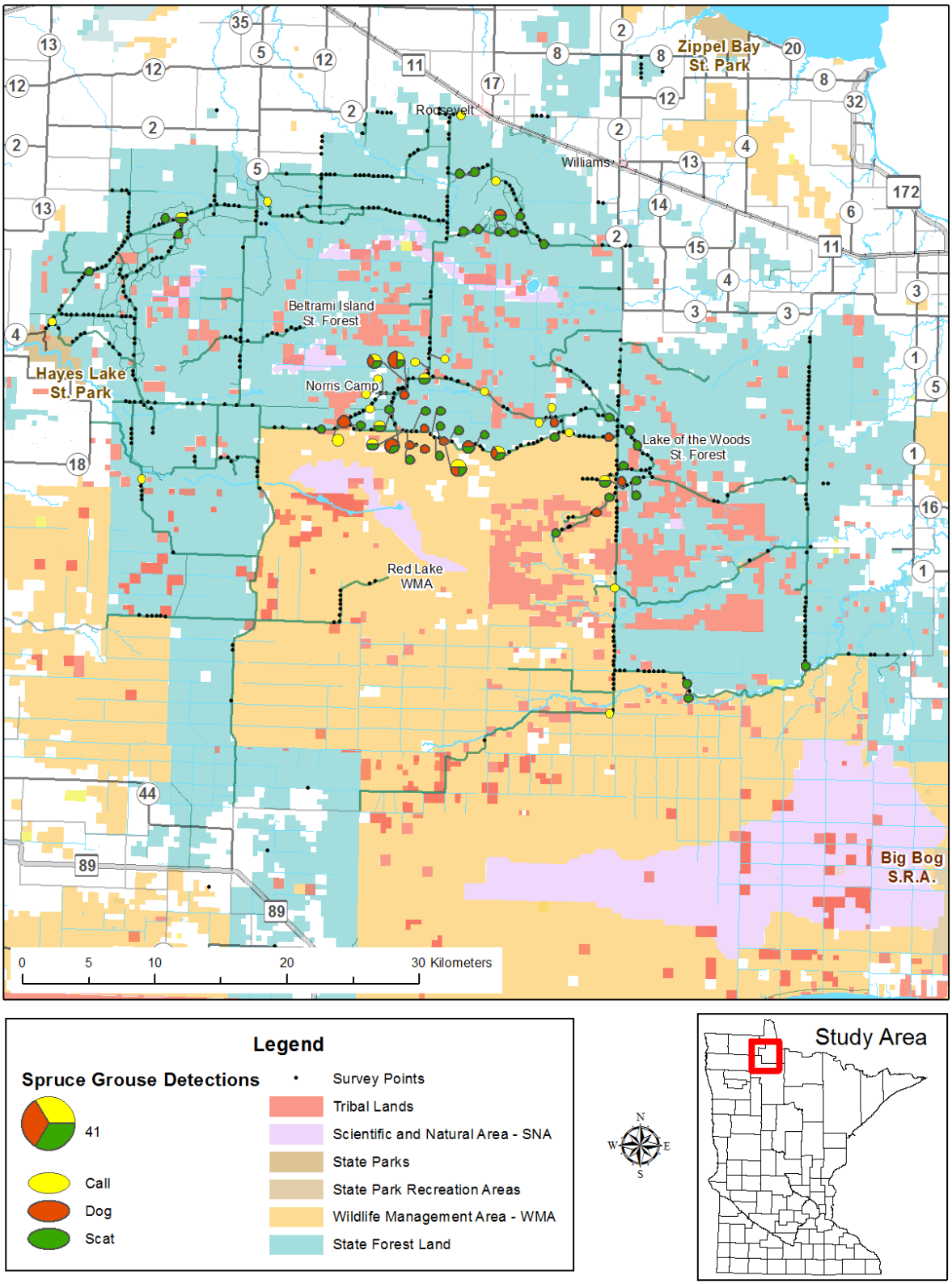

Figure 1. Spruce grouse study area at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2014. Detections and survey points along roads are depicted.

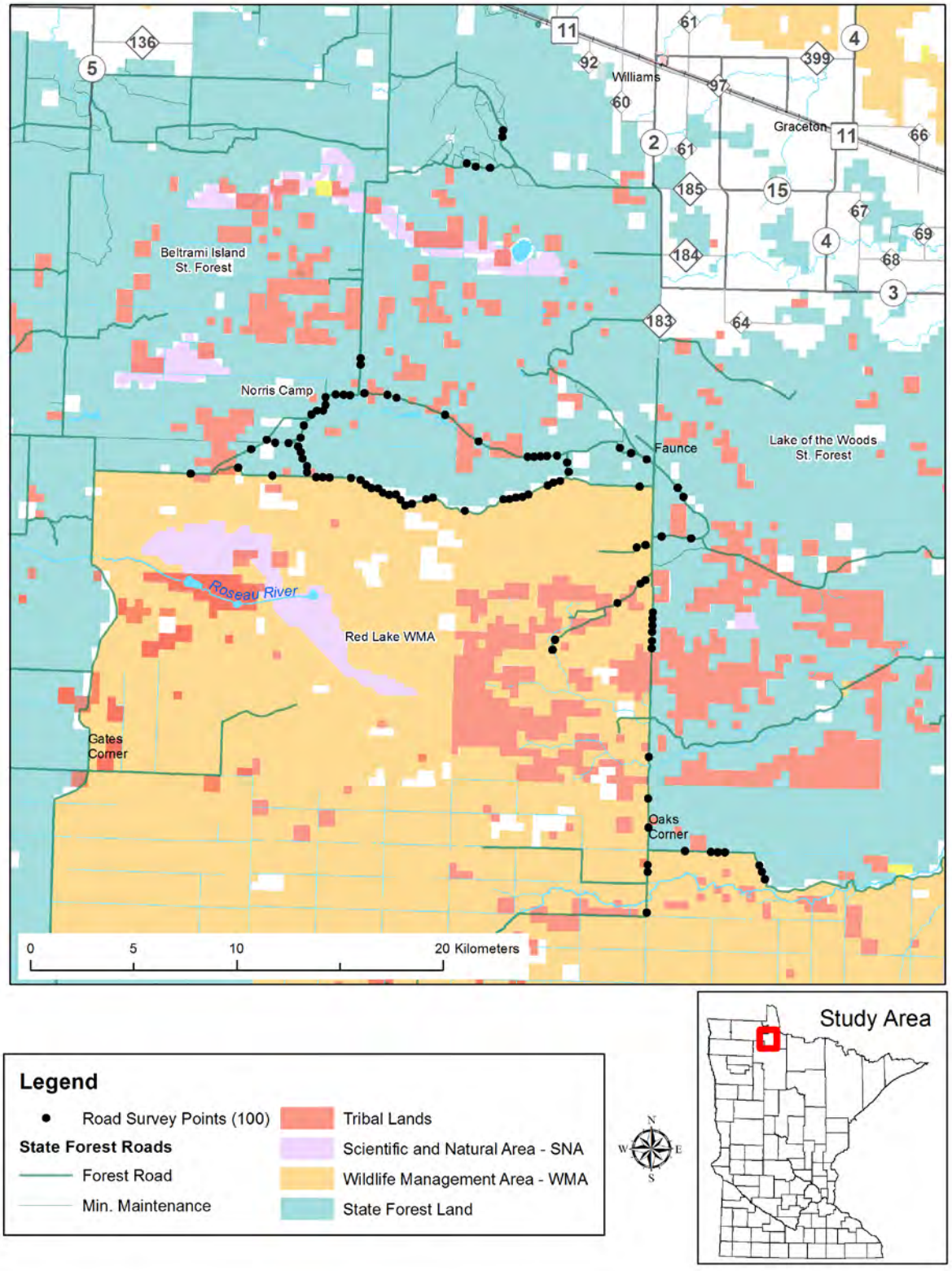

Figure 2. Study area at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2015. The study area was reduced to focus on areas where spruce grouse were detected in 2014. Off-road points were 300 m from road points and alternated sides except when access was prohibited.

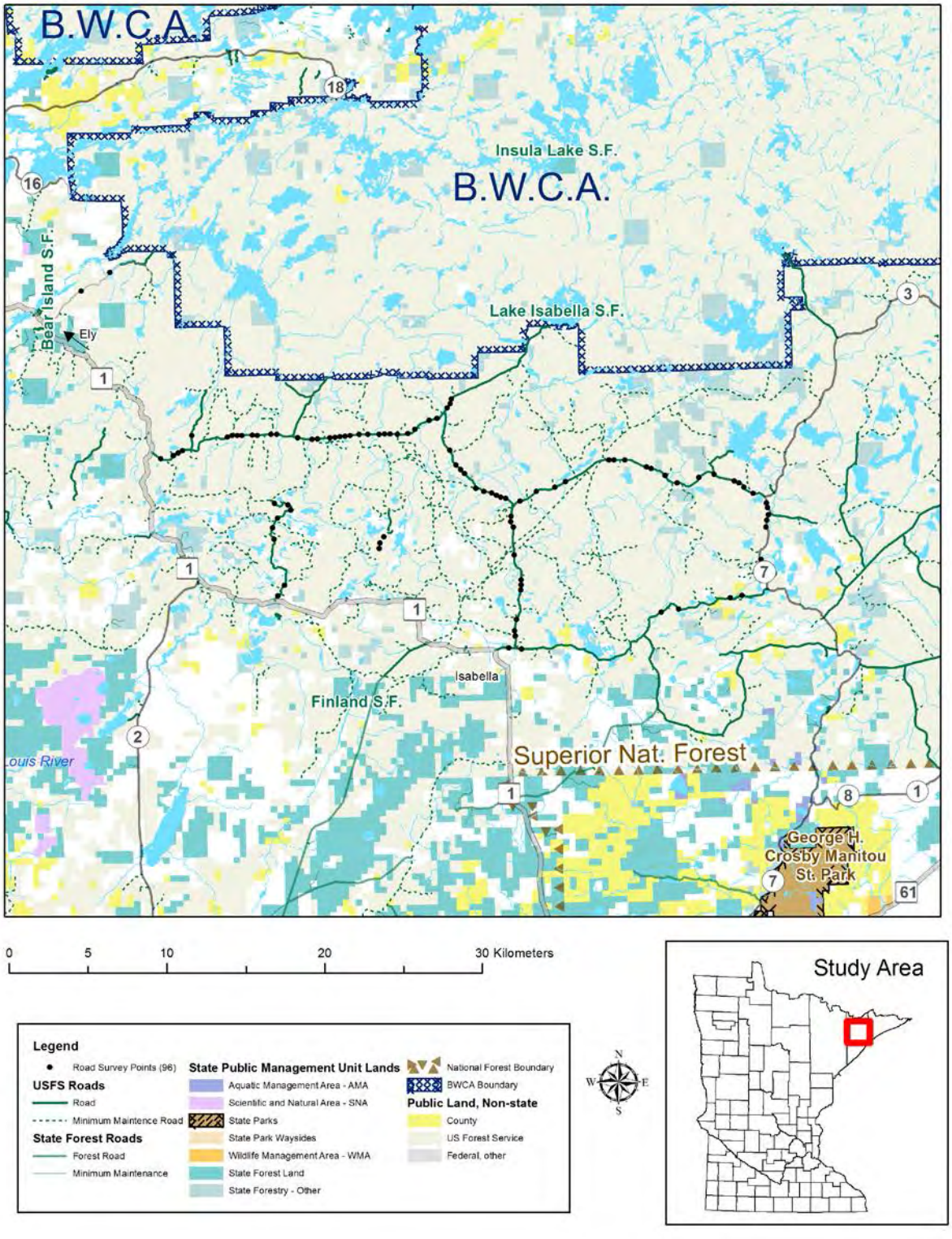

Figure 3. Study area near Isabella in 2015. Points indicate survey locations along roads. Off-road points were within 300 m of road points and alternated sides except when access was prohibited.

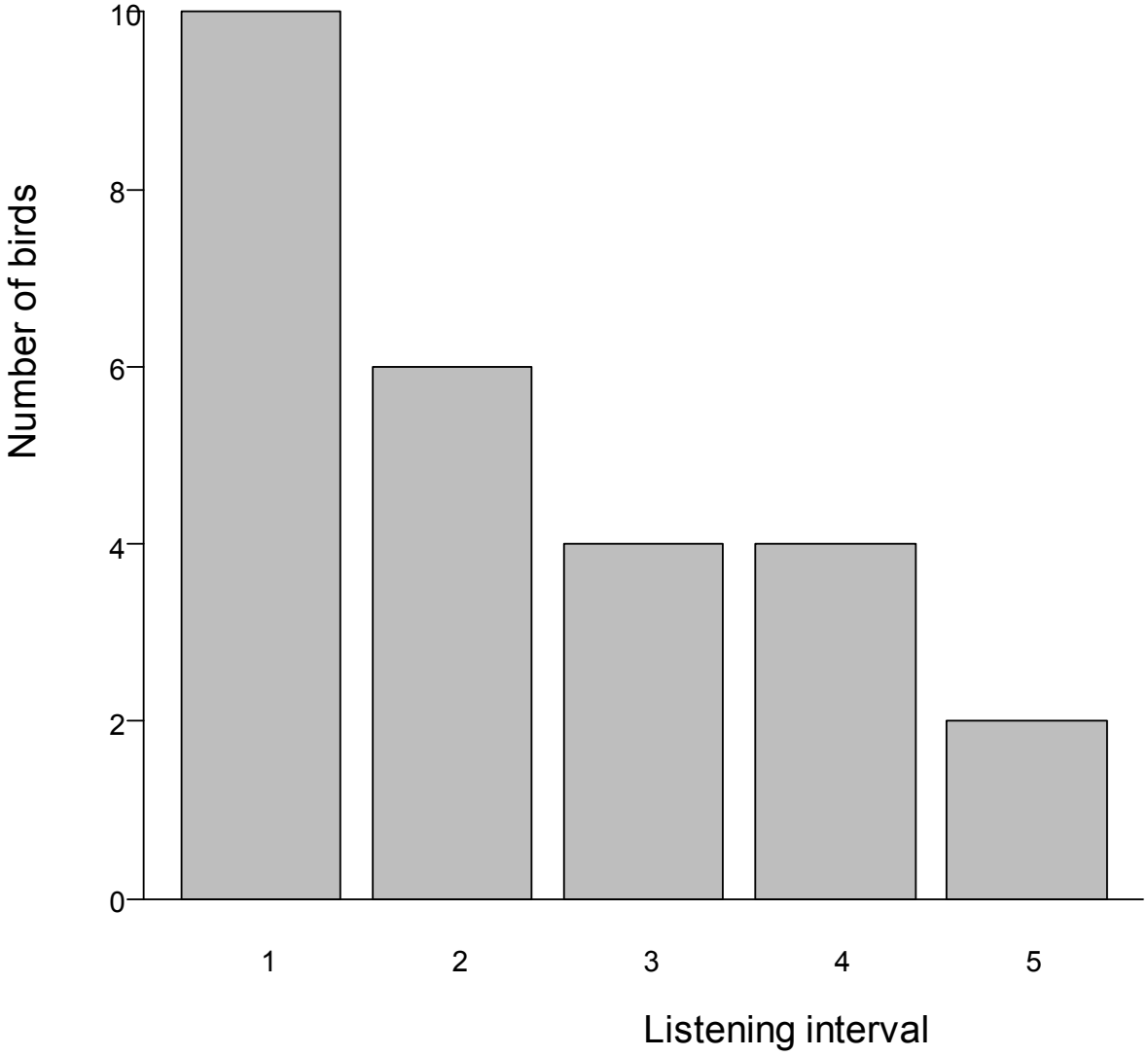

Figure 4. Distribution of initial spruce grouse detections among 3-min listening intervals of the cantus call survey at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2014.

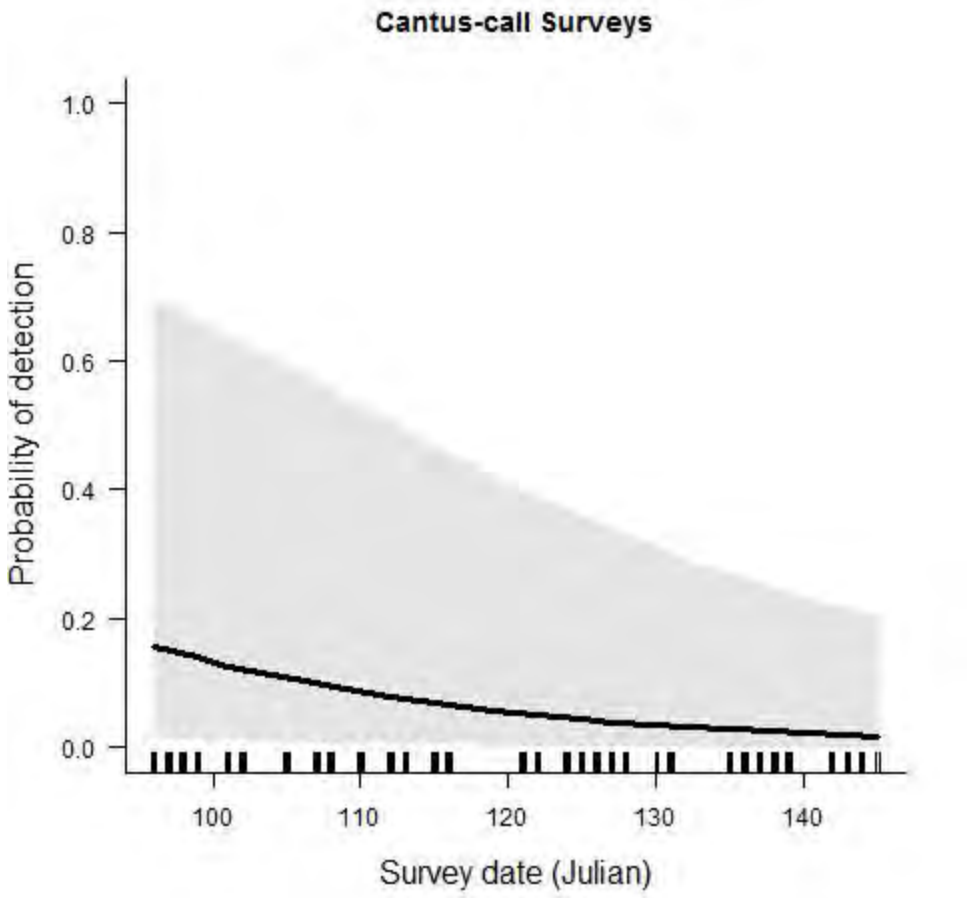

Figure 5. Mean conditional probability of detection (solid line; conditional on a bird being present and available for detection) in each listening interval as a function of survey date, Red Lake Wildlife Management Area and Beltrami Island State Forest, 2014. Gray polygon denotes 95% confidence interval. The "rug" on the x-axis denotes the sample distribution.

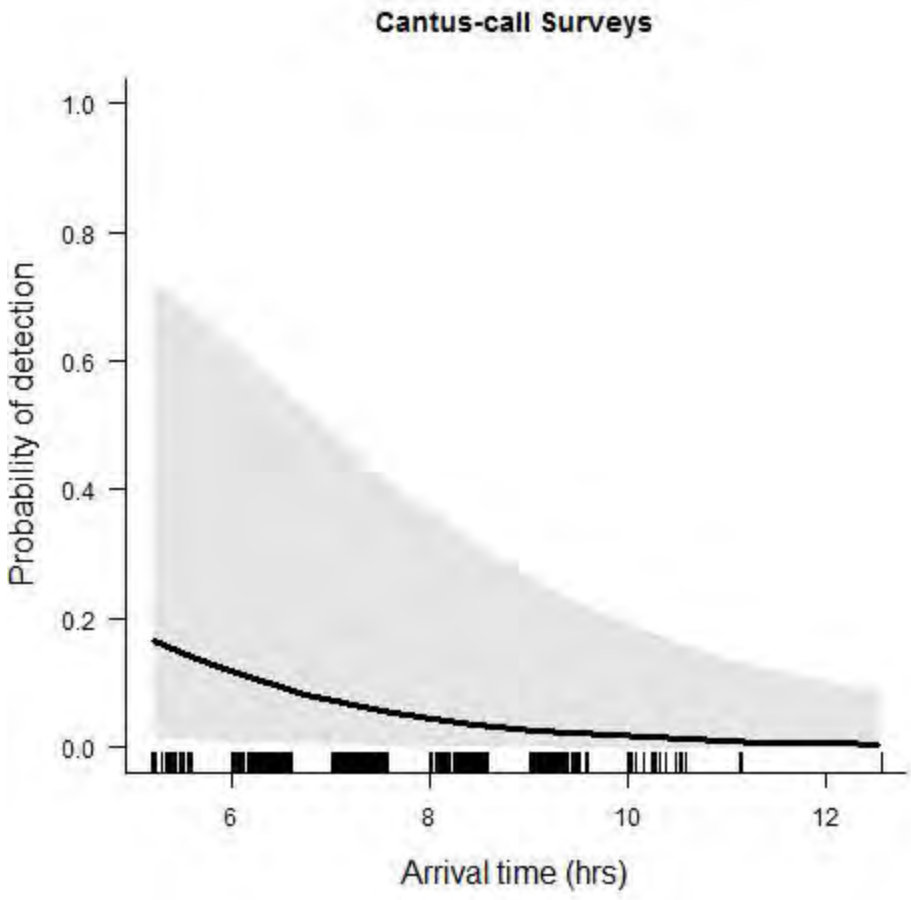

Figure 6. Relationship between spruce grouse call detections and cantus call survey arrival time (i.e., 6 = 0600 hours) at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2014. Gray polygon denotes 95% confidence interval.

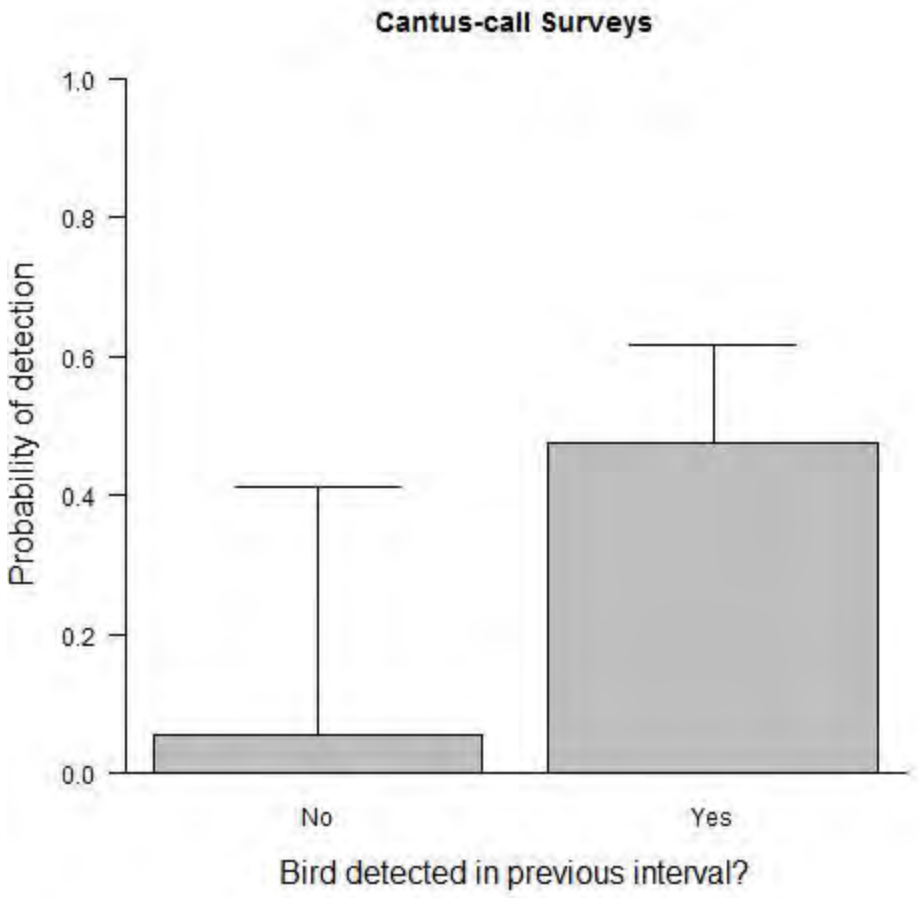

Figure 7. Probability of detection increased when a bird was detected in a previous listening interval [i.e., p(recapture) > p(capture)].

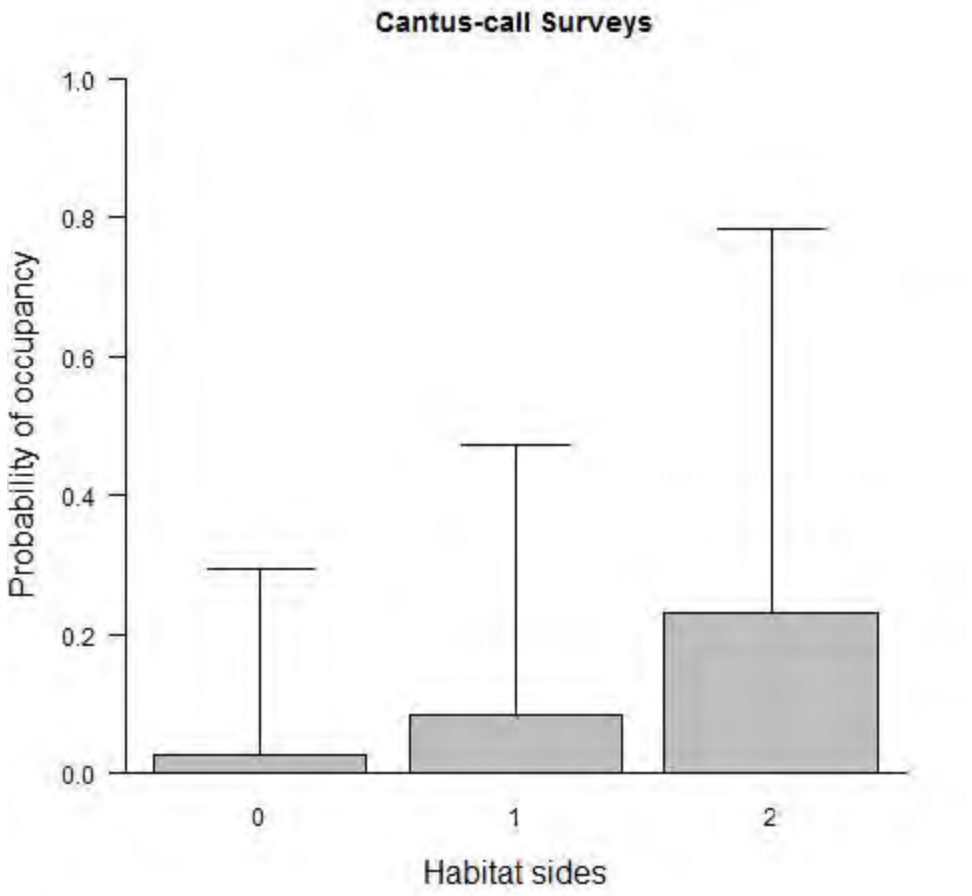

Figure 8. Relationship between the probability of spruce grouse occupancy and the presence of habitat on 0, 1, or 2 sides of the road during cantus call surveys at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2014.

ECOLOGY AND POPULATION DYNAMICS OF BLACK BEARS IN MINNESOTA

David L. Garshelis and Brian J. Dirks

SUMMARY OF FINDINGS

During April 2014–March 2015, we monitored 24 radiocollared black bears (*Ursus americanus)* at 4 study sites representing contrasting portions of the bear's geographic range in Minnesota: Voyageurs National Park (VNP, northern extreme, poorest food), Chippewa National Forest (CNF; central), Camp Ripley Training Center (southern fringe), and a site at the northwestern (NW) edge of the range. Most of the focus of this study has been in the NW site in recent years. This area had the highest reproductive rate of our 4 study sites, due to an abundance of both agricultural crops and natural foods. With a higher abundance of foods, bears matured earlier; at Camp Ripley and NW, >80% produced a surviving litter of cubs by age 4, whereas no VNP bears had cubs by age 4. Litter sizes, though, were not very different across areas; in all areas, younger bears had smaller litters. Hunting has been the primary source of mortality in all areas, although vehicle collisions are a significant source of mortality for bears wandering off Camp Ripley, which is flanked by highways.

INTRODUCTION AND STUDY AREAS

Telemetry-based research on black bears was initiated by the Minnesota Department of Natural Resources (MNDNR) in 1981, and has been ongoing since then. Objectives shifted over the years, and study areas were added to encompass the range of habitats and food productivity across the bear range. For the first 10 years, the bear study was limited to the Chippewa National Forest (CNF), near the geographic center of the Minnesota bear range (Figure 1). The CNF is one of the most heavily hunted areas of the state, with large, easily-accessible tracts of public (national, state, and county) forests dominated by aspen (*Populus tremuloides, P. grandidentata)* of varying ages. Camp Ripley Training Center, a National Guard facility at the southern periphery of the bear range, was added as a second study site in 1991. Camp Ripley is unhunted, but bears may be killed by hunters when they range outside Camp, which they often do in the fall. Oaks (*Quercus* sp.) are plentiful within Camp, and cornfields border the site. Voyageurs National Park (VNP), at the northern edge of the Minnesota range (but bordering bear range in Canada) was added as a third study site in 1997. Soils are shallow and rocky in this area, and foods are generally less plentiful than in the other sites. Being a national park, it is unhunted, but like Camp Ripley, bears may be hunted when they range outside VNP.

In 2007, we initiated work in a fourth study site at the northwestern edge of the Minnesota bear range (henceforth NW; Figure 1). This area differs from the other 3 areas in a number of respects: (1) it is largely agricultural (including crop fields, like corn and sunflowers that bears consume), (2) most of the land, including various small woodlots, is privately owned, with some larger blocks of forest contained within MNDNR Wildlife Management Areas (WMAs) and a National Wildlife Refuge (NWR); (3) the bear range in this area appears to be expanding and bear numbers have been increasing, whereas, until recently, most other parts of the bear range have had stable or declining bear numbers; and (4) hunting pressure in this area is unregulated (it is within the no-quota zone, so there is no restriction on numbers of hunting licenses).

OBJECTIVES

1. Quantify temporal and spatial variation in cub production and survival;
2. Compare sources of bear mortality in different parts of the bear range.

METHODS

We previously attached radiocollars with breakaway and/or expandable devices to bears either when they were captured during the summer or when they were handled as yearlings in the den with their radiocollared mother. We used aerial telemetry to locate den sites.

During December–March, we visited all radio-instrumented bears once or twice at their den site. We immobilized bears in dens with an intramuscular injection of Telazol, administered with a jab stick or dart gun. Bears were then removed from the den for processing. We measured and weighed them, assessed body condition, and took blood and hair samples. We changed or refit the collar, as necessary. We used VHF collars in CNF and VNP, mainly VHF collars at Camp Ripley, and GPS-Iridium collars (Vectronic Aerospace GmbH, Berlin, Germany and Telonics Inc., Mesa, AZ) in the NW study site and 2 bears at Camp Ripley. All collared bears had brightly-colored, cattle-size ear tags (7x6 cm; Dalton Ltd., UK) that would be plainly visible to hunters.

We assessed reproduction by observing cubs in March dens. We sexed and weighed cubs without drugging them. We evaluated cub mortality by examining dens of radiocollared mothers the following year; cubs that were not present as yearlings with their mother were presumed to have died.

We monitored heart rates of a subset of bears using a new Insertable Cardiac Monitor developed for human heart patients (Reveal LINQ™, Medtronic Inc., Minneapolis, MN). The device is small enough (4.0 x 7.2 x 44.8 mm; 2.4 grams) to be injected subcutaneously in a left peristernal location. Surgical sutures were used to close the puncture site. The device provided wireless transmission of heart and activity data to an antenna buried under the nest material in the den, which was then relayed by cell phone to a base station. These data are not presented in this report, but were reported by Laske et al. (2014). Besides providing physiological information, the heart rate and activity data also yielded birth dates of cubs, signaled by significantly increased heart rates and activity (just prior to birth), followed by a rapid decline to lower than pre-birth levels, which was maintained the rest of the denning period.

We set some trail cameras (Reconyx, Inc., Holmen, WI) outside bear dens to gain information about dates and behaviors of bears exiting dens (e.g., Do bears just come out and leave, or is it an extended process involving several days, especially when it involves new cubs? Do mothers ever leave cubs unattended and possibly exposed to predation risks during the den emergence period?).

We periodically monitored survival of bears during the summer. Mortalities also were reported to us when bears were shot as a nuisance, hit by a car, or killed by a hunter. Prior to the hunting season (1 September–mid-October), hunters were mailed a letter requesting that they not shoot collared bears with large ear tags, and this request was also made through news releases.

RESULTS AND DISCUSSION

Radiocollaring and Monitoring

As of April 2014, the start of the current year's work, we were monitoring 24 radiocollared bears: 4 in the CNF, 8 at Camp Ripley, 1 in VNP, and 11 in the NW (Table 1). We did not trap any new bears this year, but we collared 2 female yearlings in a den in VNP and 1 in the NW. Since 1981 we have handled >800 individual bears and radiocollared >500, 364 of which were followed until they died (Table 2).

Reproduction

Eight collared females produced cubs in 2015, including the remaining 6 collared females

in Camp Ripley. One Camp Ripley female that was due to have cubs (based on 2-year reproductive cycle) was not checked in the den because her collar prematurely broke away, but later camera trap photos showed her without cubs.

Since 1982, within the 4 study areas, we have checked 282 litters with 723 cubs ($\bar{x}$ = 2.6 cubs/litter), of which 51.6% were male (Tables 3–6). Mortality of cubs during their first year of life averaged 21% (annual range 0–31% for years with at least 10 cubs monitored), with mortality of male cubs (25%) exceeding that of females (17%; χ^2 = 5.35, P = 0.02). The timing and causes of cub mortality are unknown.

Reproductive rates (cubs/female 4+ years old: combining litter size, litter frequency, and age of first reproduction into a single parameter) were highest in the NW study area, and lowest in VNP (Figure 2). This is somewhat ironic in terms of Minnesota's bear management, given that the NW study site is outside "core" bear range and, accordingly, is within a management zone where bear hunting license sales are unrestricted (no-quota). The NW site contains not only agricultural crops consumed by bears, but also an abundance of natural foods, especially along the edges of woodlots (Ditmer et al. 2015). Reproductive rates were higher for ≥7-year-old bears than 4- to 6-year-olds because many bears in this younger age group either had not yet reproduced or just had their first litter, which tended to be smaller. Litter sizes differed more between young bears and older bears within each area than for a given age group among areas (Figure 3).

Conversely, age of first reproduction was dramatically different among areas. By 4 years of age, >80% of bears at Camp Ripley and in the NW had produced surviving cubs (observed in the den at 1 year; Figure 4). Only 36% of bears on the CNF produced surviving cubs by 4 years old and no bears at VNP produced cubs by 4 years of age. Within the CNF, where we have 35 years of reproductive data, we noticed no consistent trends through time in the percent of bears producing cubs (whether litters survived or not) by age 4 (Figure 5). However, reproduction appeared to be highest during the first 5 years of the study (1981–1985), when nearly 60% of bears had their first litter by age 4; during every time period since then, ≤50% of females produced by age 4 (Figure 4).

Mortality

Legal hunting has been the predominant cause of mortality among radiocollared bears from all study sites (Table 2). Vehicle collisions are a significant source of mortality at Camp Ripley, which is flanked by 2 highways.

Despite our request not to shoot collared bears (with large eartags), 3 of 24 (13%) collared bears were shot by licensed hunters during September 2014. Two of these bears were the last collared adult females on the CNF; together they had produced 38 cubs since 2002.

Camera Trap Photos at Dens

We obtained camera-trap photos of bears emerging from 3 dens in NW and 2 at Camp Ripley during spring 2015. Three adult female bears in the NW first emerged during 13–18 March, and stayed at the den site for 6–18 days (Table 7). A yearling emerged from the den on the same date as its mother, whereas cubs (approximately 2 months old) emerged 1–5 days after their mother. The bears came in and out of the den several times before departing the den site. Two mothers with cubs spent considerable time watching the cubs, as they played and learned climbing skills (Figure 6). They physically pushed cubs with their paws or noses, and carried them in their mouth to keep them close to the den.

All 3 mothers periodically raked more bedding material into the den (Figure 7). One mother licked newly fallen snow (Figure 6).

At one den site, people (related to the landowner) came to take photos. The mother bear bolted from the den at their approach, and they photographed the cubs (Figure 8). This was the only time in which a mother left her cubs unattended. The mother returned 3 hours after the people left, sniffed about the area for 4 hours, then took her cubs and vacated the site at just after midnight. She may have left the site in darkness due to the threat of the people. After the bear family departed this site, a bobcat (*Lynx rufus*) visited and peered in the den (Figure 8). Bobcats

are known predators of cubs (LeCount 1987), pointing out the potential dangers associated with humans visiting dens and scaring away the mother, even for a short time.

At Camp Ripley, we obtained camera trap photos of a pack of wolves (*Canis lupus*) surrounding a den of a mother and cubs. The mother had previously been out of the den but was in the den when the wolves arrived. One bold young wolf looked into the den, but then quickly backed away (Figure 9).

We presently have no information on causes of cub mortality in Minnesota during the first year of life, but we plan to use cameras more in the future to further investigate threats, disturbances, and behaviors during the late denning period.

We retrieved cameras in the NW in August, so we witnessed a number of animals that visited the den site after the bears had left. Those that specifically investigated the den included multiple deer (*Odocoileus virginianus*), a groundhog (*Marmota monax*; which used and reconfigured a den), a ruffed grouse (*Bonasa umbellus*; which dusted itself for nearly an hour in the excavated dirt pile in front of the den), and 2 unmarked black bears.

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Table 1. Fates of radiocollared black bears in 4 study sites (Chippewa National Forest, Camp Ripley, Voyageurs National Park, and northwestern Minnesota), April 2014−March 2015.

	CNF	Camp Ripley	VNP	NW
Collared sample April 2014	4	8	1	11
Killed as nuisance				
Killed in vehicle collision				
Killed by Minnesota hunter	2	1		
Natural mortality				
Dropped collar		1		2
Failed radiocollar				2[b]
Lost contact[a]				1
Collared in den			2	1
Collared sample April 2015	**2**	**6**	**3**	**7**

[a] Due to radiocollar failure, unreported kill, or long-distance movement.
[b] Presumed collar failure, but may have been killed and collar destroyed.

Table 2. Causes of mortality of radiocollared black bears ≥1 year old in 4 Minnesota study sites, 1981–2015. Bears did not necessarily die in the area where they usually lived (e.g., hunting was not permitted within Camp Ripley or VNP, but bears were killed by hunters when they traveled outside these areas).

	CNF	Camp Ripley	VNP	NW	All combined
Shot by hunter	225	12	15	12	261
Likely shot by hunter[a]	8	1	0	4	13
Shot as nuisance	22	2	1	3	28
Vehicle collision	12	8	1	3	24
Other human-caused death	9	1	0	0	10
Natural mortality	8[b]	3	5	0	15
Died from unknown causes	4	2	0	3	9
Total deaths	**288**	**29**	**22**	**25**	**364**

[a] Lost track of during the bear hunting season, or collar seemingly removed by a hunter.
[b] Only 1 bear died of “old age”.

Table 3. Black bear cubs examined in dens of radiocollared mothers in or near the Chippewa National Forest during March, 1982–2015. High hunting mortality of radiocollared bears severely reduced the sample size in recent years.

Year	Litters checked	Number of cubs	Mean cubs/litter	% Male cubs	Mortality after 1 year[a]
1982	4	12	3.0	67%	25%
1983	7	17	2.4	65%	15%
1984	6	16	2.7	80%	0%
1985	9	22	2.4	38%	31%
1986	11	27	2.5	48%	17%
1987	5	15	3.0	40%	8%
1988	15	37	2.5	65%	10%
1989	9	22	2.4	59%	0%
1990	10	23	2.3	52%	20%
1991	8	20	2.5	45%	25%
1992	10	25	2.5	48%	25%
1993	9	23	2.6	57%	19%
1994	7	17	2.4	41%	29%
1995	13	38	2.9	47%	14%
1996	5	12	2.4	25%	25%
1997	9	27	3.0	48%	23%
1998	2	6	3.0	67%	0%
1999	7	15	2.1	47%	9%
2000	2	6	3.0	50%	17%
2001	5	17	3.4	76%	15%
2002	0	0	—	—	—
2003	4	9	2.3	22%	0%
2004	5	13	2.6	46%	33%
2005	6	18	3.0	33%	28%
2006	2	6	3.0	83%	33%
2007	2	6	3.0	67%	17%
2008	1	3	3.0	100%	33%
2009	1	3	3.0	33%	33%
2010	1	4	4.0	100%	50%
2011	1	4	4.0	25%	50%
2012	1	3	3.0	67%	33%
2013	1	3	3.0	67%	0%
2014	1	3	3.0	67%	—[b]
2015	0	0	—	—	—
Overall	**179**	**472**	**2.6**	**53%**	**19%**

[a] Cubs that were absent from their mother's den as yearlings were considered dead.
[b] Mother was killed by a hunter so status of cubs unknown.

Table 4. Black bear cubs examined in dens in northwestern Minnesota during March, 2007–2015.

Year	Litters checked	Number of cubs	Mean cubs/litter	% Male cubs	Mortality after 1 year
2007	2	6	3.0	33%	100%
2008	5	15	3.0	67%	22%
2009	1	3	3.0	33%	33%
2010	6	17	2.8	41%	13%
2011	2	4	2.0	75%	25%
2012	4	10	2.5	60%	10%
2013	3	9	3.0	67%	18%
2014	3	8	2.7	0%	33%
2015	2	5	2.5	60%	
Overall	**26**	**72**	**2.8**	**49%**	**27%**[a]

[a] Excludes the total loss of a 5-cub litter in 2007 (which was not within the designated study area).

Table 5. Black bear cubs examined in dens in or near Camp Ripley Training Center during March, 1992–2015.

Year	Litters checked	Number of cubs	Mean cubs/litter	% Male cubs	Mortality after 1 year[a]
1992	1	3	3.0	67%	0%
1993	3	7	2.3	57%	43%
1994	1	1	1.0	100%	—
1995	1	2	2.0	50%	0%
1996	0	0	—	—	—
1997	1	3	3.0	100%	33%
1998	0	0	—	—	—
1999	2	5	2.5	60%	20%
2000	1	2	2.0	0%	0%
2001	1	3	3.0	0%	33%
2002	0	0	—	—	—
2003	3	8	2.7	63%	33%
2004	1	2	2.0	50%	—
2005	3	6	2.0	33%	33%
2006	2	5	2.5	60%	—
2007	3	7	2.3	43%	0%
2008	2	5	2.5	60%	0%
2009	3	7	2.3	29%	29%
2010	2	4	2.0	75%	25%
2011	3	8	2.7	50%	25%
2012	1	2	2.0	100%	0%
2013	6	14	2.3	50%	21%
2014	1[b]	—[b]	—	—	—
2015	6	15			
Overall	**46**	**109**	**2.4**	**52%**	**21%**

[a] Blanks indicate no cubs were born to collared females or collared mothers with cubs died before the subsequent den visit to assess cub survival.
[b] Cubs heard, litter not handled. Camera set outside den indicated that all cubs died. This litter not included in total.

Table 6. Black bear cubs examined in dens in Voyageurs National Park during March, 1999–2015. All adult collared females were killed by hunters in fall 2007, so no reproductive data were obtained during 2008–2009.

Year	Litters checked	Number of cubs	Mean cubs/litter	% Male cubs	Mortality after 1 year[a]
1999	5	8	1.6	63%	20%
2000	2	5	2.5	60%	80%
2001	3	4	1.3	50%	75%
2002	0		—	—	—
2003	5	13	2.6	54%	8%
2004	0		—	—	—
2005	5	13	2.6	46%	20%
2006	1	2	2.0	50%	0%
2007	3	9	3.0	44%	—
2008	0		—		—
2009	0		—		—
2010	1	2	2.0	50%	0%
2011	1	2	2.0	0%	0%
2012	1	2	2.0	0%	50%
2013	1	2	2.0	50%	—
2014	1	3	3.0	33%	
2015	0	0			
Overall	**29**	**65**	**2.2**	**48%**	**25%**

[a] Blanks indicate no cub mortality data because no cubs were born to collared females, or collared mothers were lost from study (died or lost collar) before denning with yearlings.

Table 7. Timing of den emergence and departure from dens recorded by camera traps at 3 dens of adult female bears in NW Minnesota, 2015.

	Bear		
Parameter	4011	4067	4064
Offspring	1 yearling	2 large cubs	3 small cubs
Date of first emergence	18 March	13 March	14 March
Date of first offspring emergence	18 March	18 March	15 March
Date of departure from den site	31 March	19 March[a]	1 April
Days between emergence and departure from site	13	6[a]	18
Mean number of times outside the den per day from first emergence to departure	0.5	1.4	3.1
Mean duration (minutes) outside den during each emergence	86	48	21

[a] Mother departed with her cubs 7 hours after people visited the den and looked in (Figure 8).

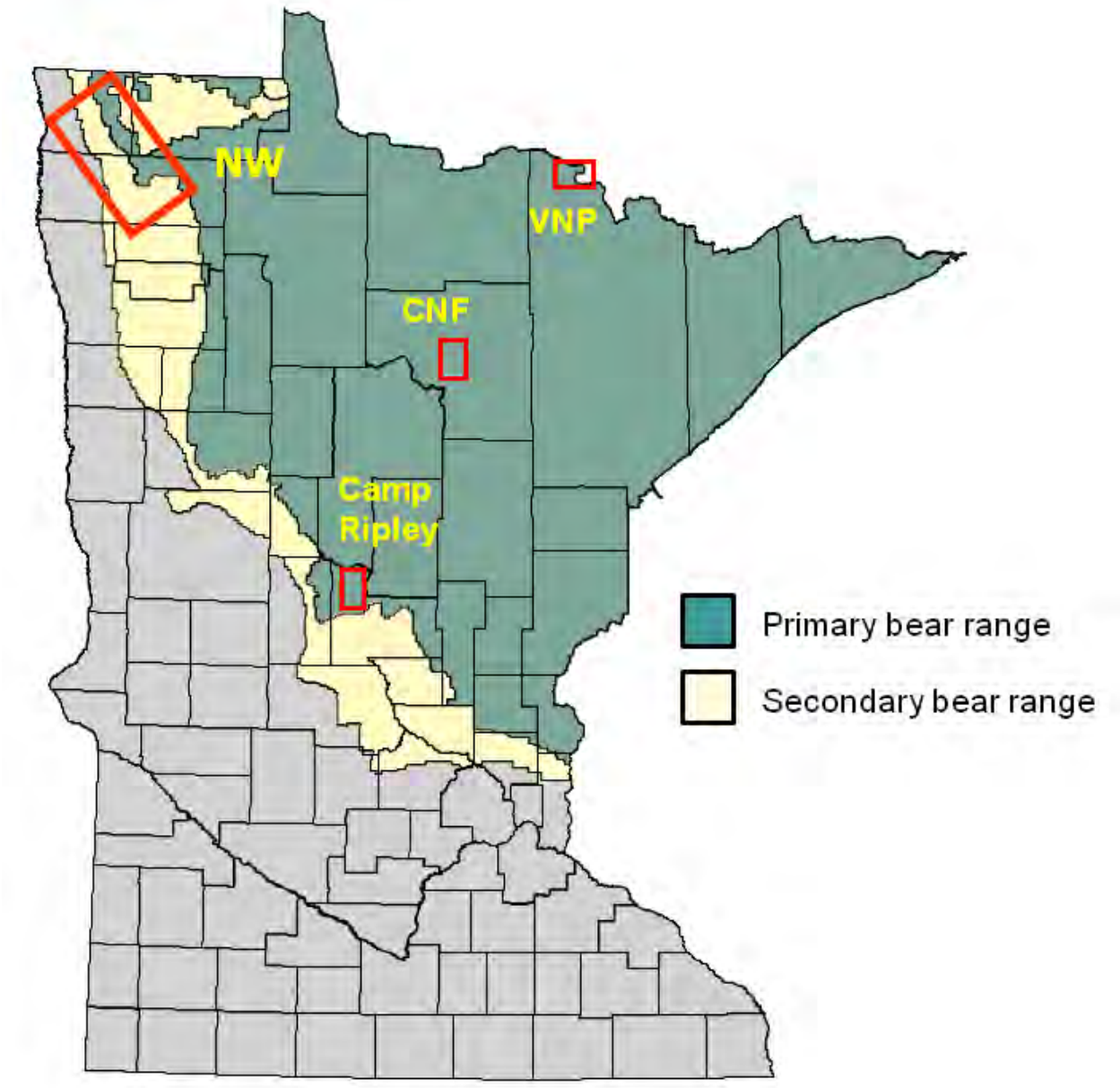

Figure 1. Location of 4 study sites within Minnesota's bear range: CNF (Chippewa National Forest, central bear range; 1981–2015); VNP (Voyageurs National Park, northern fringe of range; 1997–2015); Camp Ripley Military Reserve (near southern edge of range; 1991–2015); NW (northwestern fringe of range; 2007–2015).

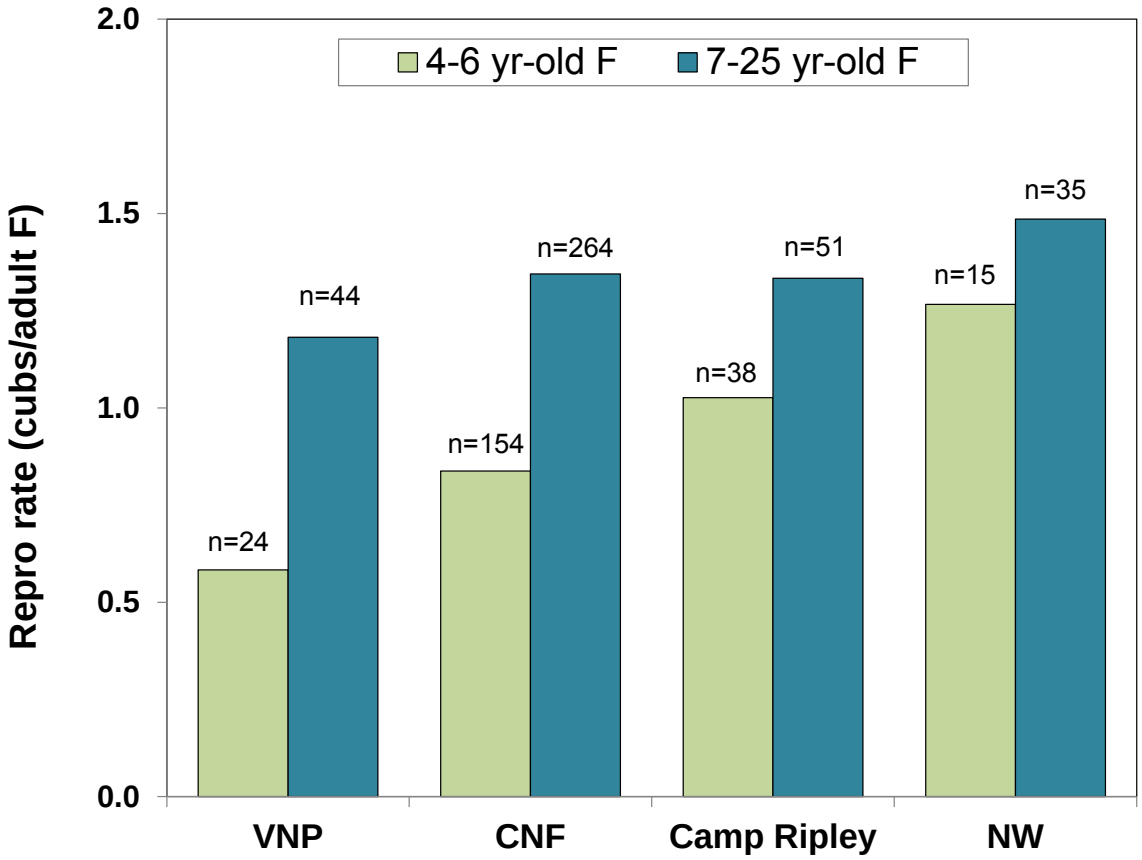

Figure 2. Reproductive rates of radiocollared bears within 4 study sites (see Figure 1) through March 2015. Data include only litters that survived 1 year (even if some cubs in the litter died). Sample sizes refer to the number of female bear-years of monitoring in each area for each age group. Some bears in CNF, Camp Ripley, and NW produced cubs at 3 years old, but are not included here.

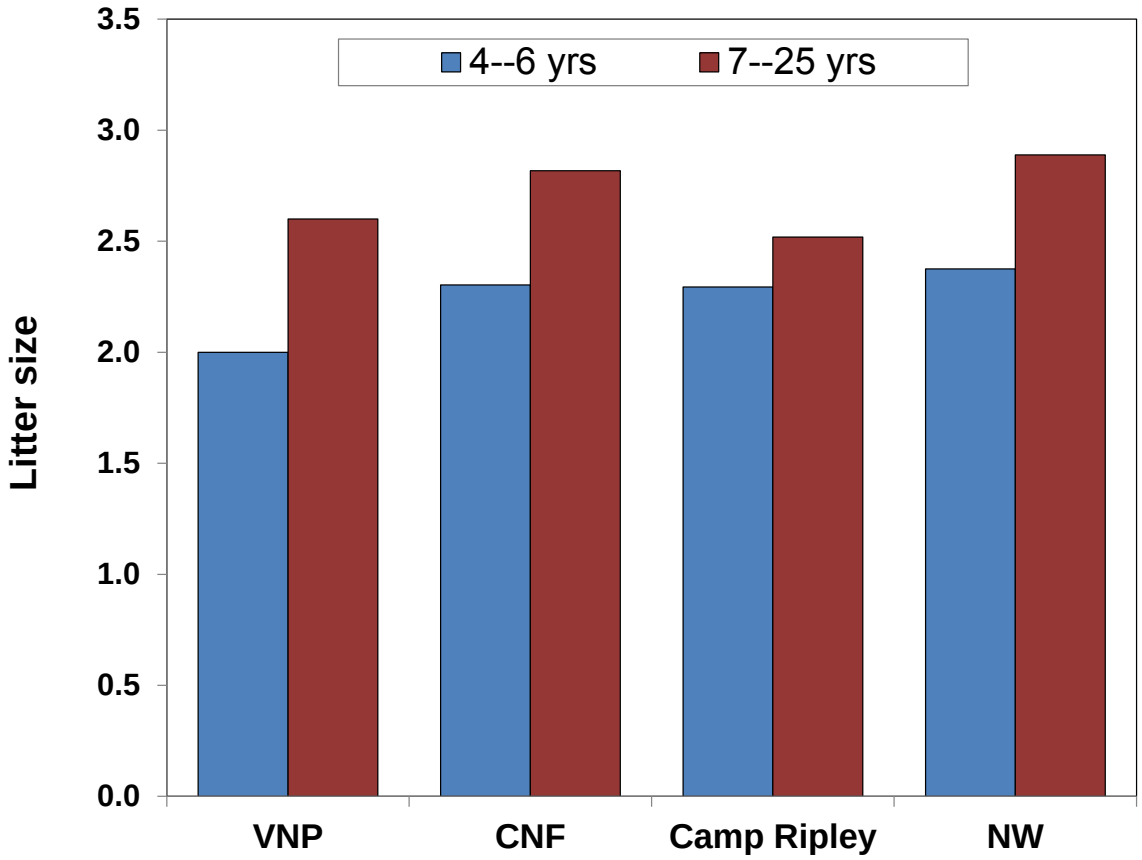

Figure 3. Mean cub litter sizes (examined in natal dens in March) of young (mainly first litters) versus older radiocollared bears within 4 study sites (see Figure 1) through March 2015. Data include only litters that survived 1 year (even if some cubs in the litter died). Sample sizes are as in Figure 2.

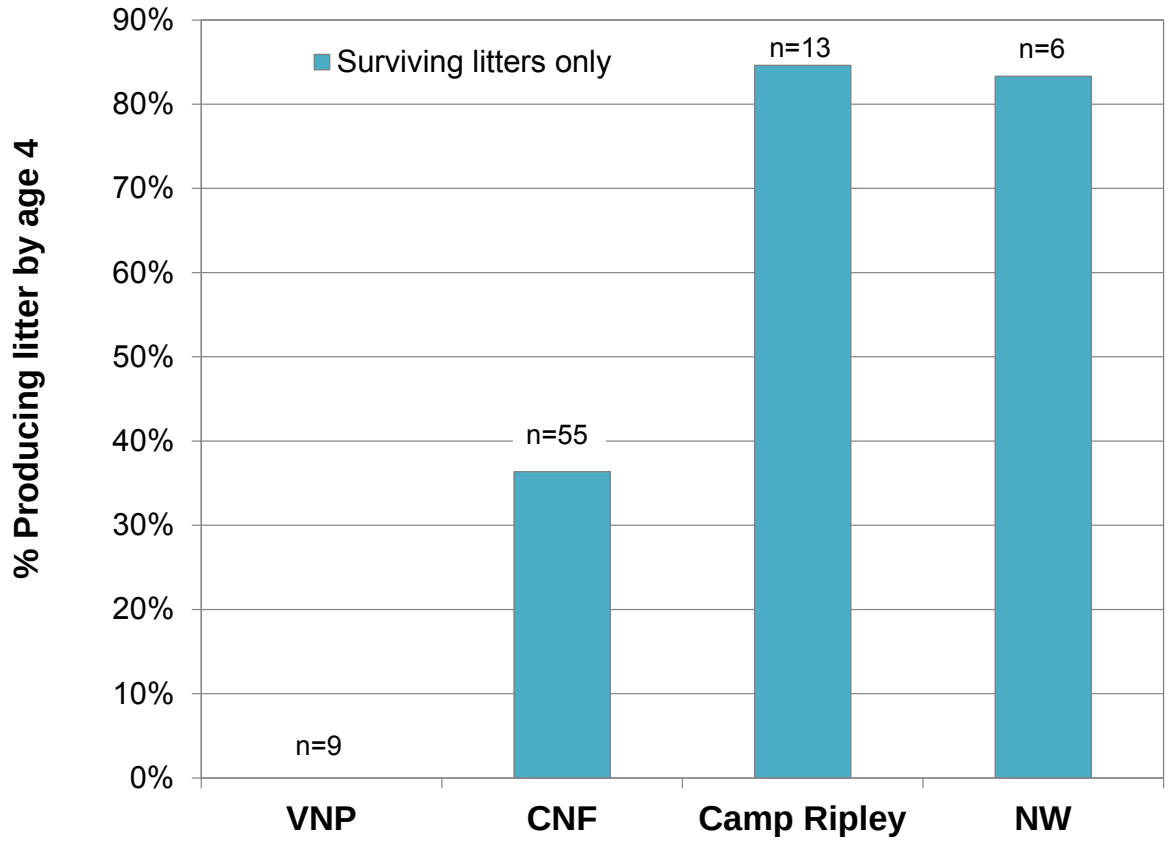

Figure 4. Percent of radiocollared females on each study site that produced a surviving litter of cubs by 4 years old. Births of cubs were detected in natal dens in March each year (through March 2015). A surviving litter was one in which at least one yearling was present in the mother's den the next winter. Note that no females in VNP produced cubs by 4 years of age.

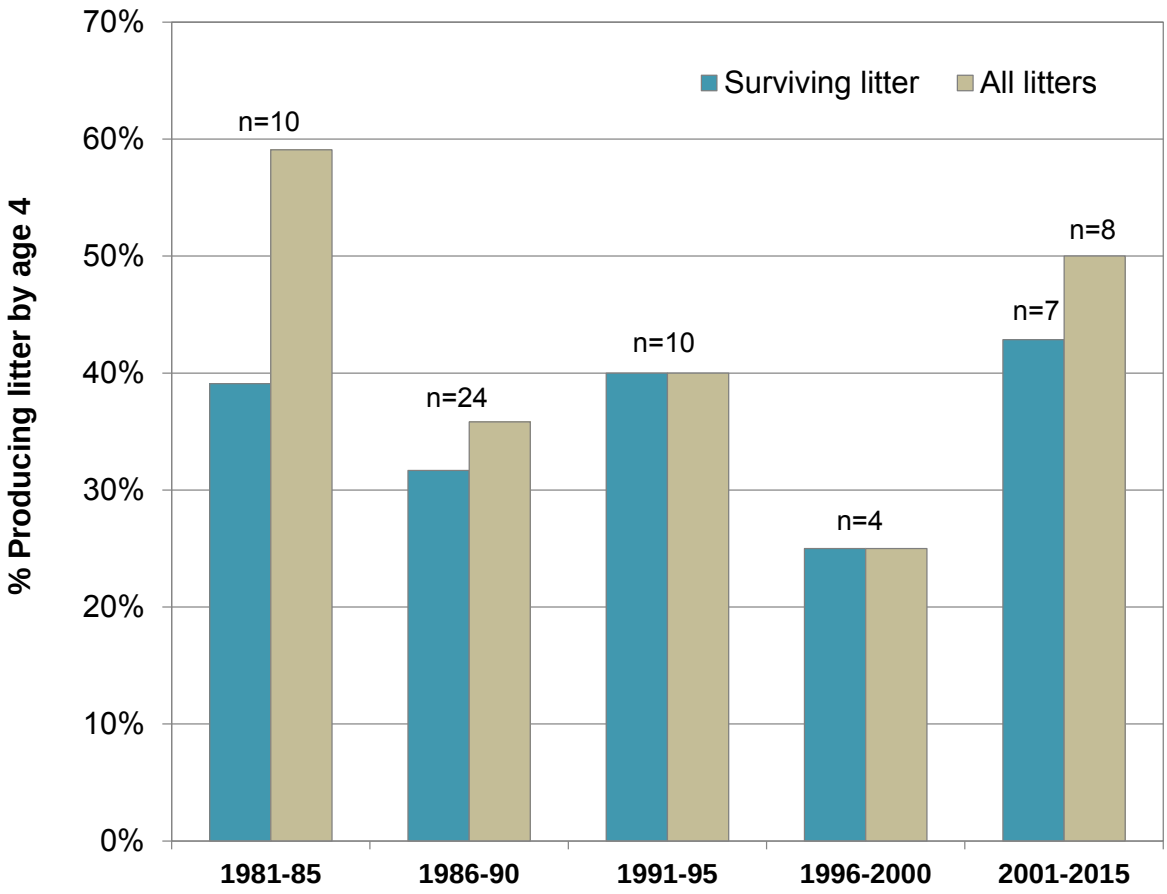

Figure 5. Percent of radiocollared females in the CNF that produced a litter of cubs by 4 years old, in different time increments (1981-2015). Births of cubs were detected in natal dens in March each year. A surviving litter was one in which at least one yearling was present in the mother's den the next winter. Sample sizes refer to the number of females that were monitored at 3 (minimum age of first reproduction) and 4 years old (s

Figure 6. Camera traps revealed the dates that bears first emerged from dens, and activities of bears at the den site during the time between den emergence and departure from the site. Mothers spent quite a bit of time watching and physically moving their cubs. After a late snowstorm one mother came out of the den to lick the snow (bottom right).

Figure 7. Camera traps at 3 dens in NW showed that each bear periodically came out in late March to pull in more bedding material.

Figure 8. Camera traps recorded visitors to dens, including people who scared the mother away (top) and photographed the cubs (the mother returned after the people left), and a bobcat, which checked out the den after the mother and cubs departed. Bobcats are a known predator of bear cubs.

Figure 9. Sequence of camera trap photos from Camp Ripley showing a radiocollared bear at the entrance of its den on March 19, 2015 and a wolf pack surrounding the den 2 days later. One bold young wolf peered in the den but was apparently scared off by the bear.

REPRODUCTIVE ECOLOGY OF FISHERS AND AMERICAN MARTENS IN MINNESOTA

John Erb, Pam Coy, and Barry Sampson

SUMMARY OF FINDINGS

As part of a larger project on *Martes* ecology in Minnesota, we began monitoring reproductive success of radiocollared fishers (*Pekania pennanti*) and martens (*Martes americana*) during spring 2009. Including the pilot year of the study, a total of 242 martens [115 females (F), 127 males (M)] and 114 fishers (65 F, 49 M) have been radiocollared. To date, age and reproductive status have been confirmed on 45 adult (≥2 years old) female martens. Pooling years, pregnancy rate has been 56% for 2-year-old martens, and 79% for martens 3 years or older. Average size of 27 marten litters is 2.96 (range = 1–4), with minimal difference between litter size of 2-year-old versus ≥3-year-old females. Based on initial data, it appears marten kits are typically born in late-April through early-May. A total of 57 marten natal or maternal dens have been located, of which 61% have been in tree [primarily aspen (*Populus tremuloides*) and cedar (*Thuja occidentalis*)] cavities, 33% in underground burrows, and 5% in hollow logs on the ground. We have also confirmed both age and reproductive status for 58 female fishers. Pooling years, pregnancy rate has been 65% for 2-year-old fishers, and 94% for fishers 3 years or older. Average size of 45 fisher litters is 2.5 (range = 1–4). Data suggests that litter size for 2-year-old fishers is lower than for older females (2.15 versus 2.7). Based on data collected to date, it appears fisher kits are typically born in early- to mid-March in the southern and central part of the Minnesota fisher range and in late-March to mid-April near the northern boundary. A total of 77 fisher natal or maternal dens have been confirmed, all but 3 being in elevated tree cavities. Cavities have been located in both live trees (73%) and snags (27%) with an overall average DBH of 20.0 in. Tree cavities used by female fishers have been located primarily in aspen (67.5%; *Populus tremuloides, Populus grandidentata)* and oak (12%, *Quercus* spp.) trees. Most female fishers appear to move kits from their natal den to at least 2 different maternal dens prior to June 1.

INTRODUCTION

American marten and fisher are native to Minnesota, but reliable documentation of their historic distribution is limited. Undoubtedly, northeastern Minnesota was a stronghold for the marten population, though notable numbers likely occurred in the northern border areas as far west as Roseau County. Limited information suggests they occurred as far south as Crow Wing County and as far southwest as Polk County. As a result of unregulated harvest, marten were considered rare in Minnesota by 1900, and extensive logging and burning around the turn of the century further contributed to the near extirpation of marten from Minnesota by the 1930s (Swanson et al. 1945). Fishers in Minnesota appear to have historically occupied a larger geographic area than martens, extending further south and west into the hardwood dominated transition zone, including southeast Minnesota (Swanson et al. 1945, Balser and Longley 1966). The impacts of unregulated harvest and habitat alteration were equally as detrimental to fisher, with populations substantially reduced by the 1930s.

Legally, fisher and marten were unprotected in Minnesota prior to 1917, after which harvest season length restrictions were implemented. These protections were removed in the mid-1920s, and remained so until all harvest was prohibited in 1929. Seasons remained closed until 1977 for fisher and 1985 for marten, when limited harvests were reinstated. While marten harvest is now legal in approximately the northern 50% of the state, most harvest occurs in counties bordering Canada, particularly in northeast and north-central Minnesota. Fisher harvest occurs in most of the northern 50% of the state, though harvest is comparatively low in extreme northeast Minnesota (Lake and Cook counties). Over the past 10 years, fisher abundance and harvest have been increasing along the southern and western edge of the 'forest zone' where forest historically transitioned to savanna and prairie and is now characterized by linear forest corridors (e.g., streams, rivers) or smaller forest patches interspersed with agriculture. Conversely, fisher abundance appears to have declined significantly over the same period in the core forested areas of north-central and northeast Minnesota. Peak statewide harvest levels have been near 4,000 and 3,500 for marten and fisher, respectively. However, due to apparent multi-year population declines for both species, harvest seasons from 2007 to the present have become progressively more conservative, with recent harvest seasons lasting only 6 days with a combined fisher/marten limit of 2 per trapper.

While both species appear to have naturally re-colonized a significant portion of their historic range, Minnesota-specific information on reproductive ecology is limited to carcass data (i.e., corpora lutea or placental scar counts) collected from harvested animals primarily from 1985 to 1990 (Kuehn 1989, Minnesota DNR unpublished data). Reproductive data is also available from other geographic areas, but questions remain on the accuracy of various methods to assess reproduction, and the amount of spatial and temporal variation in reproductive parameters. Minnesota-specific data on structures and sites used by fisher for natal and maternal dens is also lacking.

Martes pregnancy rate and litter size data are generally quantified from 1 of 4 methods: counts of corpora lutea (CL) in ovaries; counts of blastocysts (BC) in uteri; placental scar (PS) counts; or direct observation of litter size (Gilbert 1987, Mead 1994). Assuming both species are induced ovulators (but see Cherepak and Connor 1992, Frost et al. 1997), CL counts should accurately reflect copulation and ovulation rates, but all CL persist even if only 1 ovum is fertilized. Blastocyst counts reflect the number of fertilized ova, but not all BC may implant in the uterus and develop, and BC are often destroyed in poorly preserved carcasses. Hence, these 2 measures may not only overestimate litter size for parous females, but may also overestimate parturition rate (i.e., females may ovulate, 1 or more ova become fertilized, yet they fail to ultimately den and give birth). Placental scars, formed last in the reproductive process, would seem the most reliable carcass-based estimate of parturition rate and litter size. However, several authors (Gilbert 1987, Payne 1982, Strickland and Douglas 1987) have suggested that PS may not always persist long enough in mustelids to be detected during the harvest season when carcasses are easily collected. Furthermore, PS can persist in some species even if fetuses are resorbed (Conaway 1955) and detection and counts of PS may be affected by observer variability (Johnson et al. 1995). Nevertheless, PS have been reliably used in the past (e.g., Coulter 1966, Crowley et al. 1990), though others have noted that reliable results may only be obtainable when doing microscopic analysis of fresh and properly preserved and prepared uteri (Mead 1994, Frost et al. 1999).

In spite of these concerns, average litter size estimates from reproductive organs do not appear to be substantially biased. Strickland and Douglas (1987), summarizing data from 136 captive marten litters, computed average litter size of 2.9 for martens. This is within the range of average litter sizes reported from ovary or uterine analysis (2.5–3.5; Strickland et al. 1982, Strickland and Douglas 1987, Flynn and Schumacher 1995, 2009, Aune and Schladweiler 1997, MN DNR unpublished data). For fishers, the same appears to be true, with an average litter size of 2.8 from 60 captive fisher litters (reviewed in Strickland and Douglas 1987) and 19 wild litters

(York 1996), which compares favorably to estimates based on reproductive organs (2.7–3.9 (CL), 2.7–3.2 (BC), and 2.5–2.9 (PC); review in Powell 1993).

Of greater concern is the possibility that ovary, and to lesser degree uterine, analyses might consistently overestimate parturition rate, thereby also underestimating annual variability in parturition rates. Various indications of pregnancy may be detected, though not all of those females may den and produce kits in spring. For example, this might occur if BC fail to implant or fetuses are resorbed as a result of nutritional stress during the period of embryonic diapause (Arthur and Krohn 1991). Overall, CL counts have generally yielded ovulation rates for fisher of ≥95% (Shea et al. 1985, Douglas and Strickland 1987, Paragi 1990, Crowley et al. 1990, MN DNR unpublished data), while more 'direct' estimates of average parturition rate from radiomarked animals have been lower (46–75%; Crowley et al. 1990; Arthur and Krohn 1991; Paragi 1990; Paragi et al. 1994, York 1996, Truex et al. 1998, Higley and Mathews 2009), and are often highly variable. Conversely, in Minnesota, Kuehn (1989) did not detect changes in fisher pregnancy rate (from CL analysis) in spite of a 64% decline in a presumably important prey species (snowshoe hare; *Lepus americanus*).

For martens, several largely ovarian-based estimates of annual pregnancy rate have often been in the range of 80–90% (Archibald and Jessup 1984, Strickland and Douglas 1987, Aune and Schladweiler 1997, Flynn and Schumacher 1994, Fortin and Cantin 2004, MN DNR unpublished data). However, like for fishers, several marten studies have documented (also based largely on CL counts) lower or more variable pregnancy rates (Thompson and Colgan 1987, Aune and Schladweiler 1997, Strickland and Douglas 1987, Flynn and Schumacher 2009), perhaps a result of fluctuations in prey abundance (Hawley and Newby 1957, Weckwerth and Hawley 1962, Strickland 1981, Strickland and Douglas 1987, Thompson and Colgan 1987, Fryxell et al. 1999, Flynn and Schumacher 2009). We are aware of direct field-based estimates of parturition rate from radiomarked marten in only one state (Maine). Pooling samples across 4 years, the proportion of lactating adult females was 75, 81, and 92% for their 3 different study areas (Phillips 1994, Payer 1999), similar to many of the CL-based pregnancy studies.

Understanding reproductive ecology of these species also necessitates gathering information on natal and maternal den structures and selection of den sites. Natal dens are the structures where kits are born, whereas maternal dens are sites used subsequently by the female with her dependent young. Although data is absent for Minnesota, nearly all reported fisher natal dens have been in cavities of large-diameter trees or snags (Leonard 1986, Paragi et al. 1996, Powell et al. 1997, Truex et al. 1998). In northern studies, the majority of fisher natal dens have been in large diameter aspens (*Populus* spp.), and females may use 3 or more different maternal dens (Powell et al. 2003, Higley and Mathews 2009). Marten natal and maternal dens are also frequently in tree cavities (Gilbert et al. 1997), but may occur in more varied features (e.g., underground burrows, exposed root masses of trees, rock piles, large downed logs; Ruggiero et al. 1998).

Though not further discussed here, the literature is also voluminous with documentation of the importance of tree cavities, large downed logs, and other forest 'structure' for fisher and marten resting sites (see Powell et al. 2003 for a review). Initial results from this study (Joyce 2013) appear consistent with other published findings on the importance of forest structure for marten den and rest sites. Given the continuing pressure to maximize fiber production from forests (i.e., short forest rotation, biomass harvesting, etc.), the forest structural attributes critical to fishers and martens could become limiting in the future, if not already. Hence, acquiring Minnesota-specific information is critical to better inform forest management activities.

OBJECTIVES

As part of a larger project on *Martes* ecology (Erb et al. 2009), we began efforts to better describe the reproductive ecology of fisher and marten in Minnesota. Specific objectives are to:

1. Document denning chronology;
2. Determine structures used for natal and maternal dens;

3. Quantify vegetative characteristics in the area surrounding natal and maternal dens;
4. Develop a resource selection model specific to the denning season;
5. Derive field-based estimates of pregnancy rate and litter size;
6. Evaluate kit survival; and
7. Assess the potential influence of age, diet, prey fluctuations, forest attributes, and winter severity on reproductive success.

After initial evaluation of field methods during the pilot year of the study, spring 2009 marked the beginning of full-scale research activities. We defer a more complete evaluation of results until additional data are collected or additional analysis is completed. Herein we present basic information on field methods and only report preliminary findings related to denning chronology, dens structures, and pregnancy rates and litter sizes. For initial analysis related to den and rest site selection for martens, we refer the reader to Joyce (2013).

STUDY AREA

Marten research is focused on 1 study area located in northeastern Minnesota (Figure 1, Area 1), although 2 male marten were captured and radiocollared in Area 2 (Figure 1). Area 1 (approximately 700 km^2) is nearly 90% public ownership, including portions of the Superior National Forest and state and county lands. Fishers are also present in this area at low to moderate density.

Fisher research has taken place in 3 areas (Figure 1; Areas 1, 2, and 3). The work in Area 3 is a collaborative effort between Camp Ripley Military Reservation, Central Lakes Community College, and the Minnesota Department of Natural Resources. Although we include animals captured in that area in our basic summaries, we do not discuss other aspects of that project in this report. Area 2 (1075 km^2), our primary fisher study area, is approximately 67% public ownership, including portions of the Chippewa National Forest and state and county lands. Extremely few martens occupy Area 2.

METHODS

We used Tomahawk (Tomahawk Live Trap, Hazelhurst, WI) cage traps to capture both fishers (Model 108) and martens (Models 106 and 108) during winter. Traps were typically baited with deer (*Odocoileus virginianus*) or beaver (*Castor canadensis*) meat, and we placed commercial lure in or above the traps. We enclosed traps inside white plastic 'feed sacks' or burlap bags and further covered traps with natural vegetation. All traps were checked daily.

To immobilize animals, we used metal 'combs' to restrict the animal to a small portion of the trap, or restrained the animal against the side of the trap by pulling its tail through the cage mesh. Animals were injected with a hand-syringe using a 10:1 mixture of ketamine and xylazine (fisher: 30 mg/kg ketamine and 3 mg/kg xylazine; marten: 20 mg/kg ketamine, 2 mg/kg xylazine) (Kreeger et al. 2002). After processing, the xylazine was reversed with yohimbine at a dosage of 0.1 mg/kg (marten) or 0.15 mg/kg (fisher). Fisher were either ear-tagged with a monel #3 tag in one ear (National Band and Tag Co., Newport, KY) and a 2-piece plastic mini-tag (Dalton I.D. Systems, UK) in the other ear, or with a monel #3 tag in both ears. Marten were ear-tagged with a monel #1 tag (National Band and Tag Co., Newport, KY) in each ear.

During processing, we placed animals on heating pads connected to a power inverter and 12-volt battery. Portable shelters and propane heaters were also used to keep animals warm during processing. We monitored respiration, pulse, and rectal temperature during anesthesia. We weighed and sexed animals and typically removed a first pre-molar for aging. Morphological measurements taken included body length, tail length, hind foot length, and chest, neck, and head circumference. We removed guard hair samples for possible genotyping, and for evaluating the use of stable isotope analysis for deciphering food habits (Ben-David et al. 1997). To assist with determining which female fishers would likely produce kits, blood samples were drawn when

possible to measure serum progesterone levels (Frost et al. 1997). Antibiotics were administered subcutaneously to all animals prior to release as a precaution against infection (Kreeger et al. 2002) from minor wounds that may have occurred while in the trap, and because of certain invasive procedures utilized during handling (ear-tagging, removal of tooth).

During the pilot year, we deployed several radiocollar designs on fishers, including an Advanced Telemetry Systems (ATS; Isanti, MN) M1585 zip-tie collar (43 g), an ATS M1930 collar (38 g), and a Lotek Wireless Inc. (Newmarket, ON, CA) SMRC-3 collar (61 g; deployed on adult males only). Since the pilot year, we have primarily deployed ATS M1940 (43 g) or Sirtrack (Havelock North, New Zealand) TVC-162 collars (45 g) on fishers. The majority of martens have been fitted with Holohil Systems Ltd. (Carp, ON, CA) MI-2 collars (31 g). We retrofitted each collar with a temperature data logger (I-button model DS1922L; Maxim Integrated, San Jose, CA) to provide ancillary information on winter activity and spring den attendance patterns, as well as to provide information on time of death for other study objectives.

We ground-tracked collared females to locate possible den structures. When a suspected den structure was located, we deployed remotely-activated cameras (Reconyx PC-85, RC-55, HC600, or XR-6; Reconyx, Inc, Holmen, WI) to monitor female activity. We considered a female to have given birth if kits were confirmed via sound or video/camera, if the female repeatedly used the same den, or if other reliable evidence (e.g., obvious lactation, placental scars, or kit bite marks on collar) was obtained when an animal was subsequently handled as a mortality or recapture. Litter size was ascertained via visual confirmation in most cases, though we also utilized placental scar counts on any females that died during summer or fall, and for which other methods failed to produce a count. To confirm or count kits at dens located in tree cavities, we used an MVC2120-WP color video camera (Micro Video Products, Bobcaygeon, Ontario), attached to a telescoping pole if necessary, and connected to a laptop computer. Dens were only examined when the radiomarked female was not present. If video inspection equipment did not work at a particular den structure, we deployed remote cameras in an effort to obtain pictures of kits when they emerged or were moved by the female (Jones et al. 1997).

When a natal or maternal den was confirmed, we recorded den location (i.e., above, on, or below the ground) as well as various location-specific details (e.g., tree species, log or tree diameter, burrow entrance attributes, etc.). We note that since birth is never observed, and kits may be moved to new dens within days following birth, distinguishing natal dens from maternal dens can rarely be done with certainty. Hence, we pool natal and maternal dens for purposes of general summaries herein.

We are also collecting more detailed information on vegetative characteristics of the site surrounding each den structure, with a goal of developing a biologically meaningful den site selection model using methods and metrics that should be available from existing and periodically collected forest sampling data (e.g., see Zielinski et al. 2006). Following the United States Forest Service's Forest Inventory and Analysis (FIA) protocol, we quantify vegetative characteristics in a 1-acre (120-ft. radius) area surrounding the den structure by sampling in 4 circular subplots, each being 0.04-acre (24-ft. radius) in size. One subplot is centered on the den structure, with the other 3 subplots centered 120 feet from the den at 360°, 120°, and 240°. Within each subplot, 3 24-ft. coarse woody debris sampling transects are established, originating from the subplot center, and oriented at 30º, 150º, and 270º. Deviating from FIA protocol, we also establish 3 (not 1, as with FIA) 0.003-acre (6.8-ft. radius) circular micro-plots for estimating sapling density, each micro-plot situated at the end of the 3 coarse woody debris sampling transects. Details of vegetation sampling methods within each subplot will be outlined in subsequent years as results become available. Herein, we simply note that we are collecting quantitative data on (1) mean DBH and basal area of live trees, overall and by species; (2) percent overhead (angular) canopy; (3) sapling density; (4) understory cover density; (5) density and volume of snags; (6) volume of coarse woody debris; (7) number of stumps, root masses, and slash piles; (8) distance to improved road; and (9) distance to water. Canopy structure will also be categorized based on number and distribution of canopy layers. Lower-resolution LIDAR data (1 ppm) will also be

analyzed in all study areas, along with higher-resolution (8 ppm) data for a portion of the marten study area.

To better understand any observed fluctuations in reproductive parameters, we are also collecting data on factors that may influence reproductive success, including winter severity and prey fluctuations. In each study area, a temperature monitor was placed on the north-facing side of a tree in each of 6 cover types. Each sensor records temperature every 30 minutes from 1 December to 1 June. At approximately 10-day intervals from 1 December to 1 April, we also recorded snow depth and 2 measures of snow compaction at 3 locations along transects situated in each of 6 cover types. Two snow compaction tools were constructed using PVC pipe, one each with an end-cap similar in diameter to a typical marten and fisher track in the snow. Each pipe length was then adjusted to ensure the pipe-specific load (g/cm^2) was similar to marten and fisher foot-loading measures (females) reported by Krohn et al. (2004). Depth of snow compaction was recorded by dropping each load tool from 1 in. above snow level and measuring compaction depth.

Prey sampling transects have also been established in both study areas. Prey sampling is being conducted primarily to document between-area differences in prey abundance, annual within-area fluctuations in prey, and ultimately to assess whether fisher or marten habitat use, diet, survival, or reproductive success is correlated with prey dynamics. Prey-sampling transects (approximately 125 in each study area) consist of 10 sampling locations (2 parallel lines of 5 stations) spaced 20 m apart, with transects distributed in 6 cover types throughout each study area. Transects are generally oriented perpendicular to roads or trails, with the first plot 30 m off the trail. In spring, we count snowshoe hare pellets in a 1-m^2 plot at each sampling station (McCann et al. 2008). During fall, small mammal snap-trapping occurs for 2 consecutive days at the same sampling stations, similar to protocols used on an existing small mammal survey in Minnesota (Aarhus-Ward 2009). During both spring (hare pellet sampling) and fall (small mammal trapping), we also count the number of red squirrels (*Tamiasciurus hudsonicus*) observed or heard along each transect. Rather than using 10-min point counts (e.g., Mattson and Reinhart 1996, Bayne and Hobson 2000) with our small mammal and hare pellet stations as the sampling points, we record the number of unique squirrels detected per transect (summarized per unit time) while checking pellet plots and small mammal traps. Information on white-tailed deer and ruffed grouse (*Bonasa umbellus*) populations may be available from existing surveys or population models.

RESULTS AND DISCUSSION

Including the pilot year of the study, a total of 242 martens (115F, 127M) and 114 fishers (65F, 49M) have been radiocollared. Because tooth aging has not yet been completed for all animals, some of which may be only 1 year of age (i.e., not capable of producing kits), we present results only for animals known to be ≥2 years of age during spring den visits, or those of unknown age but for which we have confirmed parturition at the time of this writing (i.e., until age is known, we do not include animals that we have confirmed to be nulliparous). As of this writing, spring 2015 reproductive status assessment is largely complete.

Treating females that were alive during multiple parturition periods (years) as independent units, and excluding females known to be <2 years of age, we have confirmed age and reproductive status for 45 female martens (Table 1). Pooling years, pregnancy rate has been 56% for 2-year-old martens (*n* = 16), and 79% for martens 3 years or older (*n* = 29, Table 1). We have been able to confidently assess litter size for 27 marten litters, for which average litter size is 2.96 (range = 1–4); litter size averaged 3.0 for 2-year-olds (*n* = 8) and 2.94 for ≥3-year-old (*n* = 18) martens (Table 1). Data suggest most marten kits are born in late-April and early-May with a few litters being born in mid-April and mid-May.

A total of 57 natal or maternal dens have been confirmed for martens (Table 2). For temporal reference, 43 (75%) of the marten dens were documented to be used from mid-April through 1 June, with the low number (*n* = 14) of maternal dens located after this a result of time

constraints and increasing difficulty in finding dens in summer. Of the 57 dens, 61% were in tree cavities, 33% were in underground burrows, and 5% were in hollow logs on the ground (Table 2). Of the 35 dens in tree cavities, 74% have been in live trees whereas 26% have been in snags. Pooling live trees and snags, most tree-cavity dens used by martens have been in aspen (*n* = 14) and cedar (*n* = 12), with 1–3 dens located each in tamarack (*Larix laricina*), red maple (*Acer rubrum*), black ash (*Fraxinus nigra*), and white pine (Pinus strobus, Table 2). Average DBH for all den trees with cavities was 16.9 in. (range = 10.4–30.0, Table 2). Of the 19 underground dens, 9 were characterized as being in soils with an abundance of medium to large rocks or in a crevice of a rock outcrop, 7 were under the base of larger trees or stumps or associated with shallow roots or sphagnum 'soils' adjacent to the base of the tree, and 3 were under 'tip-ups' (Table 2). Three dens were located in hollow logs on the ground, 2 in cedar, and 1 in an aspen. As marten kits become more mobile, females make use of den structures closer to the ground. Of the 14 dens located after 1 June, 64% were located in burrows or hollow logs on the ground and 36% were in tree cavities (1 with an entrance at ground level and another at 2 feet high). All dens located after 1 July (*n* = 6) were in burrows or hollow logs.

Similar to martens, we treat female fishers that were alive during multiple parturition periods (years) as independent units. Excluding individuals known to be 1 year of age during the parturition period, we have confirmed both age and reproductive status for 58 female fishers (Table 1). Pooling years, pregnancy rate for female fishers has been 65% for 2-year-olds (*n* = 23), and 94% for fishers 3 years or older (*n* = 35, Table 1). We have been able to confidently assess litter size for 45 fisher litters. Overall average litter size is 2.5 (range = 1–4); litter size averaged 2.15 for 2-year-olds (*n* = 13) and 2.7 for ≥3-year-olds (*n* = 29, Table 1). Based on data collected to date, it appears fisher kits are typically born in early- to mid-March in the central and southern portion of their Minnesota range (Figure 1; Areas 2 and 3) and in late-March to mid-April further north (Figure 1, Area 1).

A total of 77 fisher natal or maternal dens have been located to date (Table 3). For temporal reference, 64 (83%) of the fisher dens confirmed were documented to be used in March and April, with the few maternal dens located in May (*n* = 7) or after 1 June (*n* = 6) a result of time constraints and increasing difficulty in finding dens in summer. Of the 77 dens confirmed, all but 3 were in elevated tree cavities; the remaining 3 maternal dens were in large hollow logs either on or suspended above the ground (Table 3). Of the dens in tree cavities, 73% have been in live trees whereas 27% have been in snags. Pooling live trees and snags, most tree cavity dens used by fishers have been in aspen (*n* = 50) and oak (*n* = 9), with 1–5 dens located each in sugar maple (*Acer saccharum*), red maple, white cedar, white pine, and American elm (*Ulmus americana*, Table 3). Average DBH for fisher den trees was 20.0 in. (range = 13.6–29.1, Table 3). Similar to martens, most female fishers appear to move their kits from their natal den to 1 or more maternal dens in the first 8 weeks following birth.

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Table 1. Parturition rate and litter size for radiocollared[1] female fishers and martens in Minnesota from 2008 to 2015.

	Parturition Rate		Litter Size		
Species*Age	# females	% with litters	# litters	Average	Range
Martens					
2-year-olds	16	56	8	3.0	2 - 4
≥3-year-olds	29	79	18	2.94	1 - 4
All	48[2]	69	27[3]	2.96	1 - 4
Fishers					
2-year-olds	23	65	13	2.15	1 - 4
≥3-year-olds	35	94	29	2.7	1 - 4
All	59[2]	81	45[3]	2.5	1 - 4

[1] Excludes unknown-aged nulliparous females and all 1-year-olds. Multiple years for same female treated as independent.
[2] Includes females with age ≥2, but otherwise unknown age.
[3] Includes known litters from unknown-aged females.

Table 2. Natal and maternal den structures (*n* = 57) used by radiocollared female martens in Minnesota from 2008 to 2015.

Den Structure	# dens	% of total	Average DBH (in.)	DBH Range (in.)
Above-Ground, All Tree Cavities	35	61.4	16.9	10.4 – 30.0
Cavity, live tree	26	45.6	17.5	10.4 – 30.0
Cavity, snag	9	15.8	15.2	11.6 – 20.4
All Aspen cavities	14	24.6	15.9	10.4 – 23.8
All Cedar cavities	12	21.0	17.0	10.8 – 21.5
All Tamarack cavities	3	5.3	17.6	16.2 – 19.9
All Red Maple cavities	3	5.3	17.2	15.9 – 19.0
All Black Ash cavities	2	3.5	16.1	14.3 – 17.8
All White Pine cavities	1	1.8	30.0	
Below-Ground Dens	19	33.3		
Burrow, under base of tree	7	12.3	14.3	9.0 – 18.6
Burrow, rocky soils/outcrop	9	15.8		
Burrow, under tip-up	3	5.3		
Hollow log	3	5.3		

Table 3. Natal and maternal den structures (*n* = 77) used by radiocollared female fishers in Minnesota from 2008 to 2015.

Den Structure	# dens	% of total	Average DBH (in.)	DBH Range (in.)
Above-Ground, Tree Cavities	74	96.1	20.0	13.6 – 29.1
Cavity, live tree	54	70.1	20.3	13.9 – 29.1
Cavity, snag	20	26.0	19.2	13.6 – 26.1
Aspen cavities	50	64.9	19.8	13.6 – 29.1
Oak cavities	9	11.7	20.1	15.1 – 28.0
White pine cavities	5	6.5	23.1	19.0 – 25.6
Sugar Maple cavities	2	2.6	20.6	19.1 – 22.1
Red Maple cavities	5	6.5	20.1	18.0 – 23.6
Cedar cavities	2	2.6	17.1	13.9 – 20.3
American Elm cavities	1	1.3	19.2	
Hollow Log	3	3.9	15.7	13.0 – 18.3

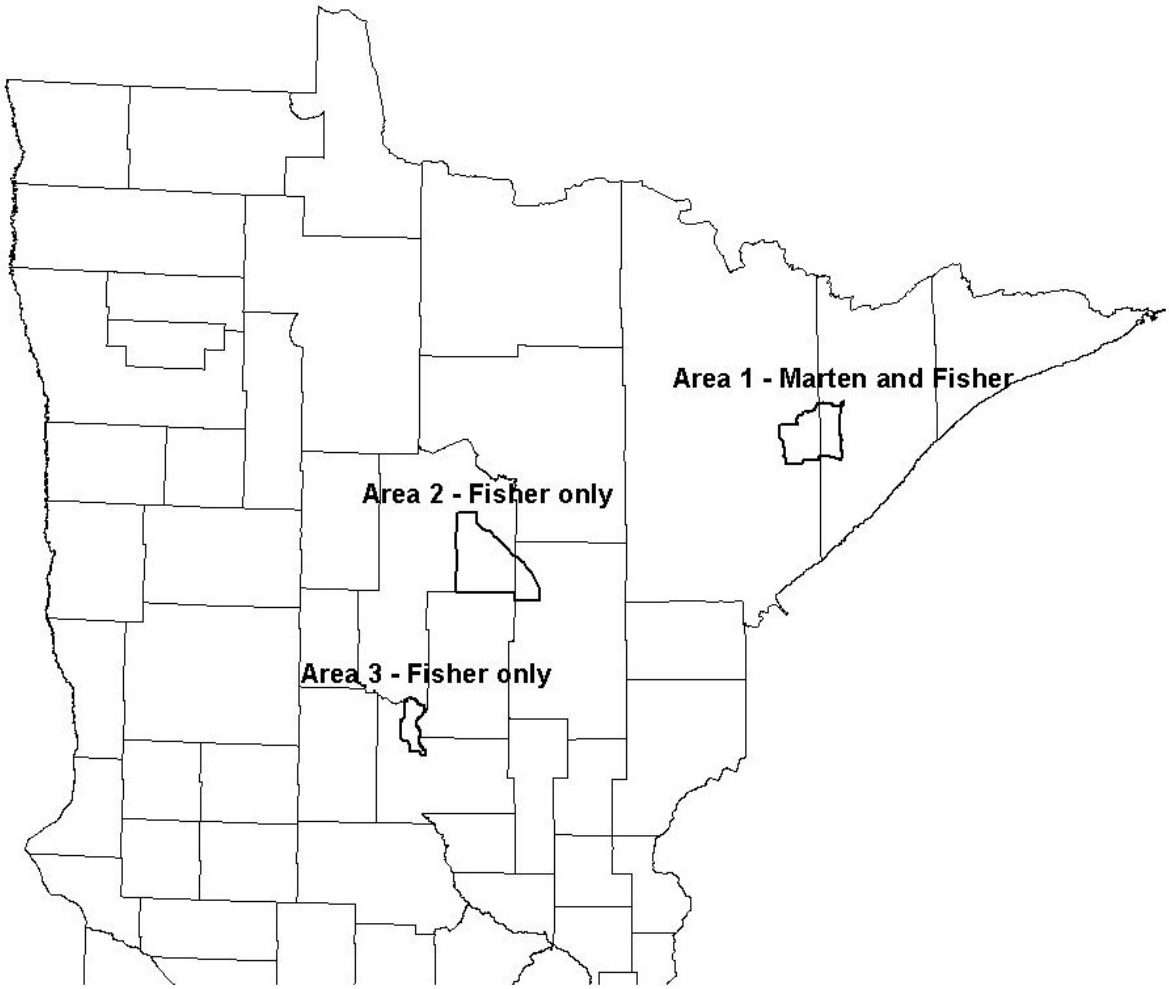

Figure 1. Fisher and American marten study areas in Minnesota, 2008–2015.

SURVIVAL AND CAUSES OF MORTALITY FOR FISHERS AND MARTENS IN MINNESOTA

John Erb, Pam Coy, and Barry Sampson

SUMMARY OF FINDINGS

As part of a larger project on *Martes* ecology in Minnesota, we began monitoring survival of radio-collared fishers (*Pekania pennanti*) and martens (*Martes americana*) during winter 2007-08. Radio-collaring efforts have now ended. Including the pilot year of the study, a total of 242 martens [115 females (F), 127 males (M)] and 114 fishers (65F, 49M) were radio-collared. An additional 6 animals (3 martens, 3 fishers) were ear-tagged only. Of the 242 martens radio-collared, 6 (2F, 4M) are still actively being monitored, radio-contact has been lost on 83, 9 (8F, 1M) whose collars are inaccessible have either slipped their collars or died, and 144 deaths have been confirmed (of which 12 were censored due to death within 2 weeks of capture). Of the 132 non-censored marten deaths (60F, 72M), most have been from legal fur trapping (*n* = 50; 37M, 13F) and predation (*n* = 62; 37F, 25M). Approximately 91% of the marten predation deaths have been attributed to mammalian carnivores and 9% to raptors. Although natural mortality of martens >0.6 years of age has occurred in most seasons, it is highest in spring and lowest in fall. No significant sex bias has been observed in overall mortality; female martens make up 47% of our sample and 45% of the known deaths. However, marten harvest mortality (including accidental trapping) has been male-biased (70% male) while natural mortality has been female-biased (56% female).

Of the 114 fishers radio-collared, 9 are still being monitored (5F, 4M), radio contact was lost on 38, 11 (6F, 5M) have either slipped their collars or died, and 56 deaths (33F, 23M) have been confirmed. Of the 56 fisher deaths, most have been from predation (*n* = 26; 20F, 6M) and fur trapping (*n* = 17; 7F, 10M; 8 in-season, 9 accidentally out-of-season). Three fishers have been car-killed, 7 died from unknown but apparent natural mortality, and human-caused versus natural death could not be determined for 3 fishers. Similar to martens, natural mortality for fishers is highest in spring and lowest in fall. Of 20 female fishers predated, 18 were killed by other mammalian carnivores, 1 by a raptor, and 1 by an unknown predator. Conversely, 4 of the 6 male fisher predation mortalities were attributed to raptors (all bald eagles). Of particular note, 19 of the 20 female fishers killed by predators were adults, and 15 of them were killed while they still had dependent young, indirectly resulting in the death of all their offspring. The deaths of these 15 nursing females and their litters represent approximately 29% of the reproductive 'opportunities' for adult female fishers monitored during the kit-rearing season since the study began. Because the magnitude of this mortality would not likely have been sustainable for an extended period, we suggest that survival patterns have probably changed in the last 10 to 15 years. We continue to explore several hypotheses, but suspect a partial explanation may be that cumulative changes in the environment have had both direct (e.g., reduction in denning habitat quality) and indirect (e.g., weather and habitat more favorable to competing bobcats) effects on survival of female fishers in the core of Minnesota's fisher range.

INTRODUCTION

American marten and fisher are native to Minnesota, but reliable documentation of their historic distribution is limited. Undoubtedly, northeastern Minnesota was a stronghold for the marten population, though notable numbers likely occurred in the northern border areas as far

west as Roseau County. Limited information suggests they occurred as far south as Crow Wing County and as far southwest as Polk County. As a result of unregulated harvest, marten were considered rare in Minnesota by 1900, and extensive logging and burning around the turn of the century further contributed to the near extirpation of marten from Minnesota by the 1930s (Swanson et al. 1945). Fishers in Minnesota appear to have historically occupied a larger geographic area than martens, extending further south and west into the hardwood dominated transition zone, including southeast Minnesota (Swanson et al. 1945, Balser and Longley 1966). The impacts of unregulated harvest and habitat alteration were equally as detrimental to fisher, with populations substantially reduced by the 1930s.

Legally, fisher and marten were unprotected in Minnesota prior to 1917, after which harvest season length restrictions were implemented. These protections were removed in the mid-1920s, and remained so until all harvest was prohibited in 1929. Seasons remained closed until 1977 for fisher and 1985 for marten, when limited harvests were reinstated. While marten harvest is now legal in approximately the northern 50% of the state, most harvest occurs in counties bordering Canada, particularly in northeast and north-central Minnesota. Fisher harvest occurs in most of the northern 50% of the state, though harvest is comparatively low in extreme northeast Minnesota (Lake and Cook counties). Over the past 10 years, fisher abundance and harvest have been increasing along the southern and western edge of the 'forest zone' where forest historically transitioned to savanna and prairie and is now characterized by linear forest corridors (e.g., streams, rivers) or smaller forest patches interspersed with agriculture. Conversely, fisher abundance appears to have declined significantly over the same period in the core forested areas of north-central and northeast Minnesota. Peak statewide harvest levels have been near 4,000 and 3,500 for marten and fisher, respectively. However, due to apparent multi-year population declines for both species, harvest seasons from 2007 to the present have become progressively more conservative, with recent harvest seasons lasting only 6 days with a combined fisher/marten limit of 2 per trapper.

While both species appear to have naturally re-colonized a significant portion of their historic range, Minnesota-specific information on survival and causes of mortality is limited. Except for harvest data, we are aware of only 1 published field study in Minnesota. Specifically, Mech and Rogers (1977) opportunistically radio-collared 4 marten and reported survival and home range information for those animals. This information is specific to marten, now nearly 30 years old, and based on a very limited sample size. Gathering cause-specific mortality information can be useful for informing population models, detecting unknown mortality agents, and focusing management activities on issues of concern.

Krohn et al. (1994) estimated 11% annual non-harvest mortality for adult fisher in Maine, while York (1996) estimated 19% and 7% annual non-harvest mortality (including 4% poaching mortality on males) for adult male and female fisher, respectively, in Massachusetts. Excluding the first 4–5 months of life, juvenile fisher non-harvest mortality rates have been estimated to be 28% in Maine (Krohn et al. 1994), and 0% (females) and 23% (males) in Massachusetts (York 1996). While mortality may be higher in the first months of life than the rest of the year, if we assume a similar non-harvest mortality rate during the first 4–5 months of life, we calculate that annual non-harvest mortality for juvenile fishers would be approximately 56% in Maine. Combining minimum summer survival estimates for kits with telemetry estimates of survival the rest of the year, York (1996) estimated approximately 67% (males) and 22% (females) annual non-harvest mortality for juvenile fishers in Massachusetts. Kelly (1977, in Paragi et al. 1994) reportedly estimated 18% annual mortality of juveniles and 44% annual mortality for adult fisher in New Hampshire. More recently, Koen et al. (2007) estimated annual mortality rate (including harvest mortality) of fishers in Ontario to be 55–67% for males, and 29–37% for females. While non-harvest mortality of adult fishers is often presumed to be 'low', it has not always proven to be the case. Furthermore, there is limited data on which to assess the amount of geographic or temporal variation in non-harvest mortality of fisher.

Natural mortality, particularly via predation, appears more common with martens. Marten survival data is available from Wisconsin (McCann et al. 2010), Maine (Hodgman et al. 1994, 1997), Ontario (Thompson 1994), Oregon (Bull and Heater 2001), British Columbia (Poole et al.

2004), Alaska (Flynn and Schumacher 1995, 2009), Quebec (Potvin and Breton 1997), and Newfoundland (Fredrickson 1990). Although we do not summarize details of these studies here, a couple conclusions are worthwhile. First, when comparing across studies, annual adult non-harvest mortality rates varied from 0.07 to 0.48. Juvenile data was rarely separated, but a few studies pooled ages, and mortality rates also were within the above interval. While this variability may be attributable to both sampling and biological variability, the wide range suggests that it is risky to assume results from any area are applicable elsewhere. Secondly, at least 1 study (Maine; Hodgman et al. 1997) has documented significantly higher natural mortality for females compared to males, and other researchers have postulated this to be common given the typical male-biased harvest, 50:50 sex ratio at birth, and often balanced adult sex ratio (Strickland et al. 1982, Strickland and Douglas 1987). Due to male-biased harvest and our *assumed* sex-related equality in non-harvest mortality, our marten population model previously projected a very female-biased population, contradicting our preliminary capture results and suggesting that our model inputs were overestimating female survival, underestimating male survival, or incorrectly assuming a 50:50 birth sex ratio.

OBJECTIVES

As part of a larger project on *Martes* ecology in Minnesota (Erb et al. 2009), we began monitoring survival and causes of mortality for fishers and martens. After initial evaluation of field methods during the pilot year of the study, winter 2008-09 marked the beginning of full-scale research activities. Although details are not discussed here, we are also collecting data on various potential correlates to survival (e.g., prey dynamics, winter severity, diet, habitat use, activity patterns, and body condition). Our primary objectives are to:

1. Determine causes of mortality;
2. Estimate cause- and sex-specific mortality rates;
3. Document seasonal patterns of mortality; and
4. Examine potential effects of winter weather, prey fluctuations, competitor density, activity patterns, and habitat on survival probability.

Herein we present basic descriptive information regarding number of captures and number and causes of deaths. We defer a more comprehensive statistical analysis until a later time.

STUDY AREA

Marten research is focused on 1 study area located in northeastern Minnesota (Figure 1; Area 1), though 2 martens have been captured and radio-collared in Area 2 (Figure 1). Area 1 (approximately 700 km^2) is approximately 90% public ownership, including portions of the Superior National Forest and state and county lands. Fishers are also present in this area at low to moderate density.

Fisher research has taken place in 3 areas (Figure 1; Areas 1, 2, and 3). The work in Area 3 is a collaborative effort between Camp Ripley Military Reservation, Central Lakes Community College, and the Minnesota Department of Natural Resources. Although we do include animals captured in that area in our basic summaries, we do not discuss other aspects of that project in this report. Area 2 (1075 km^2), our primary fisher study area, is approximately 67% public ownership, including portions of the Chippewa National Forest and state and county lands. Extremely few martens occupy Area 2.

METHODS

We used Tomahawk (Tomahawk Live Trap, Hazelhurst, WI) cage traps to capture both fishers (Model 108) and martens (Models 106 and 108) during winter. Traps were typically baited with either deer (*Odocoileus virginianus*) or beaver (*Castor canadensis*) meat, and commercial lure was placed in or above the traps. We enclosed traps inside white plastic 'feed sacks' or burlap bags and further covered traps with natural vegetation. All traps were checked daily.

To immobilize animals, we used metal 'combs' to restrict the animal to a small portion of the trap, or restrained the animal against the side of the trap by pulling its tail through the cage mesh. Animals were injected with a hand-syringe using a 10:1 mixture of ketamine and xylazine (fisher: 30 mg/kg ketamine and 3 mg/kg xylazine, marten: 20 mg/kg ketamine and 2 mg/kg xylazine; Kreeger et al. 2002). After processing, the xylazine was reversed with yohimbine at a dosage of 0.1 mg/kg (martens) or 0.15 mg/kg (fishers). Fishers were either ear-tagged with a monel #3 tag in one ear (National Band and Tag Co., Newport, KY) and a 2-piece plastic mini-tag (Dalton I.D. Systems, UK) in the other ear, or with a monel #3 tag in both ears. Martens were ear-tagged with a monel #1 tag (National Band and Tag Co., Newport, KY) in each ear.

During processing, we placed animals on heating pads connected to a power inverter and 12-volt battery. Portable shelters and propane heaters were also used to keep animals warm during processing. We monitored respiration, pulse, and rectal temperature during anesthesia. We weighed and sexed animals and typically removed a first pre-molar for aging. Morphological measurements taken included body length, tail length, hind foot length, and chest, neck, and head circumference. We removed guard hair samples for possible genotyping, and for evaluating the use of stable isotope analysis for deciphering food habits (Ben-David et al. 1997). To assist with determining which female fishers would likely produce kits, blood samples were drawn when possible to measure serum progesterone levels (Frost et al. 1997). Antibiotics were administered subcutaneously to all animals prior to release as a precaution against infection (Kreeger et al. 2002) from minor wounds that may have occurred while in the trap, and because of certain invasive procedures utilized during handling (ear-tagging, removal of tooth).

During the pilot year, we deployed several radiocollar designs on fishers, including an Advanced Telemetry Systems (ATS; Isanti, MN) M1585 zip-tie collar (43 g), an ATS M1930 collar (38 g), and a Lotek Wireless Inc. (Newmarket, ON, CA) SMRC-3 collar (61 g; deployed on adult males only). Since the pilot year, we have primarily deployed ATS M1940 (43 g) or Sirtrack (Havelock North, New Zealand) TVC-162 collars (45 g) on fishers. The majority of martens have been fitted with Holohil Systems Ltd. (Carp, ON, CA) MI-2 collars (31 g). We retrofitted each collar with a temperature data logger (I-button model DS1922L; Maxim Integrated, San Jose, CA) to provide ancillary information on winter activity and spring den attendance patterns, as well as to provide information on time of death for other study objectives.

Radio-locations were obtained year-round from fixed-wing aircraft at approximately weekly intervals, with intensive ground telemetry primarily during certain focal periods (e.g., denning season). When a radiocollar emits a mortality signal, we usually investigate and recover the animal or collar within 1–2 days. To determine cause of mortality, we use a combination of field investigation and animal necropsy. Starting in the second year of the project, we also began collecting forensic samples (hair by wound, wound swabs) from all animals exhibiting signs of being predated, particularly if a mammalian predator is suspected (Wengert et al. 2013). Forensic samples were submitted to either the University of California-Davis Veterinary Genetics Laboratory or Integral Ecology Research Center (Blue Lake, CA) for analysis. If non-predation natural causes are suspected after initial analysis (i.e., no visible trauma), carcasses in suitable condition were submitted to the University of Minnesota's Veterinary Pathology Lab for a full pathological exam.

RESULTS AND DISCUSSION

Including the pilot year of the study, a total of 242 martens (115 F, 127 M) and 113 fishers (65 F, 48 M) have been radiocollared. An additional 6 animals (3 martens, 3 fishers) were ear-tagged only. Tooth aging has not yet been completed for all animals; however we note that because capture operations took place during winter, all animals were a minimum of 7 months of age at initial capture. We have yet to derive formal estimates of survival rate. Instead, we provide a simple overview of the fate of collared animals in this summary.

Twelve martens died within 14 days post-release and will ultimately be censored from survival analysis. Cause of death for these 12 martens was predation (n = 7), capture-related complications (n = 4), and 1 whose collar became lodged in a rocky crevice after release.

Excluding these 12 animals, 6 (3%) of the 230 collared martens are actively being monitored, radio contact has now been lost on 83 (36%; *n* = 75 missing, *n* = 8 slipped collars), the status of 9 (4%) is uncertain due to unrecoverable collars or collars found with no other evidence, and 132 (57%) have died (Table 1). Of the 132 non-censored deaths, most have been from legal fur trapping (*n* = 50) and predation (*n* = 62, Table 2). Ten animals died of other natural causes, including being crushed by a tree, perforation and blockage of the intestine from a piece of bone, starvation related to an intestinal polyp, and 7 from unknown but assumed natural causes (Table 2).

Of the 62 non-censored marten predation deaths, 58 could be attributed to either avian or mammalian predation. Evidence suggests 53 (91%) were killed by mammalian predators and 5 (9%) by raptors. Although predation deaths have occurred in nearly all months, predation and overall natural mortality is highest in the spring and lowest in the fall (Figure 2). Forensic (DNA) analysis of samples collected from predated marten (mammalian predation only) is still incomplete. To date, field evidence and DNA analysis suggests bobcats (*Lynx rufus*) as the most common mammalian predator, with red fox (*Vulpes vulpes*), fisher, and lynx (*Lynx canadensis*) also confirmed in several cases.

Excluding martens censored within 14 days of capture, our sample of radiocollared marten has been comprised of 47% females. In comparison, female martens have accounted for 45% of the total marten deaths, 30% of the total deaths due to harvest, and 56% of the predation deaths; although there is no apparent sex-bias to overall mortality, marten harvest has been notably male-biased whereas natural mortality has been female-biased.

Of the 114 fishers radiocollared, 9 (8%) are still being monitored, radio contact has been lost with 38 (33%; *n* = 18 missing; *n* = 20 collars broke, slipped, or removed), the fate of 11 (10%) is uncertain due to unrecoverable collars or collars found with no other evidence, and 56 (49%) have died (Table 1). General cause of death (human versus natural) could be assigned to 53 of the 56 fisher deaths. Of these, 20 (38%) were attributable to humans (8 trapped during legal season, 9 accidental trapping, and 3 car-killed) whereas 33 (62%) were attributable to natural causes (26 predated, 7 unknown natural cause; Table 2). The seasonal pattern of natural mortality for fishers has been similar to that of martens, being greatest in spring and lowest in fall (Figure 3).

Of the 26 predated fishers, 20 were females (Table 2). Of the 20 females predated, only 1 was attributed to an avian predator [Great-horned owl (*Bubo virginianus*) suspected]. Conversely, 4 of the 6 male fisher predation deaths were attributed to raptors (all bald eagles; *Haliaeetus leucocephalus*), though we can't rule out scavenging in 1 case where only the radiocollar was retrieved directly underneath an active eagle nest. We are awaiting forensic DNA analysis on many fishers killed by mammalian predators. However, similar to martens, field evidence and forensic DNA analysis suggests bobcats as the most common predator, with canids (wolf or coyote) suspected in at least 2 fisher deaths.

Of particular note, 19 of the 20 female fishers killed by other predators were adults (≥2 years old), and 15 of those 19 were nursing females whose deaths resulted in complete litter loss. The deaths of these kit-rearing females and their litters represented 29% of the parous females and litters monitored during spring and early summer since the study began.

We suspect that 2 broad factors may explain the high mortality of kit-rearing female fishers during late-winter and spring: increased activity and increased vulnerability (independent of activity level). Given the potential for negative energy balance during parts of winter, compounded in early spring by the added energy demands of gestation and lactation, female fishers may need to increase activity in spring to meet energy demands. Combined with the need to locate suitable (and multiple) natal or maternal dens, this activity, much of which may be in localized areas near den trees and hence more predictable and detectable to other carnivores, may increase predation risk. Preliminary data from temperature data loggers attached to radiocollars suggests that fishers do spend increasing amounts of time (compared to winter) outside of den and rest sites during late-winter and spring. Secondly, independent of their activity level, fishers may be more vulnerable in spring because concealment cover is diminished (i.e., before 'green-up') and interspecific competition may be high due to potential prey for carnivores being at the low point in

the annual cycle. Collectively, this may yield a period of high energetic demand that overlaps with a high risk competitive environment for female fishers. Progressing into summer and fall, concealment cover is maximal, prey abundance (for all carnivores) is maximal, energetic demands of female fishers decrease as kits are weaned, and female movements may be less restricted (i.e., predictable) with mobile kits.

Regardless of the explanation, it seems unlikely that the level of predation we have observed on nursing female fishers during the study would be sustainable for long periods, which may partially explain the decline in fisher abundance in core areas over the previous decade. However, many of the correlates to the timing of predation mortality that we have mentioned are not new challenges for adult female fisher, and since 1977 the core fisher population appears to have only been in decline only over the last 10 years or so, suggesting that other more recent changes may be altering dynamics. Possible explanations for the observed and presumably new mortality pattern for female fishers continue to be assessed, including potential declines in fisher habitat quality in core fisher range and changes in habitat and weather that may have contributed to an increase in competing bobcats. Wengert et al. (2014) also recently documented high bobcat predation rates on female fishers in California during spring, suggesting the pattern may not be unique to Minnesota. Overharvest of fisher, particularly in the 4 years preceding the start of this study, may also have contributed to the apparent decline in fisher abundance but does not explain the high natural mortality of female fishers, and to some extent female martens, that we have observed during this study.

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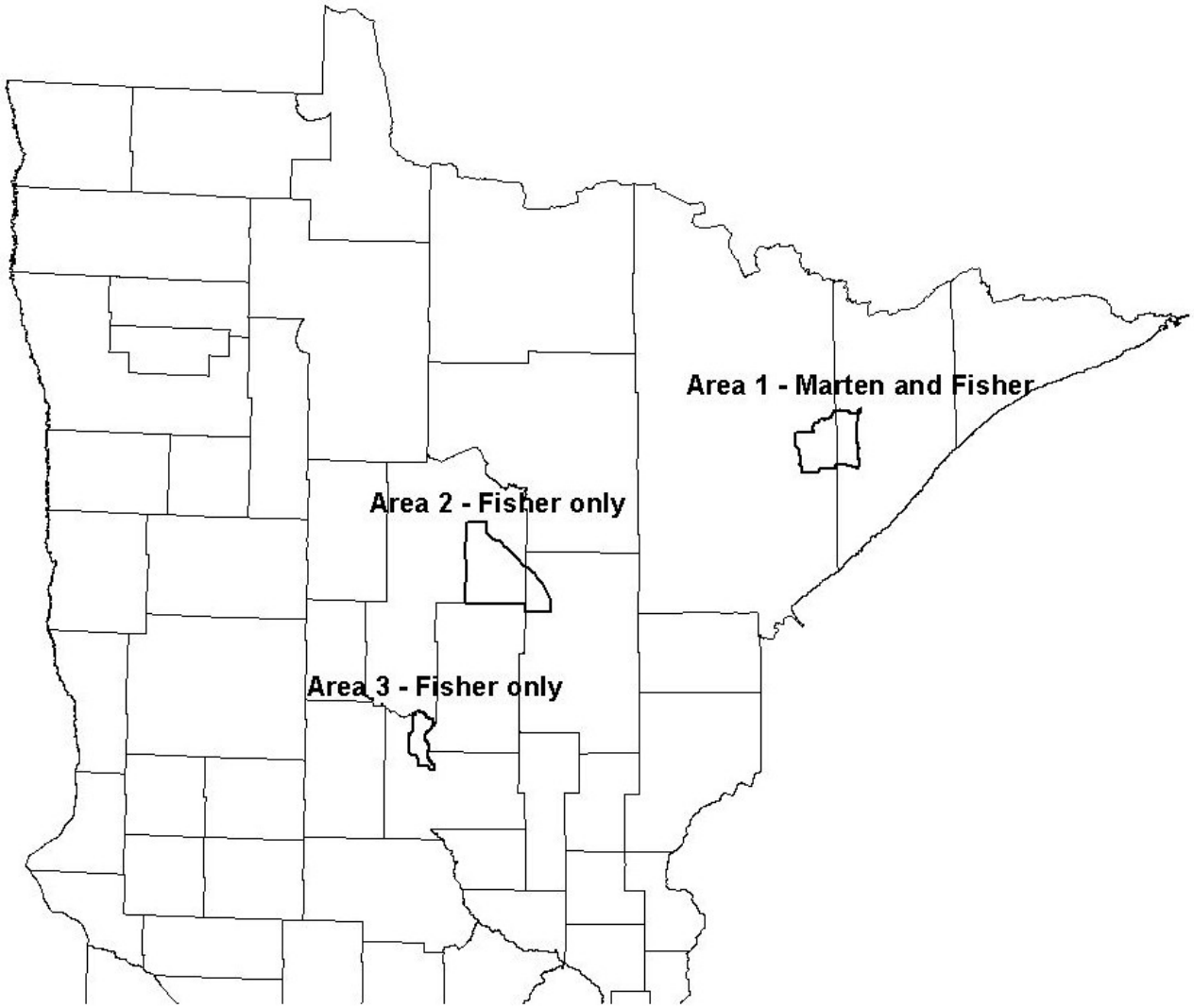

Figure 1. Fisher and marten study areas in Minnesota 2008–2015.

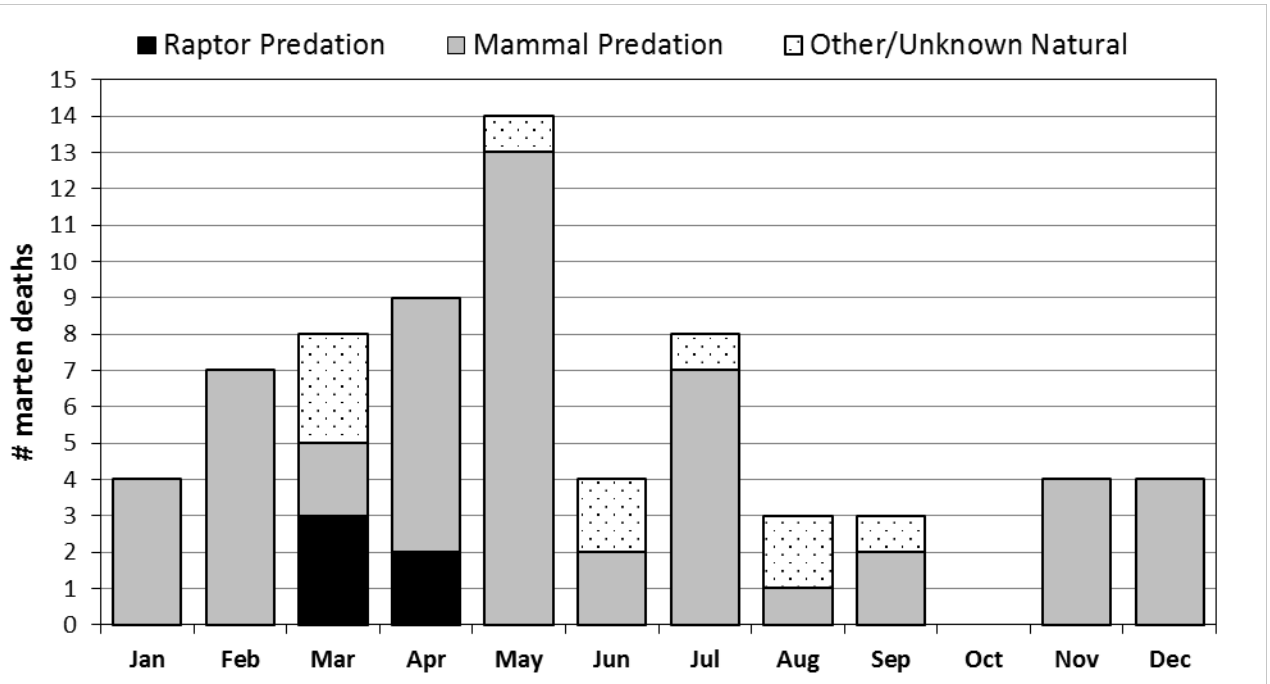

Figure 2. Seasonal timing of natural mortality for martens in northeast Minnesota, 2007–2015.

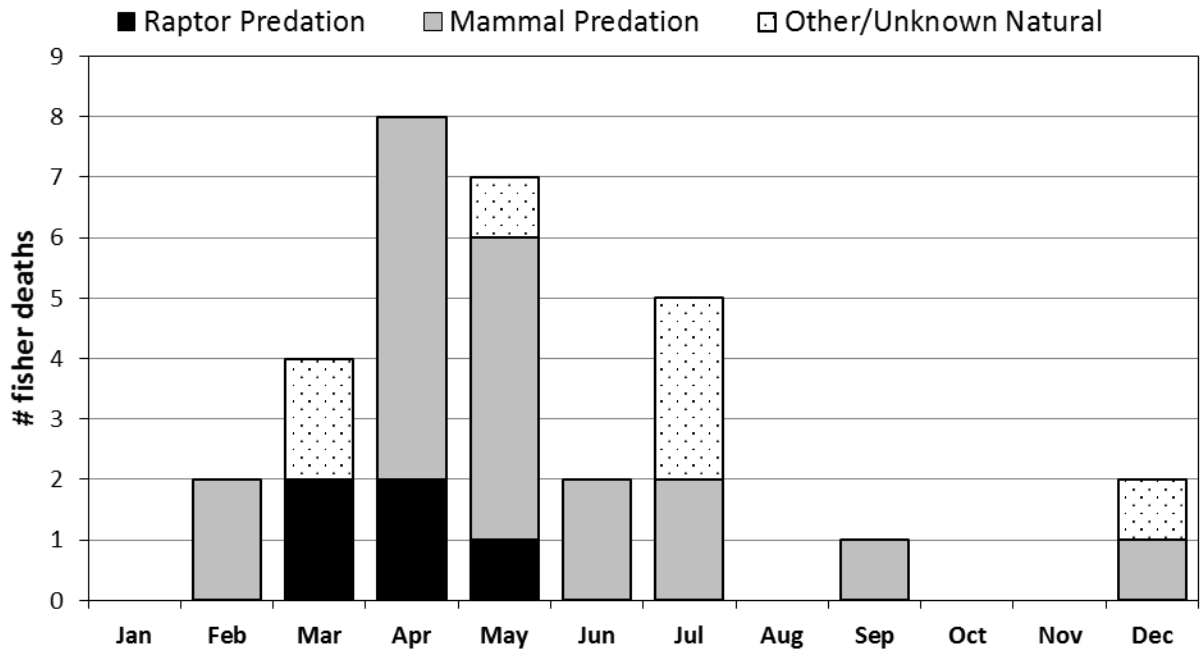

Figure 3. Seasonal timing of natural mortality for fishers in northeast Minnesota, 2007–2015.

Table 1. Number and status of fishers and martens radiocollared[1] in Minnesota from 2007 to 2015.

Sex*Species	# Active	# Deaths	# Missing	# Slipped Collars	Unknown[2]	Total
Male Martens	4	72	43	4	1	124
Female Martens	2	60	32	4	8	106
Male Fishers	4	23	8	9	5	49
Female Fishers	5	33	10	11	6	65

[1] Excludes radiocollared animals that died within 2 weeks of capture and release.
[2] Unknown represents collars not yet retrieved from tree cavities or underground locations (presumed dead or slipped collars), or retrieved but with uncertainty whether the animal slipped the collar or had died.

Table 2. Cause of death for fishers and martens radiocollared1 in Minnesota from 2007 to 2015.

Sex*Species	Predation	Natural Accident	Disease/ Illness	Unknown Natural	Car-Killed	Trapped In Season	Trapped Out of Season	Collar Complication	Unknown[2]	Total
Male Martens	25	2	1	4	0	37	2	1	0	72
Fem. Martens	37	0	0	3	1	13	3	2	1	60
Male Fishers	6	0	0	4	2	4	6	0	1	23
Fem. Fishers	20	0	0	3	1	4	3	0	2	33

[1] Excludes radiocollared animals that died within 2 weeks of capture and release.
[2] Unknown represents animals where evidence was insufficient to assign to natural versus human-related cause.

AN ALTERNATE METHOD TO DETERMINE MOOSE CALVING AND CAUSE-SPECIFIC MORTALITY OF CALVES IN NORTHEASTERN MINNESOTA

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SUMMARY OF FINDINGS

Adult survival and recruitment are important drivers of large herbivore population dynamics. The northeastern Minnesota moose (*Alces americanus*) population has been exhibiting a downward trend since 2006. Our research was initiated because neonatal and seasonal survival rates and specific causes of mortality (e.g., predation, undernutrition, disease) of calves are largely unknown. Due to the Governor of Minnesota's Executive Order 15-10 (28 April 2015), we were unable to continue handling or collaring neonates in 2015. Beginning 1 May 2015 we monitored 60 adult female moose fitted with global positioning system (GPS) collars (16 confirmed pregnant subsequent to capture during February 2015 by serum progesterone concentrations, 43 unknown, 1 not pregnant) for long-distance calving movements followed by localization. Additionally, we used movement patterns of collared cows (i.e., long distance flees, repeated returns to a focal point) to investigate potential calf mortalities. We conducted habitat surveys at cow locations prior to their calving move, during calving, during peak lactation, and at calf mortality. We observed 50 of 60 cows localize, indicative of calving. Of these 50 cows, 13 were confirmed pregnant, and 37 that had been collared in 2013 or 2014 were of unknown pregnancy status. Median calving date in 2015 was 10 May (mean = 11 May, range = 29 April–14 June), 4 days earlier than in 2013 and 8 days earlier than in 2014. Seventy-six percent of births occurred during 3–15 May. We retrieved calf remains from suspected calf mortality sites and estimated proximate causes of mortality on site. Mean elapsed time between estimated time of death and mortality investigation ranged from 45.5 to 179.3 hours; response times were affected by efforts to ensure patterns were indicative of calf mortality, accessibility, and dam presence. Nine confirmed calf mortalities have occurred during 3 May–2 June; causes included 6 wolf (*Canis lupus*) kills, 1 bear (*Ursus americanus*) kill, and 2 unknown predator kills. Preliminary analyses of habitat data suggest cows may trade off forage for cover at calving sites. Identifying specific causes of calf mortality and understanding their relations to various landscape characteristics and other extrinsic factors should yield insight into mechanisms contributing to the declining moose population in northeastern Minnesota and serve as a basis for an ecologically sound management response.

INTRODUCTION

The moose is an iconic species of northern Minnesota, which has afforded valuable hunting and viewing opportunities (Minnesota Department of Natural Resources [MNDNR] 2012). The MNDNR has listed moose as a Species of Special Concern (http://files.dnr.state.mn.us/natural_resources/ets/endlist.pdf). Recently, the northwestern population declined precipitously to less than 100 moose due to a variety of natural factors (Murray et al. 2006). The northeastern population is in decline and is experiencing adult mortality rates similar to those of the

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northwestern population as it decreased (Lenarz et al. 2009, 2010; Butler et al. 2013; Carstensen et al. 2014).

Large herbivore population growth (λ) is most sensitive to variation in adult survival (Gaillard et al. 1998, 2000; Lenarz et al. 2010), but differences in temporal variation of juvenile survival may be important in accounting for between-year variation in λ (Gaillard et al. 2000). Fecundity and calf survival ultimately determine recruitment rates which are important to more fully understanding population dynamics (Van Ballenberghe and Ballard 2007). When viable populations of predators are present, predation can be a primary cause of mortality of temperate ungulate neonates (Linnell et al. 1995, Carstensen et al. 2009, Severud et al. 2015). Less is known about other specific ultimate and proximate sources of moose calf mortality or contributing factors. It also is unclear when predation is compensatory or additive to other sources of mortality (Franzmann et al. 1980, Linnell et al. 1995), although a recent study documented additive effects of predation on moose calves in Alaska (Keech et al. 2011). The degree of predation's impact on population-wide calf survival rates depends on the extant predator guild and relative densities of predator and prey (Eriksen et al. 2011, Patterson et al. 2013).

After the calves' first summer, the magnitude of mortality from wolves is variable (Patterson et al. 2013). Wolves are more adept at killing calves in deep snow (DelGiudice et al. 2009), but wolves in an Alaskan study were responsible for calf mortalities in fall (Keech et al. 2011). Typically, bear-caused (*Ursus* spp.) mortality of calves is greatest closer to their parturition, more immediately following emergence of bears from winter dens (Bastille-Rousseau et al. 2011). Cows in poor nutritional condition may defend calves less vigorously (Patterson et al. 2013). Further, risk of predation is not independent of maternal care and experience (Ozoga and Verme 1986). The importance of natural non-predatory causes of calf mortality, likely varies during different times of the year, such as malnutrition and exposure in spring, or malnutrition and tick-related deaths in winter (Patterson et al. 2013). The extent to which diseases drive calf mortality is not well understood, although diseases have led to poor recruitment in moose (O'Hara et al. 2001, Murray et al. 2006). Juvenile animals are more predisposed to parasites than adults, and pathology related to parasite infection may be an important source of mortality for moose calves (Jenkins et al. 2001, Murray et al. 2006). Further, small calves may not be tall enough to efficiently nurse, leading to malnutrition (Murray et al. 2006). Drowning and climate have been known to affect moose calves more than predation in some regions (Crête and Courtois 2009). In winter, temperature and snow depth can be more important causes of mortality than predation (Keech et al. 2011).

Pregnant cow moose tend to move relatively long distances prior to localizing to give birth (Severud et al. 2015). This "calving movement" is typically much longer than movements between foraging and bedding sites. Following a long movement, calving localizations as measured by GPS collars resemble mortality localizations. A cow with calves may stay within a 1.7-ha area for up to 7 days (range = 1–18 days; McGraw et al. 2014).

During calving, cows may trade off forage availability for hiding cover (Bowyer et al. 1999). Cover may affect vulnerability to predation (Griffith and Youtie 1988). As nutritional demands for lactation increase and calves begin to incorporate browse into their diet, forage becomes more important. Lactation is a high energy-demanding phase of reproduction, requiring two to three times more energy than gestation (Robbins 1993). Milk production peaks 21–31 days post-parturition for moose cows (Schwartz and Renecker 2007).

OBJECTIVES

1. Evaluate monitoring of movement behavior of GPS-collared adult female moose to determine timing and location of calving and calf mortalities
2. Evaluate the relative importance of cover and forage availability as calf mobility and maternal nutritional demands change throughout the summer

METHODS

Our study area is the same as that of the Environmental and Natural Resources Trust Fund (ENRTF)-supported study in the Arrowhead region of northeastern Minnesota focused on survival and cause-specific mortality of adult moose (also see DelGiudice et al. 2015). As part of the adult moose mortality study, 111 (84 females, 27 males), 37 (25 females, 12 males), and 32 (20 females, 12 males) moose were captured and fitted with Iridium GPS collars (Vectronic Aerospace GmbH, Berlin, Germany) during January 2013, February 2014, and February 2015, respectively (Butler et al. 2013, Carstensen et al. 2014, this volume). Blood was collected and analyzed for serum progesterone; ≥2.0 ng/mL was indicative of pregnancy. We monitored cow movements during pre-parturition and calving, with particular attention afforded to pregnant cows. We looked for movement patterns indicative of calving, primarily a long-distance movement followed by localization (Bowyer et al. 1999, McGraw et al. 2014, Severud et al. 2015).

We began monitoring 60 collared adult female moose (16 confirmed pregnant by progesterone concentrations, 43 unknown [captured and collared in 2013 or 2014], 1 not pregnant) on 1 May 2015. Based on an 89% pregnancy rate of total tested cows in 2015 (16 of 18 cows; Carstensen et al., this volume), we assumed 53 (37 unknown + 16 known) cows to be pregnant in 2015. Cow collars were programmed to collect hourly locations during May and June and to transmit these locations 4 times per day. An automated R program (J. D. Forester, University of Minnesota, unpublished data) generated emailed reports 2 times daily (0500, 1700 hours; Figure 1). Reports contained a file (pdf) displaying various movement and location metrics for each collared cow, including table (csv format) and map (kml format) files with all recent locations of each animal. The pdf reports contained a rough map of northeastern Minnesota with all current cow locations, along with a summary table of all animal locations and distances moved in the last 24 and 48 hours. The metrics for each cow included the date and time of the last location, movement path of the last 5 days, movement path of the last 24 hours overlaid on Google Earth imagery, a plot showing 3-hour average distances moved, and each cow's data on a single page (Figure 1). The distance plot showed peaks in movements that we then monitored for possible dampening of movements (localization). If the cow moved <100 m during 36 hours following a long-distance movement (dam-calf bonding time), the program flagged that cow as "localized." Additionally, a blue line representing predictions from a regression based on 2013 calving movements showed if a calving move may have occurred in the last 12 hours. Larger spikes indicated higher likelihood of a calving event. A gray line showed relatively large or small movements over the past 12 hours. When a cow was flagged as calving, we also checked her movement path on the Vectronic Aerospace website (https://www.vectronic-wildlife.com; Figure 2).

We monitored cow movement patterns daily, looking for long distance movements over a relatively short timeframe ("flees"). We also looked for cows fleeing and returning to focal areas, which had been observed in 2013 and 2014 in response to calves preyed upon by wolves or bears (Figure 3; T. R. Obermoller, unpublished data).

When we observed a flee and return, we dispatched an investigative team to search for evidence of calf mortality, and if found, to determine the cause of death (Ballard et al. 1979, Severud et al. 2015). To avoid possible investigation-induced abandonment, approaches were delayed if the dam was still in the area. Our primary field objective was to recover the entire carcass and deliver it to the University of Minnesota's Veterinary Diagnostics Laboratory (VDL) for necropsy. If the carcass could not be extracted and transported, we performed a detailed field necropsy. If scavenged or fed upon, fresh organ and tissue samples were collected and shipped or transported to the VDL as feasible (Butler et al. 2011). Cause of mortality was assigned based upon the preponderance of evidence (e.g., feeding pattern, predator tracks, scat, hair, sign of a struggle). In cases where conclusive evidence was lacking, we collected predator scat for hair analysis (Y. C. Ibrahim, Grand Portage Band of Chippewa, personal communication) or swabbed bite wounds for saliva that could be used to identify the predator (B. R. Patterson, Ontario Ministry of Natural Resources, personal communication). Care was

taken to haze off predators and scavengers when approaching a potential mortality site; bear repellent spray and firearms were available as a last resort for protection, but their use was not necessarily anticipated (Smith et al. 2008, 2012). We postponed the investigation when predators were sighted on the carcass; return was dependent on the age and size of the carcass as an indication of how long the predator or scavenger might feed.

For a subset of cows that made a calving movement followed by localization in 2013–2015, we collected site characteristics at the pre-calving site (location immediately preceding the calving movement) and presumed calving site (averaged coordinates over a 40- to 48-hour time period immediately following the calving movement, adjusted on site as confirmed by calving evidence). We similarly surveyed locations where calf mortalities were indicated by GPS locations of the dam (2013 and 2014) or confirmed by site evidence (e.g., calf bone fragments, hooves, hair, predator sign in 2015). Calf mortalities occurring at the calving site were treated as having identical habitat conditions. When calf mortalities occurred outside of the calving site, new habitat data was collected.

Peak lactation of moose dams occurs 21–31 days postpartum (Schwartz and Renecker 2007). In 2015, if we found evidence indicating a calf had survived ≥26 days (pellets and tracks), we collected site characteristics at the corresponding cow's peak lactation location. We used the nearest GPS location from each collared cow with a known calf at 26 days post-calving. If the location was in the middle of a long distance movement, we used the nearest grouping of ≥3 locations, which were usually 1 hour apart. We conducted all habitat work to match phenological conditions (i.e., leaf off and leaf on) to the time the initial location was recorded.

Habitat plots were centered at each cow's GPS location closest to the time of interest, unless that location was refuted by visual evidence. This typically occurred at calving and mortality sites, when we were able to see where a cow had calved or where a calf had died. In these cases, plot centers were placed in the middle of the cow's calving bed or at the primary location of calf remains or sign of a struggle. In the center of each plot, we collected an averaged waypoint using a handheld GPS unit and a GeoExplorer II mapping system (Trimble Navigation Limited, Sunnyvale, CA), recorded the elevation from the base map on our handheld GPS unit, and used a spherical convex densiometer to determine canopy density. We also measured the prevailing slope and aspect using a clinometer and compass.

Canopy density (in addition to being measured at the plot center) and horizontal visibility were recorded 15 m from the plot center in each cardinal direction. We used a 2-m cover pole to determine horizontal visibility, recording the visible percentage (0, 25, 50, 75, or 100%) of each of 19 bands from the center (Poole et al. 2007). To estimate calf hiding cover, we held a cardboard cut-out of a standing moose calf silhouette at the center of the plot and recorded the percentage of the cut-out that could be seen from 15 m away at a 1-m height in each cardinal direction. The observer then moved towards the cut-out, maintaining a 1-m height, and recorded their distance from the calf when visibility reached 25, 50, 75, and 100%.

We recorded trees, saplings and shrubs, exposed root masses, and coarse woody debris (CWD) within a 3-m (to match previously collected data) and 11-m radius (for later use in extrapolation using LiDAR) from the central point. Trees were defined as anything upright (<45° lean) with a DBH ≥10 cm. Saplings and shrubs were defined as DBH <10 cm. We determined the species and DBH of each tree, alive or dead, within the plots, and counted saplings and shrubs by species.

For exposed root masses, we measured the maximum height of root masses ≥60 cm wide. Coarse woody debris had to be at least 90 cm long within the plot radius and have a diameter ≥7.6 cm at its widest point. We included fallen logs and snags with >45° lean, but did not include CWD assigned a decay class of 5 (Maser et al. 1979). We recorded CWD when ≥3 feet of the tree was within the plot. If these conditions were met, we measured the DBH at either end of the CWD, maximum height from ground, total length, whether it had fallen due to natural or human factors, and the overall decay class (1–4).

RESULTS

During 2015 we observed 49 of 60 cows (82%) display calving behavior (i.e., calving movement followed by localization for >36 hours). An additional cow localized without making an observable calving movement (50 of 60, 83%). Thirteen of the 16 confirmed pregnant cows localized (87.5%); 36 of 43 cows of unknown pregnancy status localized (84%). Median localization date was 10 May 2015 (mean = 11 May, range = 29 April–14 June; Figure 4), with 76% of the localizations occurring during 3–15 May 2015 (Table 1). In 2013, mean date of cow localization (assumed to have calved within 12 hours) was 14 May (median = 14 May, range = 2 May–2 June), whereas in 2014 mean localization date was 19 May (median = 18 May, range = 5 May–16 June). Cows that calved in each of the 3 years (2013–2015) on average calved earlier in 2015 (Figure 5).

During 2015 we investigated 31 instances of cows making movements indicative of a calf mortality (i.e., flees alone or flees coupled with return visits). In 9 of these cases (29%) we found calf remains. We did not find calf remains in any cases where cows did not make a return visit (0 of 7 cases). Our success rate when using flees and returns to indicate mortality was 36% (9 of 25 cases). In all 9 of these cases, calves were ≤23 days old at time of death (mean = 13.2, range = 2–23 days old). In the remaining cases where dams fled and returned, calf remains were not found and calves were aged 24–64 days old. Dams made 1–7 return trips to calf mortality sites in cases where we found calf remains. Mean time from death (estimated as the last location before the dam fled) to investigation was 83.1 hours (range = 45.5–179.3 hr). Mean distance between calf remains and calving site was 539 m (range = 0–2,223 m), with older calves dying farther from calving sites. Mean distance between calf remains and location the dam fled from was 23 m (range = 4–79 m), indicating dams had fled from the mortality site and likely in response to the mortality.

As of 24 August 2015, for calves born during 2015 we have documented 9 natural mortalities, with 6 additional cases pending (no direct evidence of calf mortality but predator scat [1 wolf scat, 5 bear scats] will be analyzed for presence of calf hair). We documented 6 wolf kills, 1 bear kill, and 2 unknown predator kills (scat and saliva evidence pending, calf remains located; Figure 6). Based on known calf mortalities and confirmed calf presence at peak lactation sites (tracks and scat), calf survival to 30 days was 60.9% (Figure 7).

Incidental observations of cows with their calves provided the only twinning information available in 2015. We received reported sightings on 11 cows (7 collared cows, 1 non-collared cow, 3 cows of unknown collar status). Singletons were spotted with 3 collared cows and 1 cow of unknown collar status. Sets of twins were reported 4 times—1 set with a non-collared cow, 1 with a collared cow, and 2 with cows of unknown collar status. A single set of triplets was observed with a collared cow. Another collared cow was reported to have 4 calves, but when the same cow was observed a month later, only 3 calves were seen. This same cow was spotted with a yearling calf in May 2015; it did not appear to make a calving move or localization, leading to some uncertainty in assigning parentage to her for any or all of these calves. Cows have been reported to adopt calves of other dams (White et al. 2001), and giving birth to 3–4 calves is unlikely.

As of 18 August 2015, we have completed habitat surveys of 38 pre-calving sites (21, 6, and 11 sites for 2013, 2014, and 2015, respectively), 73 calving sites (42, 12, and 19 sites), 18 peak lactation sites (all 2015), and 34 mortality sites (19, 6, and 9 sites). In 2013 and 2014 we collected habitat data from a 3-m radius, but in 2015 expanded the plot to an 11-m radius to better enable extrapolation using LiDAR. Preliminary analyses of 11-m radius data of shrubs and saplings showed a trend of higher numbers of coniferous stems compared to deciduous stems at calving and mortality sites (Figure 8). Cover pole measurements (horizontal visibility from the dam's perspective) were generally higher in pre-calving and calving sites compared to peak lactation and mortality sites, whereas there was less calf hiding cover at calving and mortality sites compared to pre-calving and peak lactation sites. Full results will be presented next year.

DISCUSSION

Tracking GPS-collared cow movements was a highly reliable way to estimate calving rates and to a lesser degree calf mortality. Due to the Governor of Minnesota's Executive Order 15-10, we were unable to confirm presence of calves shortly after birth, nor handle or collar calves. Without observing neonates at calving sites, we cannot estimate twinning rates for 2015. We also did not know when a calf had died, but used dam movements as an indication of calf mortality. Only in cases where the calf was ≤23 days old and the dam fled and made 1–7 return trips were we successful in confirming calf mortality. In a subset of those cases we were able to assign cause of death. This technique may serve as a method to estimate early neonatal mortality, but has less power to detect mortality as calves age beyond 3 weeks. This method will not reliably detect calves that succumb to forms of mortality other than predation because we have not documented cows fleeing from and returning to other mortality events (e.g., disease, drowning, abandonment).

In 2015 87.5% of pregnant females localized, compared to 88% in 2013 and 73% in 2014. Cows deemed pregnant by serum progesterone that did not localize may have experienced stillbirths, abandoned their neonates, or the calf may have died shortly after birth. Alternatively, uterine or ovarian adhesions may preclude pregnancy despite elevated progesterone levels (Testa and Adams 1998).

Our observed calving dates in 2015 began earlier than in 2013 and 2014. The later calving dates in 2014 may have been a result of the severe winter of 2013–2014 (Winter Severity Index [WSI] of >180 for 1 November 2013–31 May 2014) or of more intense monitoring into June 2014. A trend of later median calving dates with increased WSI, and vice versa, is emerging (Table 1). McGraw et al. (2014) reported a mean calving date of 14 May 2011 (range = 3–27 May), with 70% of births occurring during 9–20 May. In an Alaskan study, cow moose experimentally malnourished in late-winter lost mass and gave birth to unhealthy calves 2 weeks later than normal (Schwartz 2007). In our study area, there is emerging evidence of notable nutritional restriction of moose during winters 2013–2014; however, additional years of data are needed to better understand the population-level implications (DelGiudice and Severud, this volume).

Predation accounted for all 9 confirmed calf mortalities. DNA analysis to potentially identify predators may prove difficult since it is recommended to swab wounds ≤24 hours postmortem (B. R. Patterson, Ontario Ministry of Natural Resources, personal communication). We typically needed to wait longer to determine if the dam fled and returned before investigating. Predator scat analysis may not prove conclusive as a predator may startle a dam and her calves, yet they were able to escape. Any calf hair contained in the scat could belong to another calf. Known calf survival to 30 days in 2015 was similar to pooled 2013 and 2014 rates (Figure 7). We plan to conduct aerial surveys of cows at leaf-off (e.g., October 2015), first appreciable snow (e.g., January 2016), and last snow cover (e.g., April 2016) to estimate seasonal survival rates of calves and compare to rates during previous years.

Calving habitat may be an important determinant of neonatal survival (Bowyer et al. 1999). Our preliminary analyses of 2015 shrub and sapling data indicated dams may be trading off forage (deciduous stems) for cover (coniferous species) in calving sites (Figure 8). We will more finely analyze these data to look at browsed versus non-browsed species. Cover pole data, which may be an index of a dam's vantage point to observe incoming predators, showed pre-calving and calving sites offered more visibility. However, this relationship may be due to the phenological conditions during use, rather than a product of selection, because dams typically inhabit these sites before full leaf out. The calf model measured hiding cover, yet pre-calving and peak lactation sites offered higher levels of hiding cover. We intend to further analyze habitat data to investigate the role of CWD, trees, canopy closure, slope, and aspect on calving site selection. By using pre-calving, calving, and peak lactation sites for an individual dam, we can infer what decisions dams are making relative to habitat selection, and how those choices may affect calf survival. In addition to using habitat measurements on the ground, we intend to use LiDAR to extrapolate to other dam locations.

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Table 1. Calving date summary for GPS-collared cow moose in northeastern Minnesota, 2011, 2013–2015. Calving was inferred from cow localization.

Year	Mean	Median	Earliest	Latest	"Peak" calving	% of calves during peak	WSI[a]
2011[b]	14 May	Not reported	3 May	27 May	9–20 May	70%	150–170
2013	14 May	14 May	2 May	2 June	6–17 May	73%	120–139
2014	19 May	18 May	5 May	16 June	11–22 May	75%	180+
2015	11 May	10 May	29 April	14 June	3–15 May	76%	100–119

[a] Winter severity index (WSI) was calculated by accumulating a point for each day ambient temperature was ≤0° Fahrenheit (−17.8° Celsius) and an additional point for each day snow depth was ≥15 inches (38.1 cm), and then estimated for the entire moose study area.

[b] Data from McGraw et al. (2014).

25 Collar 12567 U

Last location: 2015-05-15 01:05:44
Max 3-h Avg. Speed (24h) = 19.9 m/h
Collar Temp = 14°C // Mortality Status = -7: Mortality, outside fence
UTM.X = 638479, UTM.Y = 5274688
Longitude = −91.15739, Latitude = 47.61079

Stat	T2h	T24h	T48h	Total
Disp.	2	53	30	758
Path	104	290	547	370777
Speed	20	12	11	65

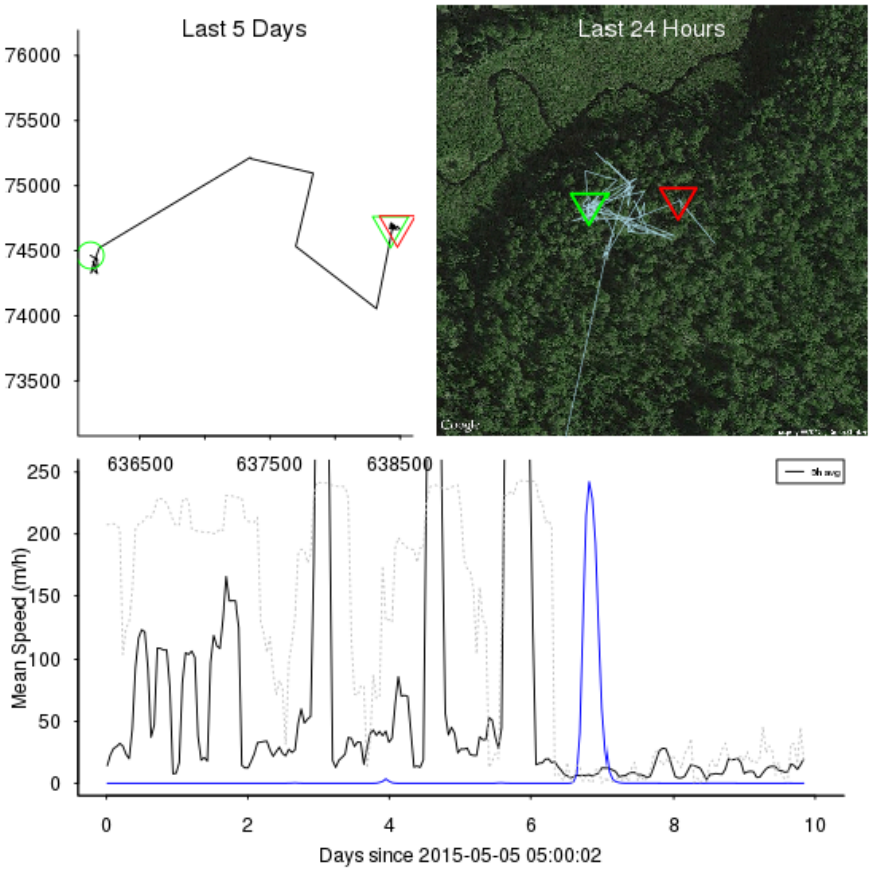

Figure 1. Example report for adult female moose number 12567 from 0500 hours on 15 May 2015, showing movement paths in northeastern Minnesota for the previous 5 days and 24 hours, and 3-hour average hourly distances moved. Green circle represents the start of the 5-day period, green triangle the start of the 24-hour period, and red triangle the most recent location. The blue line represents predictions from a regression based on 2013 and 2014 calving movement data; larger spikes suggest a higher likelihood a calving movement occurred in the past 12 hours. The light gray lines show relatively large or small movements in the past 12 hours.

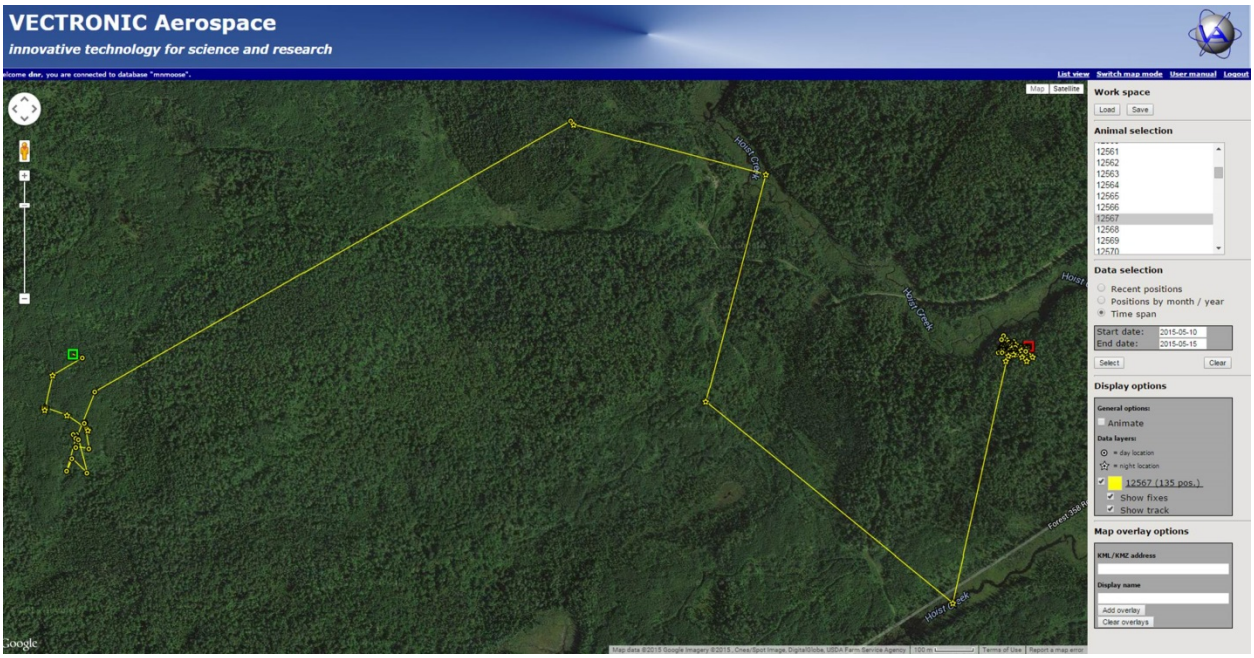

Figure 2. Vectronic Aerospace website (https://www.vectronic-wildlife.com) map interface showing the path of adult female moose number 12567, northeastern Minnesota, 10–15 May 2015. The green and red squares represent the start and end of the interval. The cow's movement pattern in the west side of the map indicates typical bedding and foraging, whereas the cluster in the east of the map indicates a tight localization following a long-distance movement.

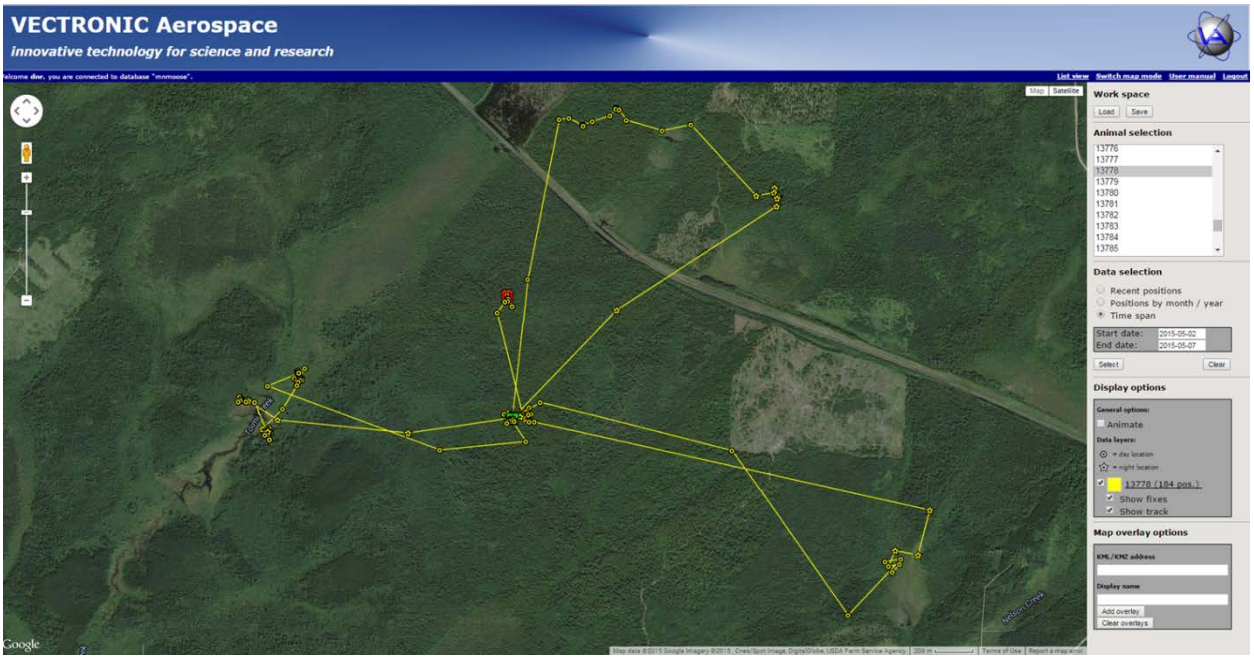

Figure 3. Vectronic Aerospace website (https://www.vectronic-wildlife.com) map interface showing the path of adult female moose number 13778, northeastern Minnesota, 2–7 May 2015. The green and red squares represent the start and end of the interval. The cow's movement pattern of fleeing and returning to a focal point is indicative of mortality of a neonate. This dam made 3 return trips before we investigated on 7 May 2015. We discovered wolf tracks and calf bone fragments at the cluster in the middle of the map, which was also the calving site. We estimated the calf was born on 1 May 2015 and was about 2 days old at time of death.

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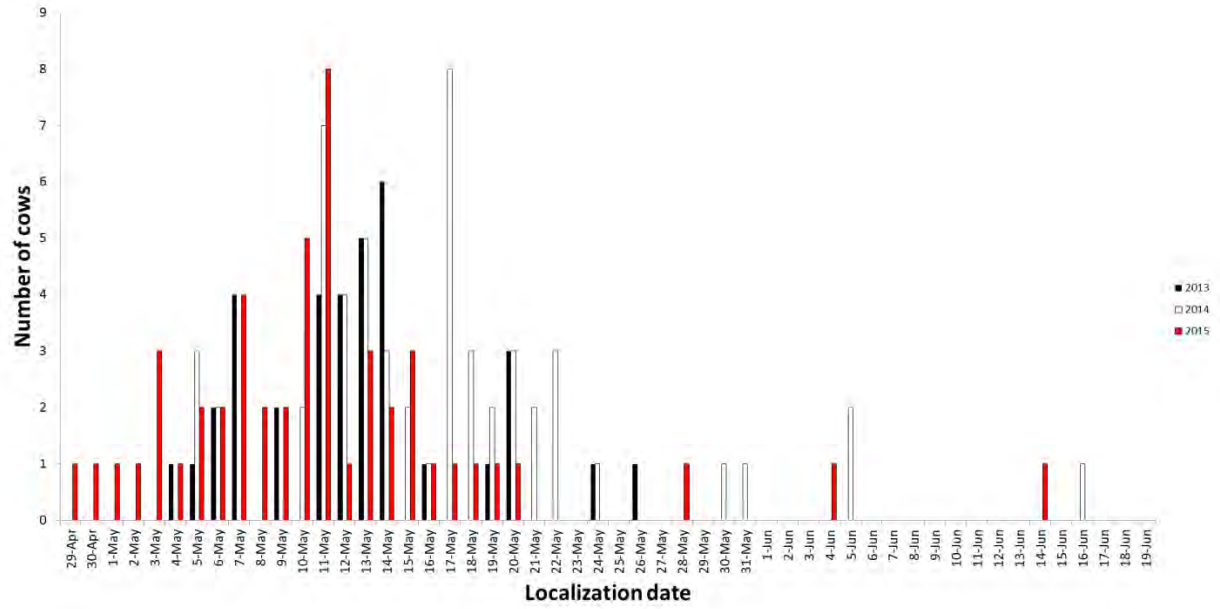

Figure 4. Temporal distribution of calving localizations of collared adult female moose, northeastern Minnesota, April–June 2013– 2015.

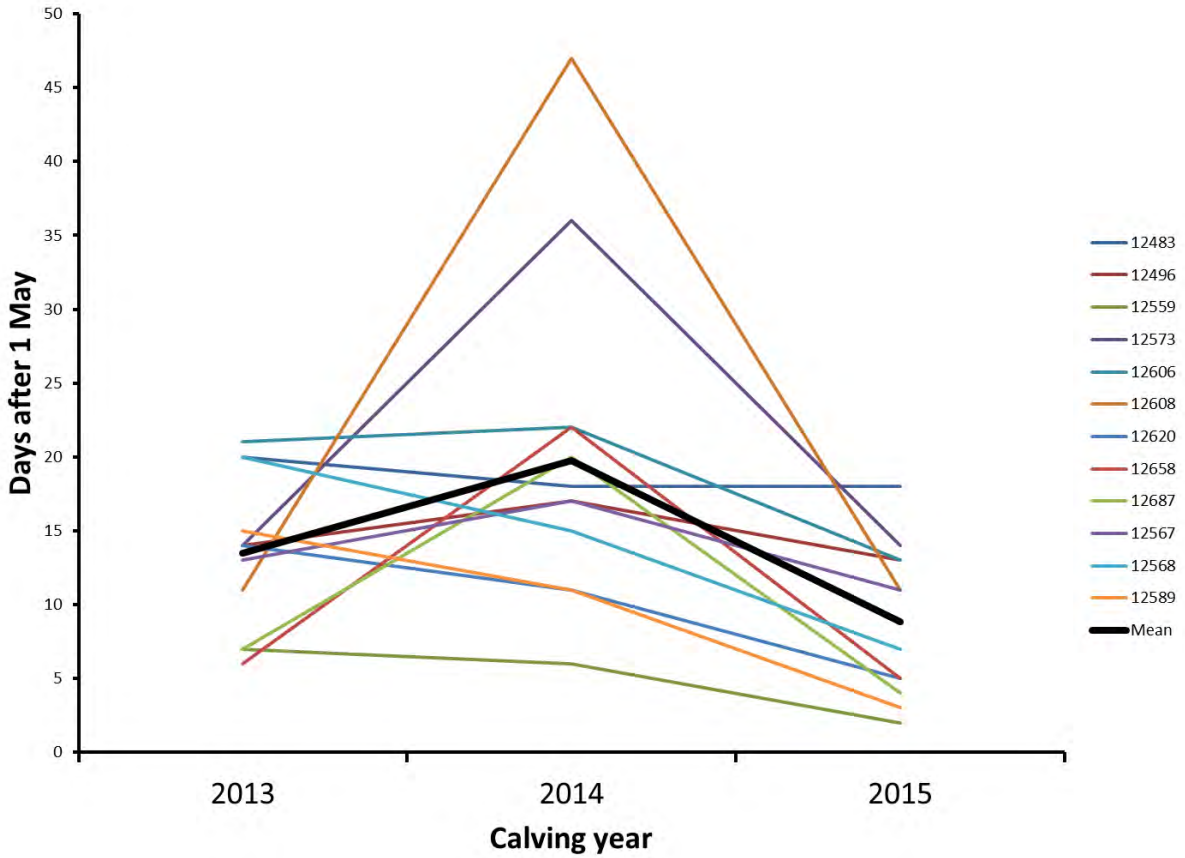

Figure 5. Temporal change in calving date (in days after 1 May) for collared female moose in northeastern Minnesota, 2013–2015. Individual cows that calved consecutively in all three years are depicted by colored lines. Mean value among these 12 cows is depicted in black.

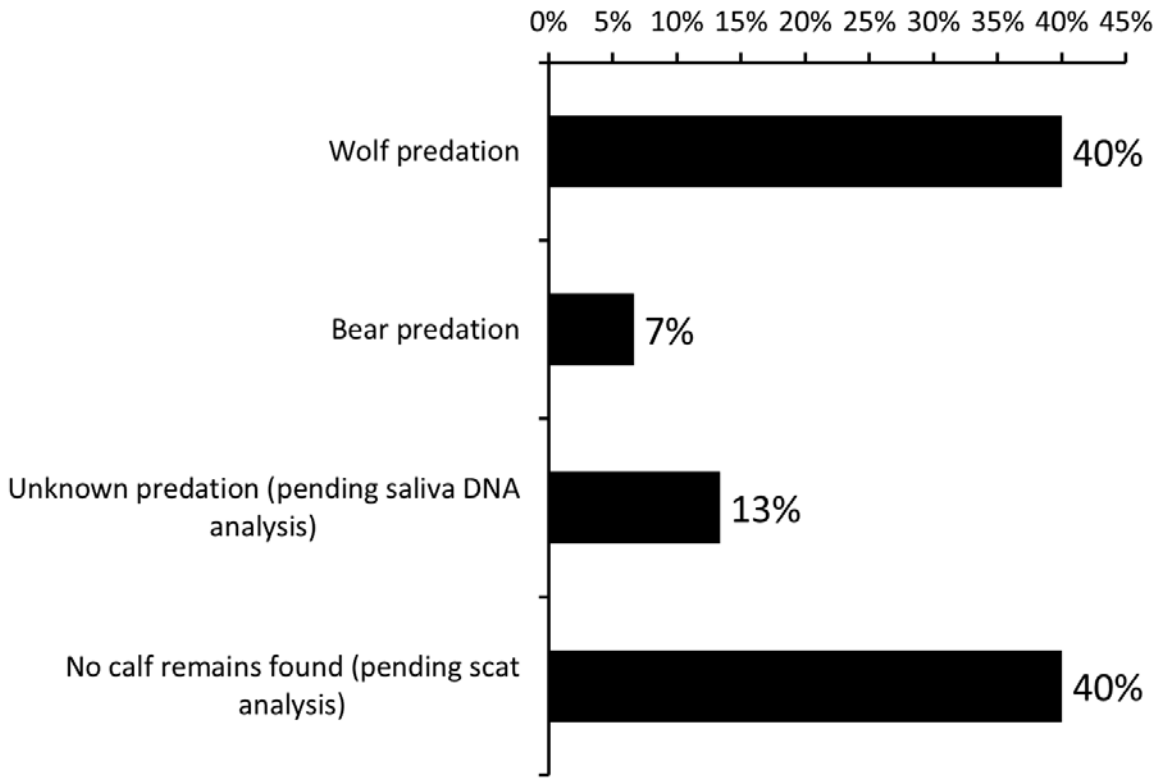

Figure 6. Cause-specific mortality of moose calves in northeastern Minnesota, May–June 2015. Mortality was indicated by dam movement patterns and confirmed through observation of calf remains. Cause was assigned based on the preponderance of site evidence.

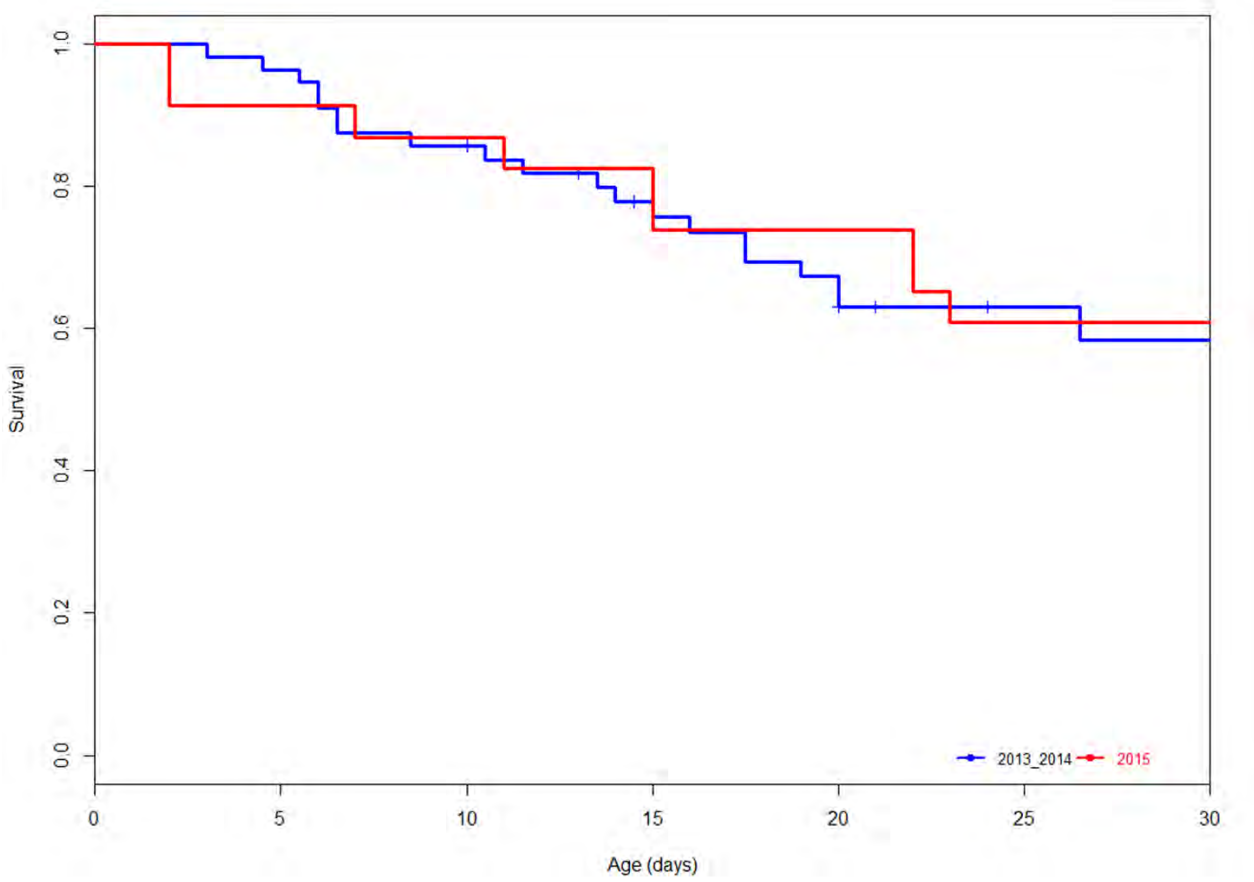

Figure 7. Kaplan-Meier survival for known moose calf mortalities, northeastern Minnesota, May–June 2013–2015. Mortality was confirmed by GPS collars (pooled 2013 and 2014, blue line) or through investigations triggered by dam movement patterns and observation of calf remains (2015, red line).

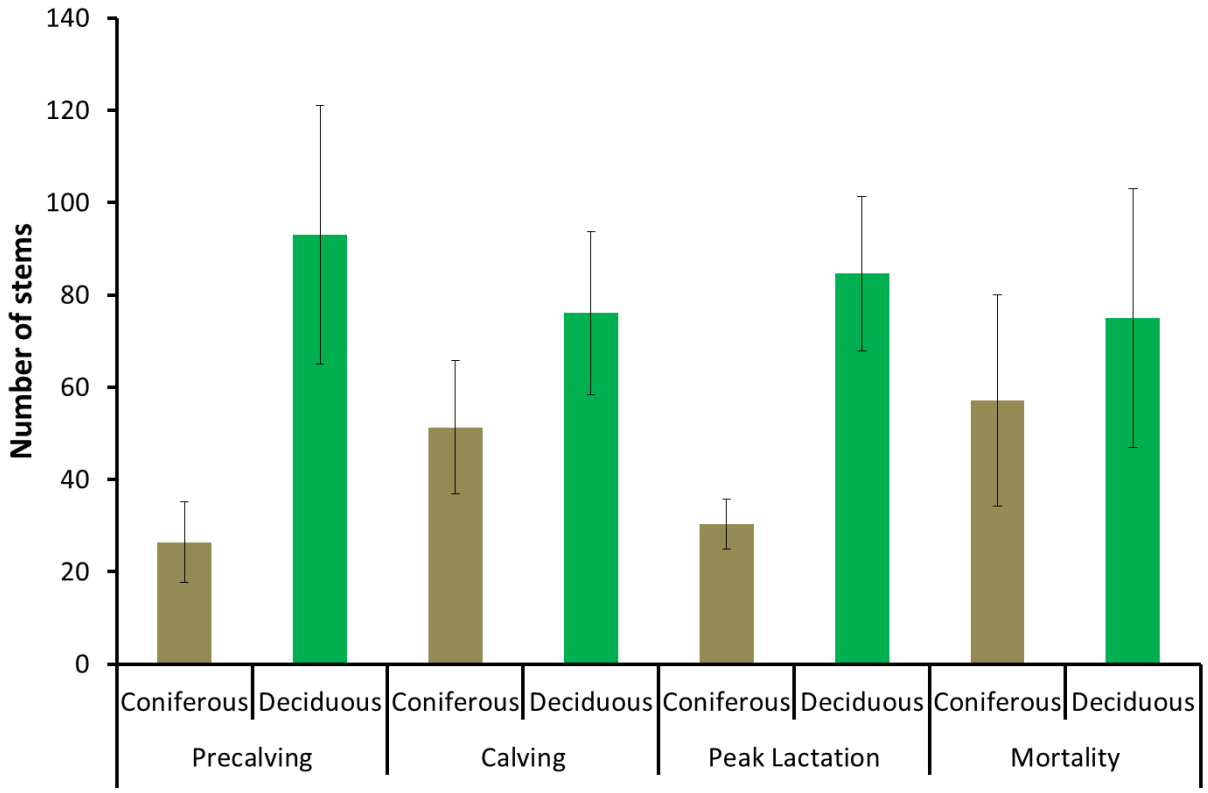

Figure 8. Mean (±SE) number of shrub and sapling stems at 11-m radius plots at moose pre-calving (*n* = 11), calving (*n* = 19), peak lactation (*n* = 18), and mortality sites (*n* = 9), northeastern Minnesota, 2015.

BLOOD PROFILES AND ASSOCIATED BIRTH CHARACTERISTICS OF FREE-RANGING MOOSE (*ALCES AMERICANUS*) NEONATES IN A DECLINING POPULATION IN NORTHEASTERN MINNESOTA, 2013

Glenn D. DelGiudice and William J. Severud[1]

SUMMARY OF FINDINGS

Steady declines in Minnesota's moose (*Alces americanus*) numbers, first in the northwest, then in the northeast, prompted 2 aggressive studies, one investigating adult survival and cause-specific mortality, and the other examining calf production, survival, cause-specific mortality, and annual recruitment. As with domestic species, blood analyses have proven to be of unique value in assessing aspects of the nutritional, hydration, reproductive, and overall health and disease status of wild animals. Herein our goal is to document the first extensive blood profiles of free-ranging moose newborns. Hind foot length was positively related to body mass at capture, particularly for 7 neonates that died within 32 days of age. We also documented that neonates captured at a younger age and smaller body size tended to live longer in the short-term. Hematological and serum constituents can range rather widely and serve as reference values when related to individual neonate and birth characteristics. We observed positive relationships between red blood cell and white blood cell counts, packed cell volumes, and hemoglobin concentration and body mass of neonates at capture. Values of hematological characteristics were not related to time to death within 30 days of capture/release, but segmented neutrophils, lymphocytes, and platelets differed between neonates that died within 32 days of birth versus those that survived beyond 32 days. The most meaningful associations of serum constituents included significant ($P \leq 0.05$) relationships between age at capture and concentrations of free thyroxine and free triiodothyronine, hormones indicative of energy status, and relationships of body mass to serum urea nitrogen and cortisol concentrations, constituents indicative of nutritional status and stress. Blood analyses will provide a more complete picture of the physiological status and condition of these moose neonates when they are in the most vulnerable phase of their lives. The better we come to understand this phase and the often obscure challenges confronted by individual calves over the long-term, the better chance we have of formulating and implementing management strategies to improve annual population recruitment, growth, and persistence.

INTRODUCTION

Steady declines in Minnesota's moose (*Alces americanus*) numbers, first in the northwest (beginning in the mid-1980s), and then in the northeast (since 2006), prompted very high frequency (VHF) telemetry studies of moose population ecology to better understand the underlying causes of the declines (Murray et al. 2006; Lenarz et al. 2009, 2010). The northwestern population had plummeted to <100 animals by 2007 (Murray et al. 2006, Lenarz et al. 2009), and presently, the northeastern population is estimated at 3,450, 61% lower than in 2006 (DelGiudice 2015). Three years ago the Minnesota Department of Natural Resources (MNDNR) followed up initial research efforts in the northeast by launching 2 aggressive studies, one investigating adult survival and cause-specific mortality (Carstensen et al. 2014), and the other addressing calf production, survival, cause-specific mortality, and annual recruitment (Severud et al. 2015). Both studies have relied on cutting-edge global positioning system (GPS) collars and monitoring technology to facilitate unprecedented examinations of cause-specific mortality and calving activity (Butler et al. 2013, Severud et al. 2015). Until these studies, greater effort had been focused on adult survival, because it has a greater impact on long-term

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dynamics of ungulate populations (Gaillard et al. 2000; Lenarz et al. 2010). But reproductive success and annual recruitment of calves cannot be discounted; low and variable recruitment can have a pronounced impact on a population's growth rate (Gaillard et al. 1998). However, because hazards are greatest within the first days, weeks, and months of calves' lives, to gain an accurate assessment of annual survival and cause-specific mortality, calves must be collared as newborns (about 2 days old). This can pose a serious challenge for the animals and researchers alike due to the vulnerability of ungulate neonates to natural mortality and highly variable risk of capture-induced abandonment and mortality (Livezey 1990; DelGiudice et al. 2006, 2015; Lenarz et al. 2010; Keech et al. 2011). Ultimate abandonment by dams, or even distancing them from their young for varying amounts of time, can lead to mortality by nutritional deprivation, if they are not killed by predators first (DelGiudice et al. 2015).

As with domestic species, blood analyses, both hematology and serum profiles, have proven to be of unique value in assessing aspects of the nutritional, hydration, reproductive, and overall health and disease status of wild animals and in understanding physioecological relationships (Benjamin 1981; Seal et al. 1981; DelGiudice et al. 1990*a*,*b*, 1992, 1994, 2007). Blood data have illuminated the variable range of "normal" and pronounced deviations from the normal condition, not otherwise observable. Most blood-sampling and analyses have involved juveniles ≥6 months old and adults (≥1 year old) of the species, but some investigations have involved neonates, both in captivity and in the wild (Tumbleson et al. 1970, Rawson et al. 1992, Kunkel and Mech 1994, Sams et al. 1995, Carstensen Powell and DelGiudice 2005). There have been no reports of blood profiles of free-ranging moose neonates.

In partial response to the decline of moose in Minnesota, herein our goal is to begin to fill this information void by documenting extensive blood profiles (*reference values* for blood constituents) of free-ranging newborn moose. Accumulating and reporting reference values from different studies of moose and other members of Cervidae relative to the circumstances of those efforts aids in the accurate biological interpretation of future blood data. We began capturing and GPS-collaring moose neonates in 2013. Our relatively brief handling protocol included blood-sampling, similar to our VHF study of white-tailed deer (*Odocoileus virginianus*) neonates (Carstensen Powell and DelGiudice 2005). However, unlike in that study, we experienced a relatively high rate of unpredictable, capture-induced abandonment of neonates (DelGiudice et al. 2015), which prompted us to reduce handling time prior to the end of capture operations.

OBJECTIVES

1. Determine potential relations between age and measures of body size (body mass and hind foot length) at capture to values of hematological and serum characteristics
2. Determine whether values of specific blood characteristics, age, or body size are indicative of conditions which predispose certain neonates to capture-induced abandonment or mortality, or early natural mortality (≤32 days of age)

STUDY AREA

Calf captures were conducted on a 6,068-km^2 study area located between 47°06'N and 47°58'N latitude and 90°04'W and 92°17'W longitude in northeastern Minnesota. This is the Northern Superior Upland region (MNDNR 2015), characterized by a variety of wetlands, including bogs, swamps, lakes, and streams; lowland stands of northern white cedar (*Thuja occidentalis*), black spruce (*Picea mariana*), and tamarack (*Larix laricina*); and uplands of balsam fir (*Abies balsamea*) and pines (*Pinus spp.*), often intermixed with trembling aspen (*Populus tremuloides*) and white birch (*Betula papyrifera*).

Most recently, the wolf (*Canis lupus*) density in northern Minnesota was estimated at 3.1 wolves/100 km^2 (Erb and Sampson 2013). Mean black bear (*Ursus americanus*) density in Bear Management Unit 31 (most of the study area) in 2008 was 23 bears/100 km^2 (Garshelis and

Noyce 2011). White-tailed deer share most of the study area with moose, are managed at pre-fawning densities of <4 deer/km^2, and are primary prey of wolves (Nelson and Mech 1986, DelGiudice et al. 2002, MNDNR 2011). Black bears and wolves also are a major source of mortality of deer neonates throughout summer (Kunkel and Mech 1994, Carstensen et al. 2009).

Lenarz et al. (2010) reported a general increase in maximum daily temperatures at Ely, Minnesota from 1960 to 2007. Mean daily minimum and maximum temperatures ranged from –5.2°C to 13.3°C and 3.3°C to 24.6°C, respectively, during April–July 2013 (http://mrcc.isws.illinois.edu/ CLIMATE/). Additional details of the study area are provided elsewhere (Severud et al. 2015).

METHODS

On 1 May 2013, Iridium GPS collars (Vectronic Aerospace GmbH, Berlin, Germany) previously placed on adult females were reprogrammed from recording 1 fix/4 hours to an hourly fix rate. We began monitoring movements of 50 and 17 GPS-collared adult female moose determined to be pregnant and nonpregnant, respectively, by serum progesterone concentrations (≥ 2.0 ng/ml, Testa and Adams 1998) from blood collected during late January–early February captures (Butler et al. 2013). A last incisor was extracted from most adults for aging by cementum annuli (Sergeant and Pimlott 1959). Additional details of adult captures are presented by Butler et al. (2013). We similarly monitored 6 collared adult females not blood-sampled during winter capture and so were assigned a pregnancy status of "unknown." Our primary monitoring objective was to record when and where individual pregnant females increased locomotor activity reflected by their "calving movement" (Severud et al. 2015). Adult location fixes, and subsequently calf fixes, were transmitted 4 and 8 times/day, respectively, to our base station (Severud et al. 2015). We used 3 different and complementary approaches for monitoring the hourly locations and movements of mothers and their GPS-collared neonates: a base station computer, a web-mapping service, and automated reports (Severud et al. 2015).

We assumed that once females made their calving movement then localized, the birthing process began, and they calved within 12 hours. We then allowed an additional 24 hours for bonding between the mother and its young; then calves were identified as "eligible" for capture. Each morning our team provided the commercial capture crew (Quicksilver Air, Inc., Fairbanks, Alaska) with a list of females (identification numbers and VHF radio frequency) and their most recent GPS coordinates. The capture crew located specified mothers and captured and collared their calves as time and conditions allowed on a daily basis.

The helicopter capture crew located the target mother from the air and then landed some distance away to allow handler(s) to disembark and approach the calves on foot. Then from overhead, with 2-way communication, the helicopter pilot guided the handler(s) into the calf or calves before again landing out of sight. The calf-handling protocol included fitting a 420-g GPS collar (GPS PLUS VERTEX Survey-1 GLOBALSTAR with expandable belt, Vectronic Aerospace GmbH, Berlin, Germany); fixing ear tags; collecting 25 ml of blood by syringe from the jugular vein into EDTA tubes for hematology and into 2 serum tubes for chemistry profiles; weighing the calf to the nearest 0.5 kg with a spring scale; measuring morphological characteristics by tape measure (e.g., ± 1 cm; hind foot length [HFL]) and rectal temperature (± 0.1°F) by digital thermometer; and a physical examination to document injuries or abnormalities. Only 4 of 31 mothers actually exhibited overt aggression, and most calves did not move more than 10 m from where they were first observed to where they were captured and handled. Additional details of captures and handling are reported elsewhere (Severud et al. 2015). The handling protocol was designed to require about 5–6 min per calf to limit separation from the mother (Keech et al. 2011). Ultimately the handling crew captured, handled, and released all observed twins together. All captures and handling protocols adhered to requirements of the Institutional Animal Care and Use Committee for the University of Minnesota (Protocol 1302-30328A) and followed guidelines of the American Society of Mammalogists (Sikes et al. 2011).

Whole blood was analyzed for hematological characteristic and serum for chemistries, metabolites, and electrolytes at the Veterinary Clinical Pathology Laboratory at the University of Minnesota (St. Paul). Hormones (total and free T_3 and T_4, cortisol) were assayed by the Diagnostic Center for Population and Animal Health at Michigan State University (Lansing).

We examined relationships between values of blood characteristics and neonate size (body mass [BM] and HFL) at capture, age (days) at capture and death, and death time since capture by simple linear regression analyses in Excel (Version 14.0.7153.5000, Microsoft Corporation 2010). Because 40% of neonate mortalities have occurred during the first 30 days of age (Severud and DelGiudice, unpublished data) during 2013–2015, we examined hematological and serum data for potential relationships with estimated age at death or death time since capture within 32 and 30 days, respectively. Additionally, we analyzed blood and morphological data for differences between those that survived $\leq$32 days versus >32 days of capture.

RESULTS

We blood-sampled and otherwise handled 16 moose neonates (8 males, 8 females) of 49 captured and GPS-collared during 8–17 May 2013 (Severud et al 2015). Eleven were twins and 5 were singletons. Mean age, BM, HFL, and rectal temperature at capture were 2.9 days ($\pm$ 0.34, range = 1.4–6.0 days), 16.8 kg ($\pm$ 0.5, range = 13.8–20.5 kg), 46.9 cm ($\pm$ 0.3, range = 45.0–49.0 cm), and 101.5 ^{o}F ($\pm$ 0.3, range = 99.9–103.4^{o}F), respectively. Blood-sampling did not include an EDTA tube (i.e., only serum) for 3 of the 16 neonates. Because capture-induced abandonments (9 of 49 neonates) occurred intermittently throughout capture operations, beginning on the first day, we discontinued blood-sampling after 12 May in an effort to make the handling protocol less invasive and reduce abandonment (DelGiudice et al. 2015). Ultimately, this modification did not have the desired effect; indeed, the frequency of capture-induced abandonment was actually higher when we did not collect blood.

Hind foot length was positively related to BM at capture for all 16 neonates (Figure 1), but this relationship ($y = 1.3632x - 47.519$) was even stronger ($r^2 = 0.690$, $P = 0.020$) for the 7 neonates that died within 32 days of age (within 30 days of capture). Age (1.4–3.2 days old), BM (13.8–20.5 kg), and HFL (45.0–48.0 cm) at capture were inversely related to time of death within 30 days of capture/release; neonates captured and blood-sampled at a younger age and smaller body size tended to live longer in the short-term (Figures 2 and 3).

Hematological and serum constituent values can range rather widely and serve as reference values when related to individual neonate and birth characteristics (Tables 1 and 2). Red blood cell (RBC) and white blood cell (WBC) counts, packed cell volume (PCV), and hemoglobin (Hgb) concentration were positively related to BM of neonates at capture (Figures 4 and 5). Hind foot lengths of these neonates were not significantly (P >0.05) related to these hematological characteristics. Values of hematological characteristics were not related to time to death within 30 days of capture/release. Hematological characteristics that differed significantly ($P \leq 0.05$) between neonates that died within 32 days of birth versus those that survived beyond 32 days, included segmented neutrophils (81.6 $\pm$ 1.3, range = 78.0–84.1 versus 70.4 $\pm$ 3.4, range = 52.1–78.0% [x10^3/uL]), lymphocytes (13.2 $\pm$ 2.1, range = 6.9–19.1 versus 23.3 $\pm$ 2.8, range = 16.1–36.0% [x10^3/uL]), and platelets (421 $\pm$ 17.6, range = 364–460 versus 650 $\pm$ 93.7, range = 357–1,065 x10^3/uL).

The most meaningful associations of serum constituents included significant relationships between age at capture and free thyroxine (T_4; $y = -2.157x + 25.460$, $r^2 = 0.381$, $P = 0.011$) and free triiodothyronine (T_3; $y = -1.700x + 15.407$, $r^2 = 0.260$, $P = 0.044$), as well as marginally significant relationships of BM to serum urea nitrogen (SUN, $y = 1.683x - 10.403$, $r^2 = 0.218$, $P = 0.068$) and cortisol ($y = 50.809x - 683.6$, $r^2 = 0.207$, $P = 0.077$) concentrations. Focusing on the 7 neonates that died within 32 days of age yielded a stronger relationship of BM to SUN ($y = 3.638x - 39.364$, $r^2 = 0.591$, $P = 0.043$) and to cortisol ($y = 128.8x - 1851.1$, $r^2 = 0.649$, $P = 0.029$) than when all were included. Sorbitol dehydrogenase was the only serum characteristic that differed significantly between neonates that died within 32 days of birth (17.0 $\pm$ 1.6, range = 11–23 U/L) versus those that survived beyond 32 days (12.3 $\pm$ 1.2, range = 6.0–16.0 U/L).

DISCUSSION

This is the first study to report blood data for free-ranging moose neonates. Although the sample sizes are limited, known circumstances of the study, afforded in part by both neonates and dams being GPS collared and by detection of a number of interesting relations, provide context and enhance our understanding of the "normal" range of 13 hematological and 27 serum characteristics. These 16 neonates ranged in age from 1.4 to 6.0 days old, yet body size and values of many of the blood characteristics, including chemistries, metabolites, enzymes, and hormones were highly variable. Because the primary focus of the overall calf study is to assess the influence of seasonal survival, cause-specific mortality, and recruitment on the population trajectory (Severud et al. 2015), we presupposed that blood data coupled with body size would reveal conditions which might predispose newborns to an early fate. Without the support of blood analyses, it seemed likely that certain conditions would not be recognized. Causes of death for neonates not surviving beyond 32 days of age, included predation by wolves (2) and black bears (1), capture-induced abandonment followed by starvation (1) or euthanization (1), natural abandonment (1), and "capture-related mortality" (1) (Severud et al. 2015).

Mean BM (16.8 kg) of blood-sampled neonates was typical (Schwartz 2007), but overall varied widely (13.8–20.5 kg). Anecdotal reports of low survival probability for ungulate neonates of small BM or underdeveloped skeletally are not uncommon (Verme 1962, Langenau and Lerg 1976, Livezey 1990, Carstensen et al. 2006, Patterson et al. 2013). Overall there was no difference in body size (BM or HFL) or age at capture between those that died within 32 days of age and those that survived longer; however, interestingly, older and larger neonates, by both BM and HFL, did not live as long during that short-term window post-capture. Although BM and HFL at capture were highly correlated (r = 0.831), particularly for neonates that died within 32 days of age, RBC counts, Hgb, PCV, and WBC counts were positively related to only BM. While HFL may be a superior metric of prenatal development (Markgren 1969, Schwartz and Hundertmark 1993), our data suggest that early post-natal BM is linked more strongly to the neonate's physiology. Similar correlations between HFL and BM have been observed for moose and white-tailed deer neonates and aging juveniles (Carstensen Powell and DelGiudice 2005, Schwartz 2007), but potential relations of hematology to BM or HFL at capture were not addressed.

The most notable of neonates dying within 30 days of capture, male singleton no. 520, initially was assigned "capture-related mortality" as the cause of death. This newborn was not abandoned, and died only 4-5 hours (first clue of a preexisting condition) after an uneventful capture and processing. Its dam was the most aggressive towards the handlers, and unlike most, did not flee with their approach (DelGiudice et al. 2015). She remained close during the capture and was with the dead calf when the handler returned to recover it. An immediate necropsy at the Veterinary Diagnostic Laboratory (University of Minnesota-St. Paul), including macroscopic and microscopic examinations, reported an empty gastrointestinal tract (starvation) as the most probable proximate cause of death and no signs of physical injury. Why this calf was not nursing or not being nursed even prior to handling is unknown, but this case is indicative of *unknown pre-existing factors* that can influence the fate of neonates whose mortalities may appear to be capture-induced due to temporal juxtaposition, but that were actually unrelated. Neonate 520 was 1 of the 2 largest (20.5 kg, 48.0 cm HFL) of the 16 neonates blood-sampled (and 49 total), was near average age (2.6 days) at capture, and had an average rectal temperature (100.9ºF). However, physiological assessment by hematological and serum profiles subsequently revealed several anomalies about this animal's unique condition. Briefly, this neonate had the highest RBC, Hgb, and PCV values of all of the neonates, strongly indicative of hemoconcentration due to severe dehydration (Benjamin 1981, DelGiudice et al. 1990*b*). Additionally, this animal exhibited a leukocytosis, segmented neutrophilia, and lymphopenia, likely reflective of the stress associated with his condition (Benjamin 1981). Most notable from its serum profile, neonate 520 exhibited seriously elevated serum urea nitrogen (SUN, 44 mg/dL) and cortisol (1,068 nmol/L) concentrations, $\geq$2–3 and 25

times higher, respectively, than those of all other neonates. These metabolite and hormone concentrations alone are indicative of severe net catabolism of endogenous protein (muscle-wasting) and stress (Benjamin 1981, DelGiudice et al. 1990*a*,*b*, 1994) associated with prolonged (as it were) starvation in this 2.6-day old. These findings were accompanied by the lowest serum free T_3 (5.4 pmol/L) and glucose (8 mg/dL) concentrations, indicative of 520's seriously low energy status, as well as by the lowest serum globulin value (1.0 g/dL), at least partially reflective of its compromised immunocompetence (Sams 1994).

Herein, we provided just a few highlights of the important information that can synthesized from samples and data obtained during the handling of moose neonates. More in-depth analyses of the hematological and serum profiles are ongoing, and doubtless, will provide a more complete picture of the physiological status and condition of these moose neonates when they are in the most vulnerable phase of their lives. The better we come to understand this phase and the often obscure challenges confronted by individual calves over the long-term, the better chance we have of formulating and implementing management strategies to improve annual population recruitment, growth, and persistence.

ACKNOWLEDGMENTS

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Table 1. Mean (± SE) values of hematological characteristics of moose neonates (1.4–6.0 days old) in northeastern Minnesota, 8–12 May 2013.

Characteristics[a]	Mean	*n*	SE	Min	Max
RBC ($x10^3$/uL)	6.1	13	0.17	5.1	6.8
Hgb (g/dL)	9.6	13	0.3	8.2	11.8
PCV (%)	31.4	13	1.1	25.7	38.9
WBC ($x10^3$/uL)	5.4	13	.37	3.9	7.8
Neutrophil segs % ($x10^3$/uL)	74.4	13	2.5	52.1	84.1
Lymphocytes % ($x10^3$/uL)	20.0	13	2.3	6.9	36.0
Monocytes % (x10^3/uL)	3.1	13	0.81	0.0	9.1
Eosinophils % ($x10^3$/uL)	2.0	13	0.49	0.0	5.9
MCV (fL)	51.8	13	1.2	45.9	57.2
MCH (pg)	15.9	13	0.3	14.1	17.6
MCHC (g/dL)	30.7	13	0.3	28.7	31.8
Platelets ($x10^3$/uL)	550	13	58.3	357	1065
Fibrinogen (g/dL)	0.5	13	0.03	0.3	0.7

[a]RBC = red blood cells, Hgb = hemoglobin, PCV = packed cell volume, WBC = white blood cells, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, and MCHC = mean corpuscular hemoglobin concentration.

Table 2. Mean (± SE) values of serum characteristics of moose neonates (1.4–6.0 days old) in northeastern Minnesota, 8–12 May 2013.

Characteristics[a]	Mean	*N*	SE	Min	Max
SUN (mg/dL)	17.9	16	2.0	10	44
Creatinine (mg/dL)	0.73	16	0.048	0.5	1.2
SUN:Creatinine	24.2	16	1.7	14	36
Ca (mg/dL)	10.1	16	0.18	8.8	11.4
P (mg/dL)	8.9	16	0.30	5.9	11.0
Mg (mg/dL)	1.7	16	0.04	1.5	2.0
Total Protein (g/dL)	4.2	16	0.17	3.3	5.9
Albumin (g/dL)	2.1	16	0.06	1.7	2.6
Globulin (g/dL)	2.1	16	0.17	1.0	3.7
Na (mmol/L)	141	16	0.8	137	150
Chloride (mmol/L)	95.3	16	0.5	92	100
K (mmol/L)	5.1	16	0.11	4.4	5.9
Bicarbonate (mmol/L)	22.3	16	1.0	15.2	32.6
Osmolality	283	16	1.7	276	304
Anion gap	28.5	16	1.0	22.0	39.0
Total Bilirubin (mg/dL)	0.39	16	0.02	0.3	0.7
Alkaline phosphatase (U/L)	294	16	21.1	170	436
GGT (U/L)	52.0	16	6.3	21	101
Sorbitol dehydrogenase (U/L)	14.6	16	1.1	6	23
Aspartate transferase (U/L)	63.0	16	4.8	42	110
Creatine kinase (U/L)	135	16	22.2	52	399
Glucose (mg/dL)	108	16	8.7	8	145
TT_4 (nmol/L)	96	16	6.2	64	156
TT_3 (nmol/L)	4.2	16	0.16	3.0	5.4
FT_4 (pmol/L)	19.3	16	1.2	11	27
FT_3 (pmol/L)	10.6	16	1.12	5.4	21.2
Cortisol (nmol/L)	172	16	60.6	42	1068

[a]SUN = serum urea nitrogen, Ca = calcium, P = phosphorous, Mg = magnesium, Na = sodium, K = potassium, TT_4 = total thyroxine, TT_3 = total thyronine, FT_4 = free thyroxine, and FT_3 = thyronine.

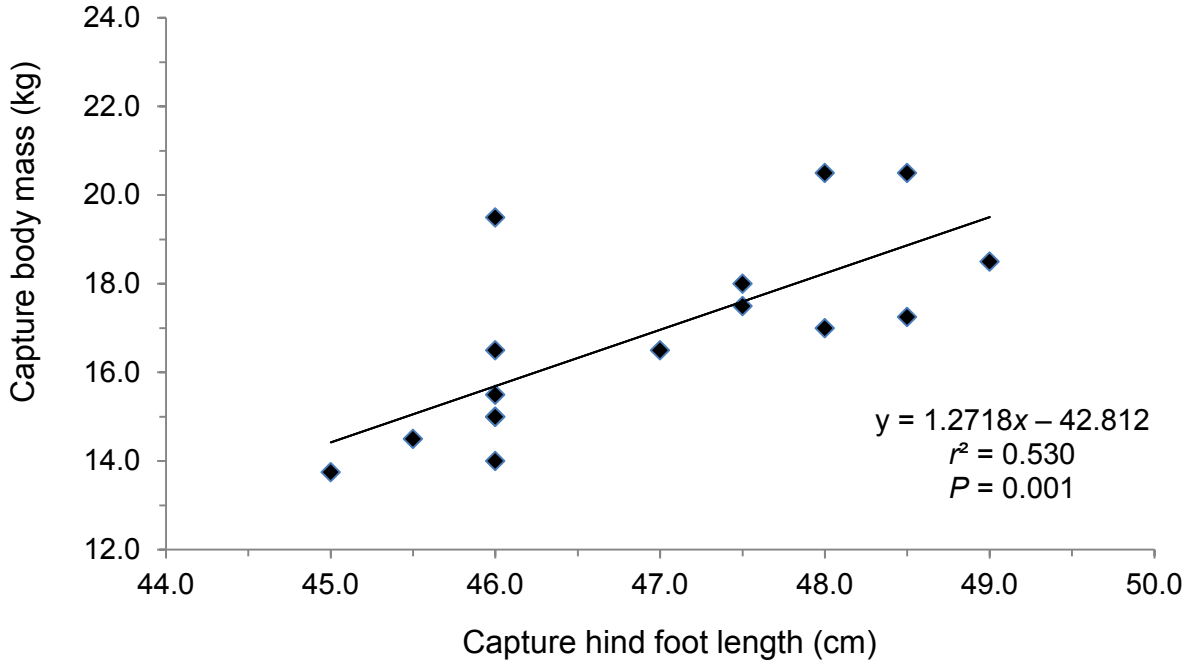

Figure 1. Relationship of hind foot length to body mass at capture for 16 free-ranging moose neonates (1.4–6.0 days old), northeastern Minnesota, 8–12 May 2013.

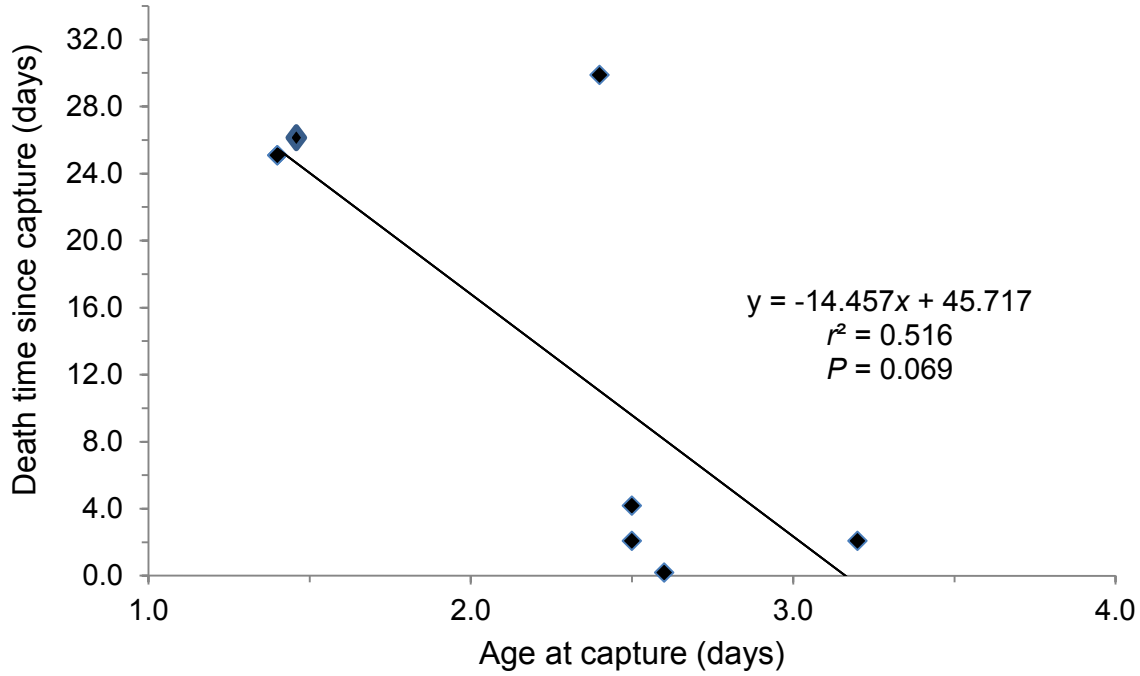

Figure 2. Relationship between age at capture and time to death since capture (within 30 days) for free-ranging moose neonates (1.4–6.0 days old), northeastern Minnesota, 8–12 May 2013.

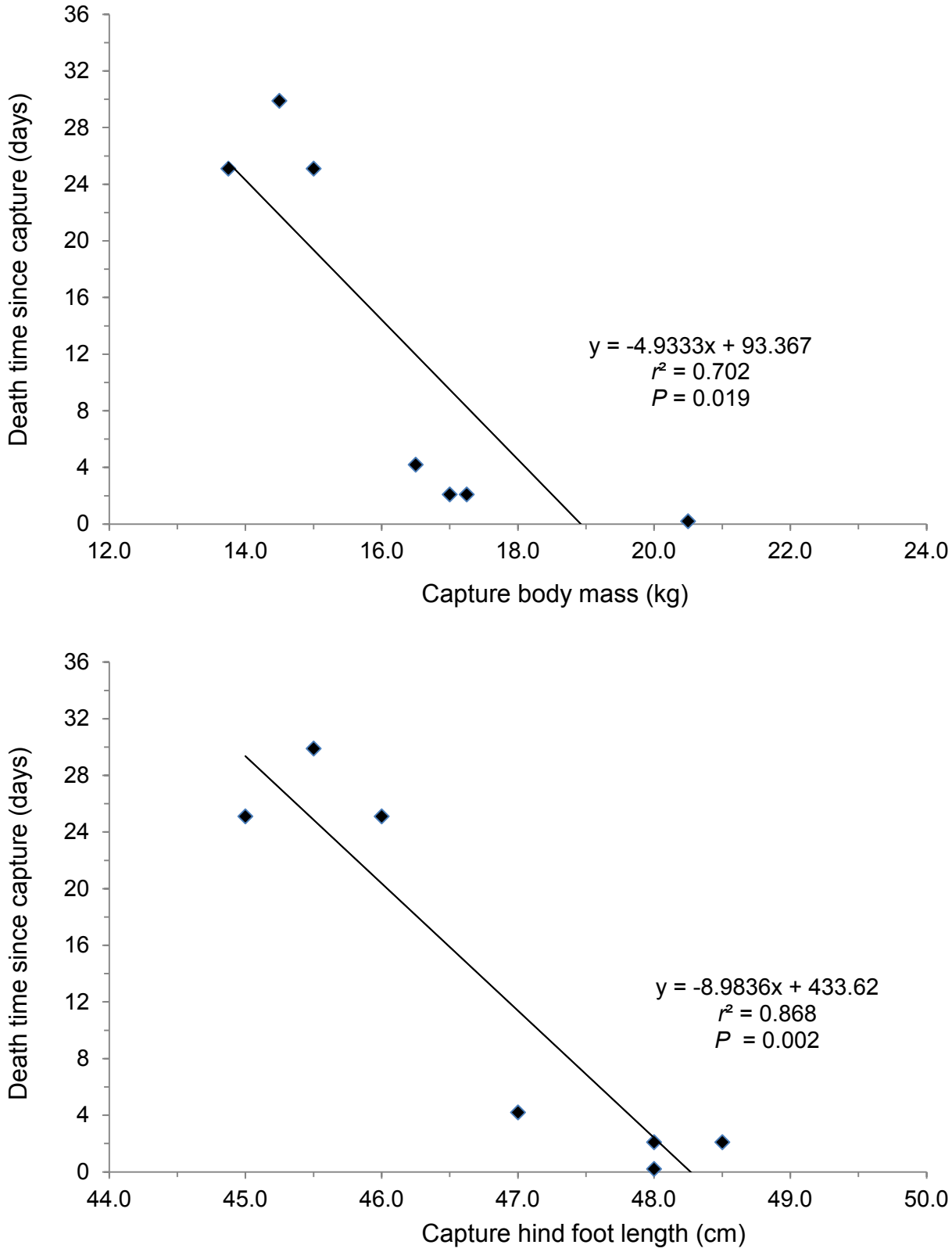

Figure 3. Relationships between body mass (top) and hind foot length (bottom) at capture and time to death since capture (within 30 days) for free-ranging moose neonates (1.4–6.0 days old), northeastern Minnesota, 8–12 May 2013.

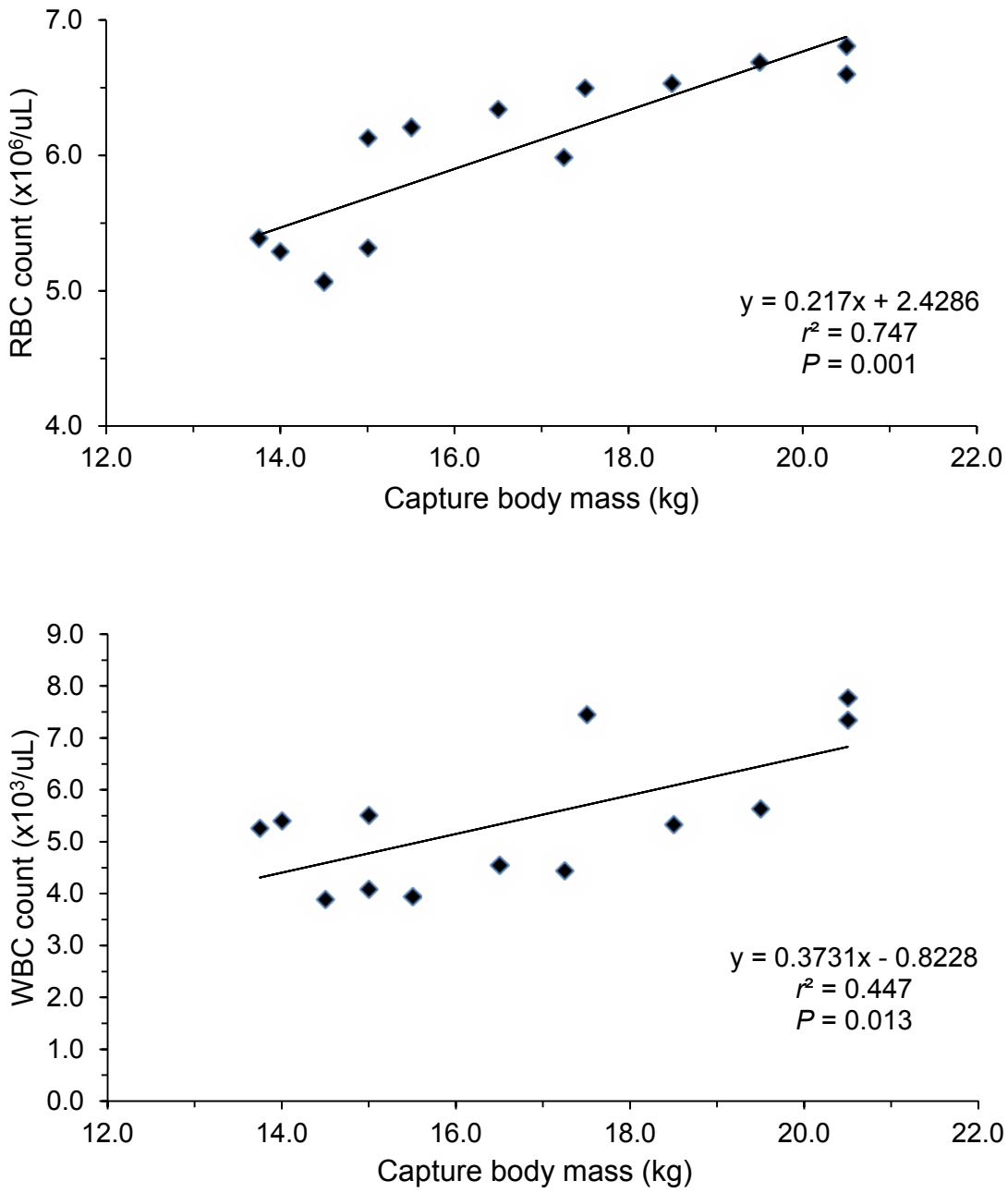

Figure 4. Relationships of body mass to red blood cell (RBC, top) and white blood cell (WBC, bottom) counts at capture of free-ranging moose neonates (1.4–6.0 days old), northeastern Minnesota, 8–12 May 2013.

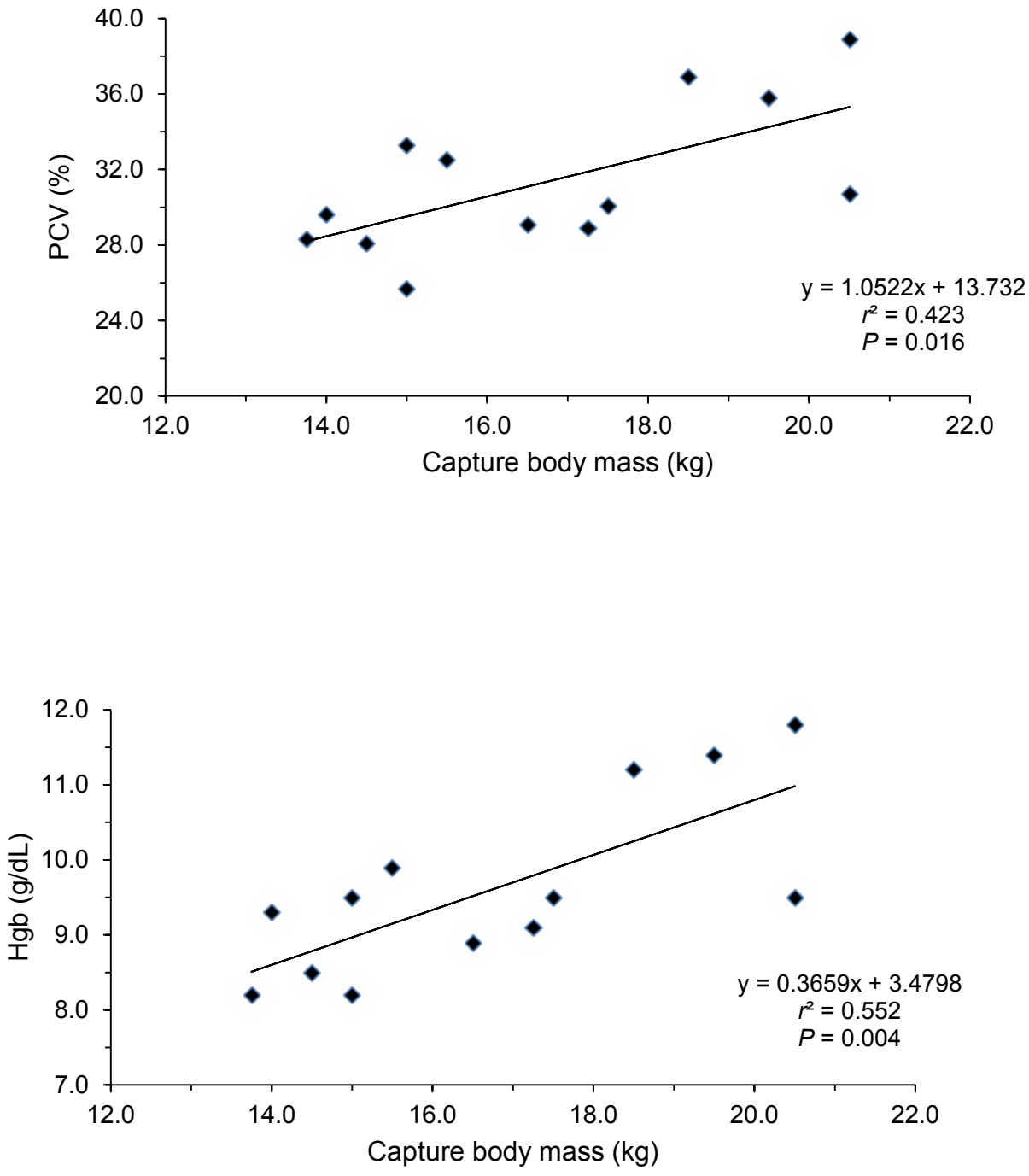

Figure 5. Relationships of body mass to packed cell volume (PCV, top) and hemoglobin (Hgb, bottom) concentration at capture of free-ranging moose neonates (1.4–6.0 days old), northeastern Minnesota, 8–12 May 2013.

MNDNR

ASSESSING WINTER NUTRITIONAL RESTRICTION OF MOOSE AND ITS RELATION TO POPULATION DYNAMICS IN NORTHEASTERN MINNESOTA, WINTERS 2013–2015

Glenn D. DelGiudice and William J. Severud[1]

SUMMARY OF FINDINGS

The moose (*Alces americanus*) population in northeastern Minnesota has declined an estimated 61% from 2006 to 2015. As in northwestern Minnesota, a number of complex ecological relationships between undernutrition, pathogens, predation, and environmental factors (e.g., habitat, ambient temperature) are likely exerting pressure on moose and contributing to this recent dramatic decline. Nutrition is centrally related to our understanding of all other aspects of wildlife ecology, including population performance. Winter nutritional restriction of moose and other northern ungulates may be physiologically assessed by serial collection and chemical analysis of fresh urine in snow (snow-urine). Urinary urea nitrogen:creatinine (UN:C) ratios have shown the most potential as a metric of winter nutritional status, have been related to moose population dynamics on Isle Royale, and have elucidated aspects of the population-level relationship of nutritional restriction with a winter tick (*Dermacentor albipictus*) epizootic and differences in habitat. During 5–6, 2-week sampling intervals (9 January–26 March 2013–2015) we collected annual totals of 123, 307, and 165 moose snow-urine samples, respectively. Overall, mean seasonal UN:C ratios were 3.7, 2.9, and 2.9 mg:mg for winters 2013–2015, respectively. The mean population UN:C ratio for winter 2013 was above the threshold indicative of severe nutritional restriction (i.e., a starvation diet) and accelerated body protein catabolism, whereas during 2014 and 2015 the corresponding values were just below the moderately severe interval (3.0–3.4 mg:mg). Additionally indicative of the unique severity of nutritional restriction in 2013, nearly one-third of all samples collected yielded UN:C ratios $\geq$3.5 mg:mg. The corresponding percentages of winters 2014 and 2015 were less than in 2013.

Perhaps the ultimate value to management of assessments of nutritional status of free-ranging animals comes when the findings can be related to the performance and dynamics of the population and other ecological factors challenging that performance. Presently, our population-level nutritional assessments are closely tracking (r^2 = 0.970) population estimates of moose from the state's annual aerial survey. Although nutritional restriction varied among the 3 winters, elevated UN:C values suggested a level of deprivation not supportive of population stability or growth, and variation in winter conditions, as indexed by the winter severity index (WSI), is not directly responsible. We also have documented that the level of severe nutritional restriction is inversely related (r = –0.91) specifically to the variation of *natural* survival rates of winter and winter-to-summer of adult global positioning system (GPS)-collared moose. Because these relationships are consistent with that of assessed nutritional restriction with the population estimates, it suggests that the study cohort of GPS-collared adult moose is indeed representative of the free-ranging population in northeastern Minnesota. While such relationships do not substantiate cause-and-effect, presently they provide the best preliminary empirical evidence indicating that inadequate winter nutrition at the population level is intricately related to the declining trajectory of moose numbers in northeastern Minnesota.

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INTRODUCTION

The moose population in northeastern Minnesota has been declining steadily since 2006. According to the annual aerial survey, conducted in mid-winter, the population has decreased 61% from an estimated 8,840 to 3,450 moose (DelGiudice 2015). The decreasing trajectory has been similar to that documented previously for moose in northwestern Minnesota, estimated at <100 moose in 2007 (Murray et al. 2006, Lenarz et al. 2009). Additionally, mean annual mortality rates of adults have been similarly high in the northwest and northeast during the declines (21%, Murray et al. 2006, Lenarz et al. 2009) and have remained elevated in most years in the northeast (R. A. Moen, unpublished data; Carstensen et al. 2014). This poses a complex and immediate management challenge, which has prompted a multi-prong approach involving studies investigating numerous aspects of moose ecology and habitat, necessary because, as in northwestern Minnesota, the recent decline is likely attributable to a number of factors. Climate change (i.e., warming temperatures) has been implicated in the decline of both populations (Murray et al. 2006; Lenarz et al. 2009, 2010). In northwestern Minnesota, malnutrition and pathogens were identified as contributing factors to the population's decrease, and in ongoing studies of survival and cause-specific mortality of global positioning system (GPS)-collared moose in northeastern Minnesota, health-related factors and predation by wolves (*Canis lupus*) and black bears (*Ursus americanus*) have had notable impacts on adult and calf survival (Carstensen et al. 2014, Severud et al. 2015).

"Knowledge of wildlife nutrition, as a component of both wildlife ecology and management, is central to understanding the survival and productivity of all wildlife populations…" (Robbins 1993). While current investigations report that disease, parasites, and predation are contributing significantly to the moose decline in northeastern Minnesota, there should be little doubt that seasonal nutrition is playing a key role and is intricately related to other environmental factors. The winter nutritional bottleneck, associated with natural reductions of forage abundance, availability, and quality, typically imposes the greatest challenge on northern ungulates (Mautz 1978, Schwartz and Renecker 2007). Further, most adult females are gravid at this time, posing additional energetic and nutritional challenges, particularly during late-winter and early-spring. Although these animals have generally adapted over millions of years to this seasonal nutritional deprivation, uniquely harsh winters coupled with other compromising extrinsic factors can have serious consequences for adult and calf survival, subsequent reproductive success, and population dynamics (DelGiudice al. 1989, 1997; Robbins 1993; Schwartz and Renecker 2007). Moose and other members of the deer family (Cervidae) may withstand losses of 33% of their peak fall body mass while they rely heavily on all of their fat reserves and up to 33% of their endogenous protein (mostly as lean body mass) to compensate for natural dietary restriction and attempt to fulfill their energy and protein requirements. However, severity of nutritional restriction of ungulates may be mediated by a variety of environmental factors, including diet composition, disease, parasites, and density of conspecifics or other nutritionally competing species (DelGiudice et al. 1997, 2001; Schwartz and Renecker 2007).

Winter nutritional restriction of moose and other northern ungulates may be physiologically assessed by serial collection and chemical analysis of fresh urine voided in snow (snow-urine; DelGiudice et al. 1988, 1997, 2001; Moen and DelGiudice 1997, Ditchkoff and Servello 2002). Urea nitrogen (interpreted as a ratio to creatinine, UN:C) is one of many chemistries investigated for its potential value as an indicator of nutritional restriction, and it has shown the most promise in studies of white-tailed deer (*Odocoileus virginianus*), moose, elk (*Cervus elaphus*), and bison (*Bison bison*). The value of UN is related to its role as an end-product of protein metabolism, both dietary crude protein and endogenous protein. How UN:C values respond to diminishing intake of crude protein and digestible energy, as well as to accelerated net catabolism of endogenous protein as dietary restriction progresses and fat reserves are depleted over time, contributes to its value as an indicator of the severity of nutritional deprivation.

On Isle Royale winter nutritional restriction of moose was assessed by serial collection and analysis of snow-urine for 7 years (DelGiudice et al. 1997). Urinary UN:C ratios, as affected by a winter tick epizootic and simultaneous nutritional restriction associated with habitat differences, were strongly related to population dynamics of moose, including significant declines and recovery to historic high numbers (DelGiudice et al. 1997). Collection and chemical analysis of snow-urine also elucidated relationships between winter nutritional restriction, winter severity, and mortality rates of deer in northern Minnesota and Maine, and elk and bison in Yellowstone National Park subjected to catastrophic fires (DelGiudice et al. 1989, 1997, 2001; Ditchkoff and Servello 2002).

For the past 3 years (2013–2015) we have been non-invasively assessing winter nutritional restriction of moose by serial collection and chemical analysis of fresh snow-urines to better understand how restriction varies annually, as winters progress, and spatially over the landscape of northeastern Minnesota. Most importantly, we are examining the potential relationships between variation of nutritional restriction, mortality rates, and long-term dynamics of the moose population to quantify the impact of winter nutrition. Our prediction is that winter nutritional restriction is critically associated with the performance and declining trajectory of northeastern Minnesota's moose population at a landscape scale. Findings will set the stage for additional work assessing nutritional relationships of moose to variations in habitat and other factors.

OBJECTIVES

1. To assess overall winter nutritional restriction, estimate the proportion of the population experiencing severe nutritional restriction (as indicated by UN:C ratios $\geq$3.5 mg:mg) and quantify how its annual variation relates to corresponding changes in population estimates.
2. To relate these assessments of winter nutritional restriction at the population level to seasonal natural survival rates of adult collared moose in a companion study.

STUDY AREA

The 6,068-km^2 study site for this research (Figure 1) is the same as that of the Environmental and Natural Resources Trust Fund (ENRTF)-supported research addressing survival and cause-specific mortality of adult moose in northeastern Minnesota (Butler et al. 2011). This area has been classified as the Northern Superior Upland region (Minnesota Department of Natural Resources [MNDNR] 2007). The MNDNR assesses winter severity (1 Nov–31 May) by a winter severity index (WSI), calculated by accumulating 1 point for each day with a temperature $\leq 0^0$ F (-17.8^0 C, temperature-day) and 1 point for each day with snow depth $\geq$15 inches (38.1 cm, snow-day), for a potential total of 2 points per day. Maximum WSI values were 120–139, $\geq$180, and 100–119 depending upon location for winters 2013, 2014, and 2015, respectively (National Oceanic and Atmospheric Administration [NOAA] 2015). Additional details of the study area are provided elsewhere (Severud et al. 2015).

METHODS

We collected fresh snow-urine specimens of moose during 9 January–26 March 2013–2015. We conducted snow-urine sampling according to a random design. In winters 2013–2015 our field team drove (by truck or snowmobile) a route of approximately 201 km (125 miles), which was divided into 4 legs to distribute the sampling throughout the study area (Figure 1). However, the annual teams were not restricted to this route, and could deviate, particularly on foot, as dictated by the presence of fresh moose sign (e.g., tracks, urine specimens, pellets). Each field team used handheld GPS units loaded with several land coverages (R. G. Wright, Minnesota Information Technology @ Minnesota Department of Natural Resources, Section of Wildlife) and a Superior National Forest map (U. S. Forest Service) to navigate in the field.

To be able to associate urine chemistry data of randomly collected snow-urines and nutritional assessments with specific temporal periods, sampling generally was conducted within 7 days of a fresh snowfall, but most often within 2–4 days. Upon observing fresh moose sign, the team tracked the individual(s) on foot as necessary until they came to a fresh specimen(s). The objective for the random collections was to sample primarily adult (>1 year old) moose (indicated by track and bed size). This was not particularly challenging, because by this time of the year calves comprised only 13–15% of the population (DelGiudice 2015). We focused primarily on the adult age class to facilitate optimum comparability of data.

Specimens were collected and handled as described by DelGiudice et al. (1991, 1997). A GPS waypoint was recorded for each snow-urine specimen collected. Date of the most recent snowfall and comments describing the presence of moose and other sign in the area also were recorded.

Snow-urine specimens were analyzed for UN (mg/dL) and C (mg/dL) by a Roche Cobas Mira autoanalyzer (Roche Diagnostics Systems, Inc., Montclair, NJ) in the Forest Wildlife Populations and Research Group's laboratory. We used 0.1 and 3.0 mg/dL as reliable thresholds for C and UN, respectively, for our autoanalyzer; values below these thresholds were excluded (C. Humpal, MNDNR, personal communication). Data were compared as UN:C ratios to correct for differences in hydration, body size, and dilution by snow (DelGiudice 1995, DelGiudice et al. 1988).

The winter sample collection period (Jan–Mar) was divided into 6, 2-week sampling intervals (1–14 Jan, 15–31 Jan, 1–15 Feb, 16–28 Feb, 1–15 Mar, and 16–31 Mar). Sample sizes for the random snow-urine collections varied by interval due to variability of weather (i.e., snow conditions), equipment availability, logistical challenges, and ease of finding samples. Most of the UN:C data are reported by the entire winter or by sampling interval as means ($\pm$ SE). Additionally, based on past work, urinary UN:C values were assigned to 1 of 3 levels of nutritional restriction: moderate or "normal," <3.0 mg:mg; moderately severe, 3.0–3.4 mg:mg; and severe, ≥3.5 mg:mg (DelGiudice et al. 1997, 2001, 2010). We report the percentage of samples with UN:C values falling within each of these categories.

RESULTS AND DISCUSSION

During 9 January–26 March 2013–2015, annual totals of 123, 307, and 165 moose snow-urine samples, respectively, were collected during 5–6 2-week sampling intervals using our designated routes. The greater number of samples collected during 2014 was largely due to the early and prolonged deep snow cover.

Overall, mean UN:C ratios were 3.7, 2.9, and 2.9 mg:mg for winters 2013–2015, respectively (Figure 2). The mean population UN:C ratio for the entire winter of 2013 was above the threshold indicative of severe nutritional restriction (i.e., a starvation diet) and accelerated body protein catabolism, whereas during 2014 and 2015 the corresponding values were just below the defined moderately severe interval (3.0–3.4 mg:mg). Additionally indicative of the unique severity of nutritional restriction in 2013, nearly one-third of all samples collected yielded UN:C ratios $\geq$3.5 mg:mg (Figure 3). The corresponding percentages of winters 2014 and 2015 were less than in 2013 (Figure 3).

Mean urinary UN:C ratios by 2-week interval of winter 2013 indicated that nutritional restriction was normal or moderate (<3.0 mg:mg) during late-January, but became severe (≥3.5 mg:mg) throughout February and early-March, and was still assessed as moderately severe (3.0–3.4 mg:mg) in late-March (Figure 4). As severe nutritional restriction of individuals progresses with winter, those animals may be under-sampled as some succumb, and those still alive urinate less, a physiological mechanism to conserve water and electrolytes. Percentage of samples with urinary UN:C ratios indicative of severe nutritional restriction peaked (73.3%) in early-February and remained relatively high through late-March (36%, Figure 5). Such elevated values (≥3.5 mg:mg) have been associated with long-term fasting in controlled nutrition studies of captive white-tailed deer and starvation of free-ranging elk, bison, and moose (DelGiudice et al. 1987, 1991, 1997, 2001). The percentage of snow-urine specimens in 2013 with UN:C ratios

indicative of moderately severe to severe nutritional restriction throughout the winter was 45.5% (Figure 3).

During all but the first 2 weeks of February 2014, the mean urinary UN:C ratios remained just below the moderately severe category (i.e., <3.0 mg:mg; Figure 4), and the percentage of samples with ratios indicative of severe nutritional restriction gradually decreased as this winter progressed (Figure 5), either due to an easing of conditions restricting access to forage or because these individuals were being under-sampled, which may be most plausible as previously explained. Although this was the most severe of the 3 winters according to the MNDNR's maximum WSI values, the adverse effects on the population of the intense and prolonged conditions of winter 2013, high spring-summer calf loss and absence of the need to lactate (Severud et al. 2015), may have allowed the surviving animals to rebound nutritionally more quickly and to fare better during winter 2014. This would not be unlike the documented effects on the nutritional status and survival of northern Minnesota deer during the consecutive severe winters of 1996 and 1997 (DelGiudice et al. 2006; G. D. DelGiudice, unpublished data). Overall in winter 2014, UN:C values of 64% of the collected snow-urine samples classified nutritional restriction as moderate (normal), whereas 36% reflected moderately severe to severe restriction, which was less than in 2013 (Figure 3). Similar to winter 2014, severe nutritional restriction of moose was not as prevalent in 2015 as in 2013, but it was up slightly compared to 2014 (Figure 3). However, a higher percentage of moose appeared to be experiencing moderate or normal restriction and a smaller percentage moderately severe than in 2013 and 2014 (Figure 3). Rapidly diminishing snow cover did not allow collection of snow-urine samples or assessments during the last 2 weeks of March 2015, certainly a positive factor relative to moose nutrition at that time.

According to maximum WSI values, winter 2014 was the most severe of the 3 in northeastern Minnesota moose range, followed by 2013, and relatively modest conditions in 2015. Although the WSI numbers have value for annual comparisons of winter conditions, this WSI formula has far greater relevance to the size and energetics of white-tailed deer than for the much larger moose (DelGiudice et al. 2002, 2006; Schwartz and Renecker 2007). Furthermore, while the accumulation of snow-days and temperature-days has proven significant relative to the survival of white-tailed deer (DelGiudice et al. 2002), actual snow depth, its temporal occurrence, and duration may be of equal or greater importance, particularly for moose (Telfer and Kelsall 1984, DelGiudice 1998, DelGiudice et al. 2002, Schwartz and Renecker 2007). The severe restriction of 2013 was most similar to that which occurred in moose during several winters (1988–1990) on Isle Royale associated with serious winter tick infestations and steep population decline (DelGiudice et al. 1997). Abundant evidence from the field in the MNDNR's ongoing studies similarly indicated that the winter tick infestation of moose in northeastern Minnesota was notably more serious during winter 2013 than in 2014 and 2015 as the population continued to decline (Carstensen et al. 2014; M. Carstensen, MNDNR, personal communication).

Perhaps the ultimate value to management of assessments of nutritional status of free-ranging animals comes when the findings can be related to the performance and dynamics of the population and other ecological factors challenging that performance (DelGiudice et al. 1997, Cook et al. 2004). Presently, our population-level nutritional assessments are closely tracking (r^2 = 0.970) population estimates of moose from the annual aerial survey (Figure 6). What is most clear is that although restriction varies among the 3 winters, elevated UN:C values suggest a level of nutritional deprivation not supportive of positive population performance, population stability, or growth. Further, variation in winter conditions is not directly responsible (DelGiudice et al. 1997). We have also documented that variation in the level of severe nutritional restriction of moose is inversely related (r = –0.91) specifically to the variation of *natural* survival rates of winter and winter-to-summer (Figure 7). Importantly, because these relationships are consistent with association of assessed severe nutritional restriction with the population estimates, it suggests that the current study cohort of GPS-collared moose is indeed representative of the free-ranging population in northeastern Minnesota. While such relationships do not substantiate cause-and-effect, presently they provide the best preliminary

empirical evidence indicating that inadequate winter nutrition at the population level is intricately related to the declining trajectory of moose numbers in northeastern Minnesota.

In addition to the multi-year occurrence of severe nutritional restriction of moose, preliminary analyses reveal a vast spatial distribution throughout moose range of collected snow-urine samples with UN:C ratios indicative of severe nutritional deprivation (Figures 8–10). Currently, no unique clusters have been identified, but additional analyses are required. The wide temporal and spatial distributions of severe nutritional restriction suggest that habitat deficiencies at the landscape scale may constitute a primary contributing factor or source of the relatively serious nutritional restriction we documented. Whether habitat deficiencies are related to forage availability and quality, vegetative species composition, or less-than-optimum arrangements of forage openings and forest stands affording seasonal thermal cover for moose should be investigated. Data from additional winter nutritional assessments are required to provide additional support for our conclusions or to refute them. But the current data set, in combination with data from other ongoing habitat and nutritional studies, should at least provide a basis for formulating management recommendations that may be implemented and evaluated in the near future.

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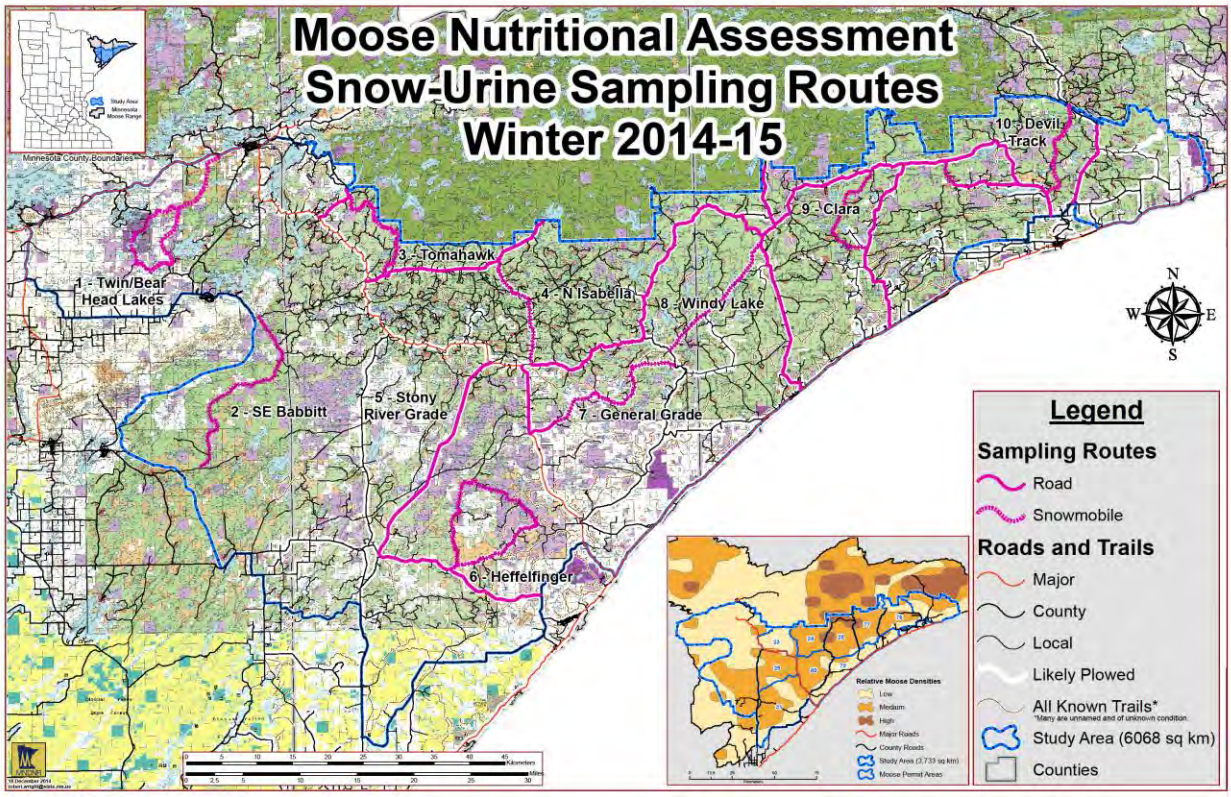

Figure 1. Map depicting the moose study area in northeastern Minnesota and the routes (roads and snowmobile trails in purple) used to distribute the sampling of fresh moose urine in snow (snow-urine) for nutritional assessments throughout the area, 9 January–26 March 2013–2015.

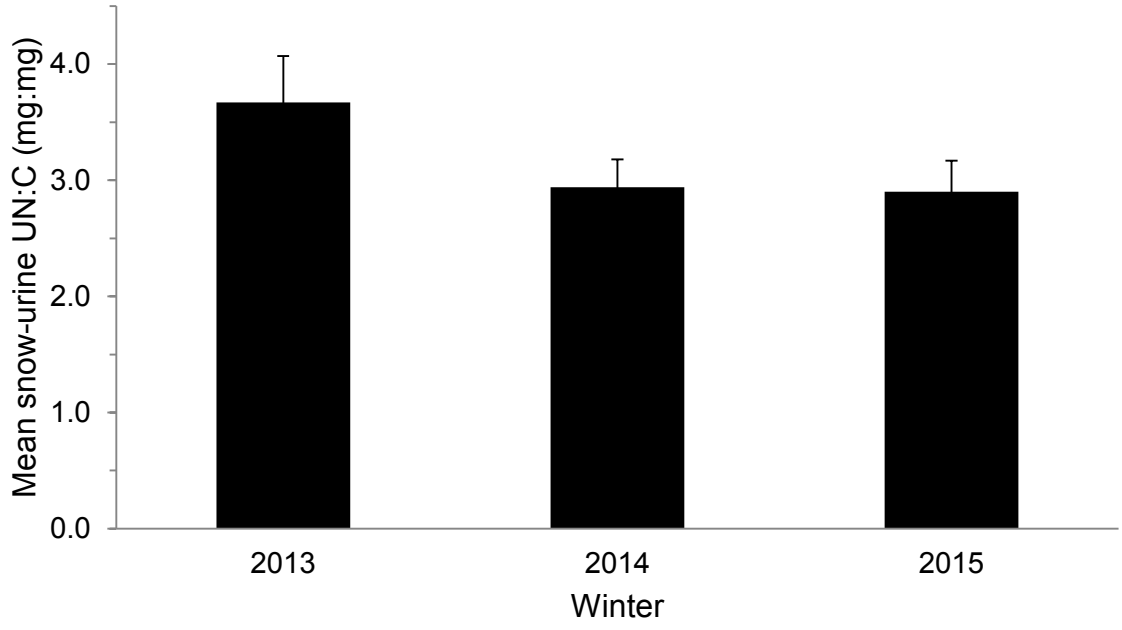

Figure 2. Overall mean (+ SE) urea nitrogen:creatinine (UN:C) ratios of samples of fresh urine voided in snow (snow-urine) by moose and serially collected for assessments of nutritional restriction throughout northeastern Minnesota, 9 January–26 March 2013–2015.

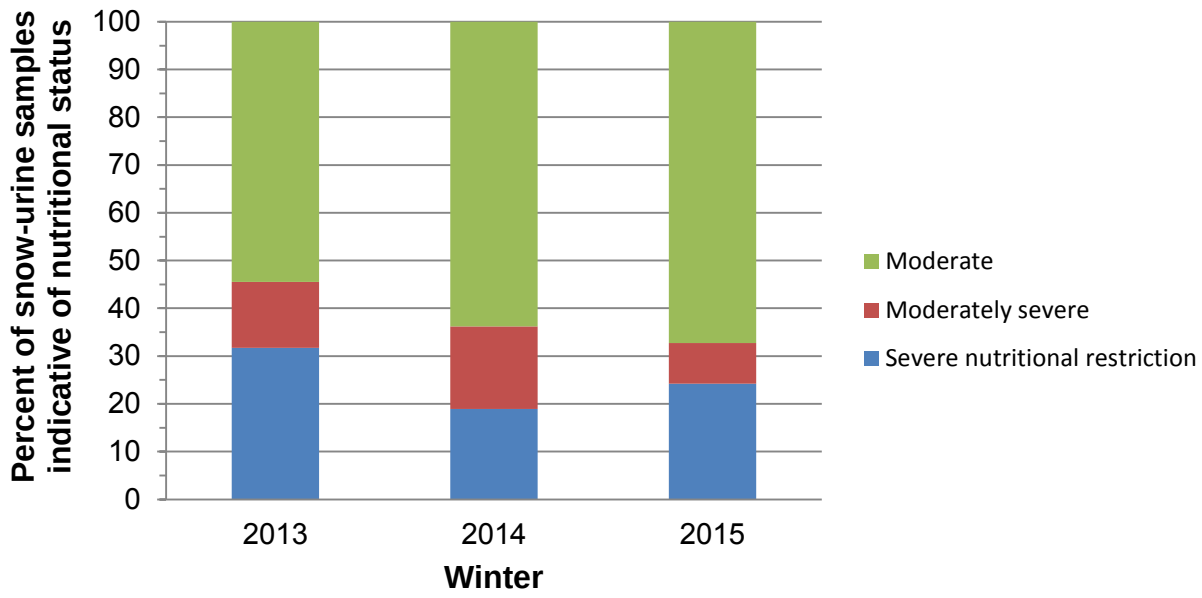

Figure 3. Overall percent of serially collected moose urine samples voided in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios indicative of moderate/normal (UN:C < 3.0 mg:mg), moderately severe (UN:C = 3.0–3.4 mg:mg), and severe nutritional restriction (UN:C ≥3.5 mg:mg) throughout northeastern Minnesota, 9 January–26 March 2013–2015.

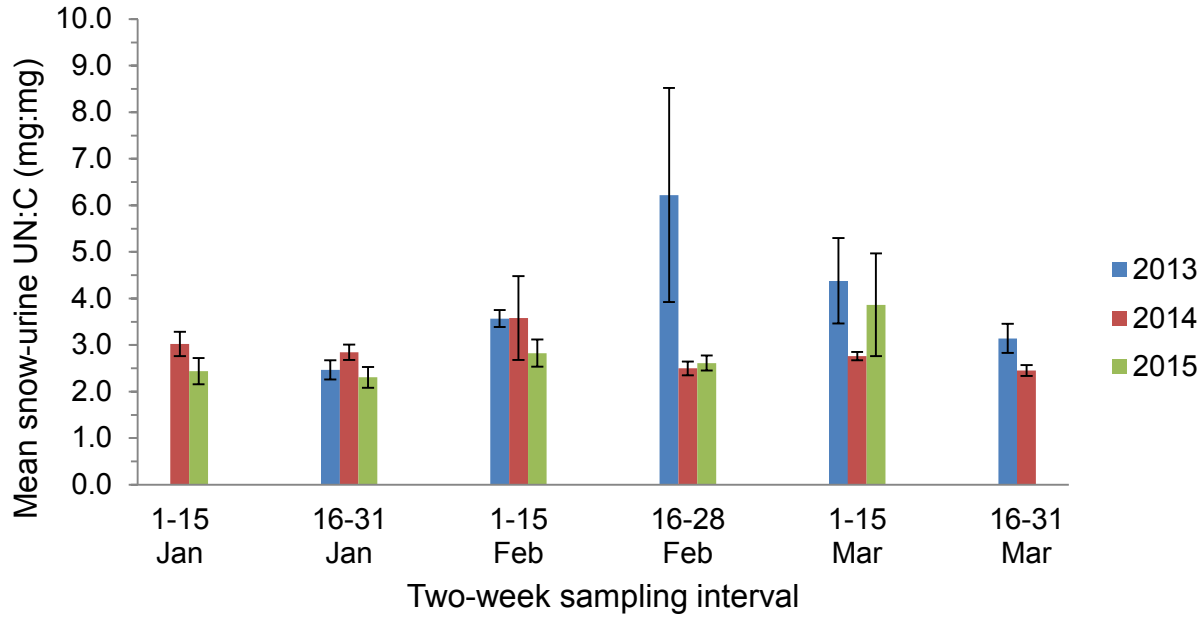

Figure 4. Mean (± SE) urea nitrogen:creatinine (UN:C) ratios of samples of fresh urine voided in snow (snow-urine) by moose and collected during 2-week sampling intervals for assessments of nutritional restriction throughout northeastern Minnesota, 9 January–26 March 2013–2015.

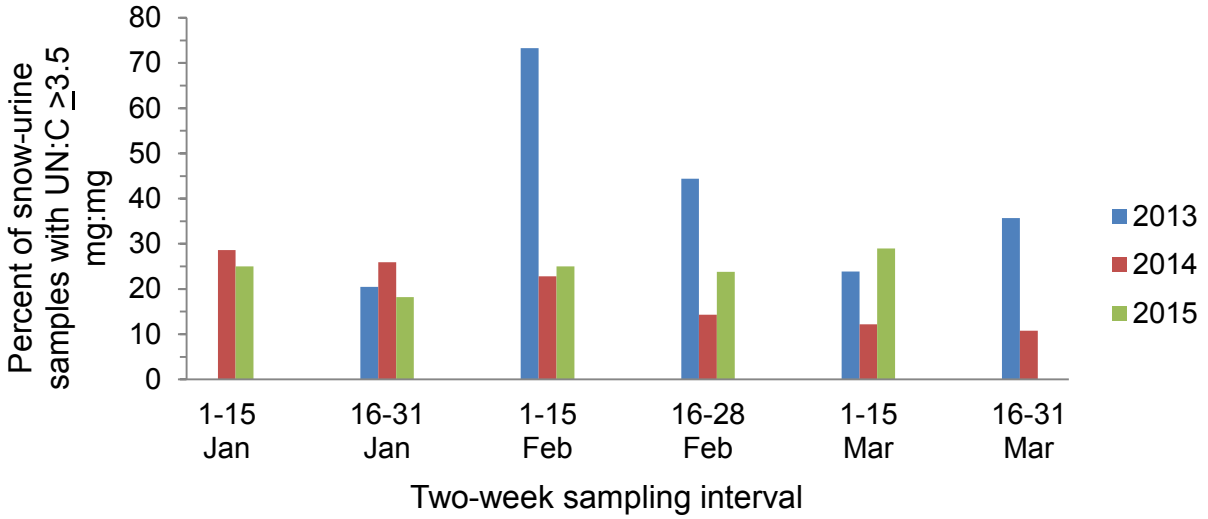

Figure 5. Percent of fresh urine samples voided in snow (snow-urine) by moose and collected during 2-week intervals with urea nitrogen:creatinine (UN:C) ratios indicative of severe nutritional restriction (UN:C ≥3.5 mg:mg) throughout northeastern Minnesota, 9 January–26 March 2013–2015.

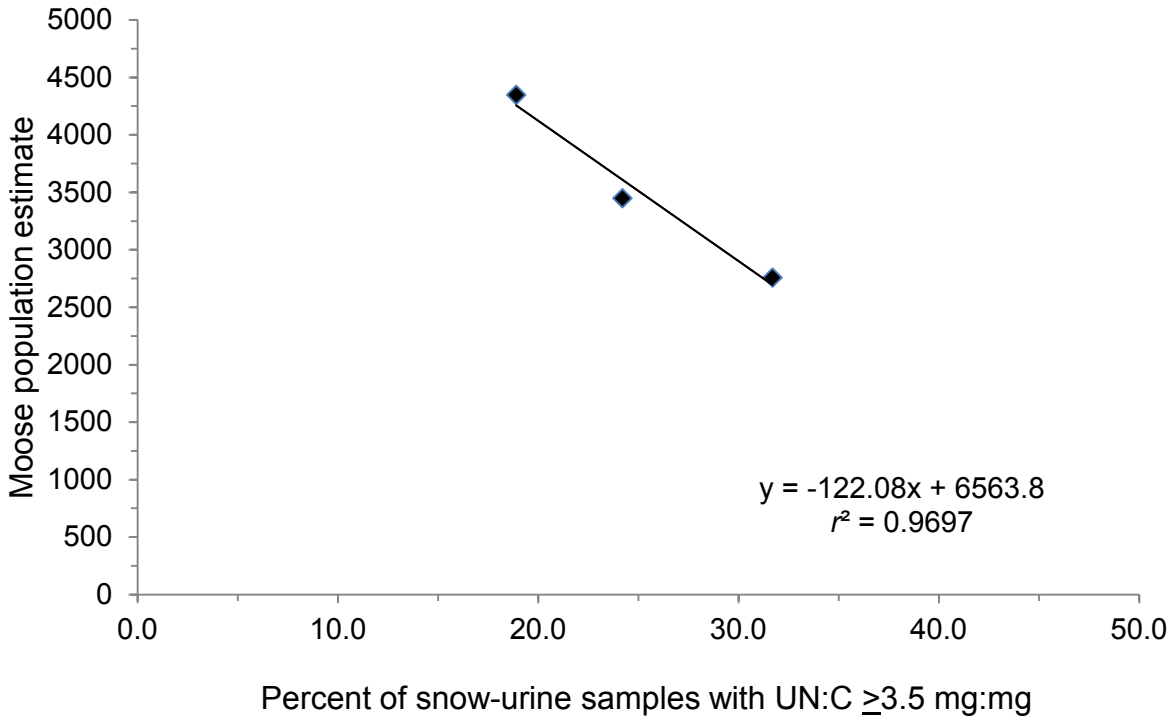

Figure 6. Relationship of the level of severe winter nutritional restriction of moose, indicated by the percentage of collected samples of urine in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios >3.5 mg:mg, to annual population estimates of moose in northeastern Minnesota, (estimates from DelGiudice 2015), 9 January–26 March 2013–2015.

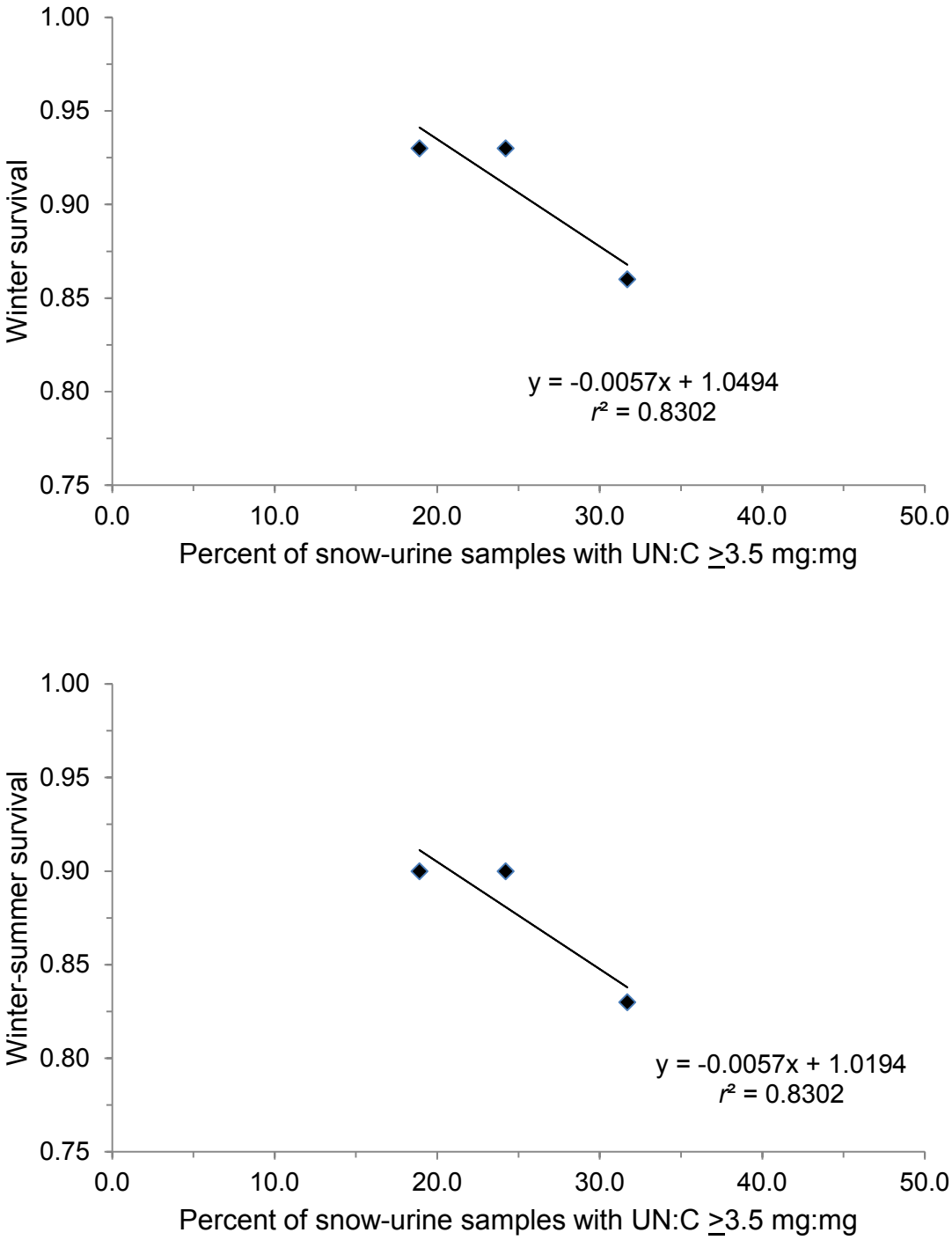

Figure 7. Relationship of the level of severe winter nutritional restriction of moose, indicated by the percentage of collected samples of urine in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios ≥3.5 mg:mg, to winter (top, 1 Nov–31 May) and winter-to-summer (bottom, 1 Nov–31 August) survival, northeastern Minnesota, 2013–2015.

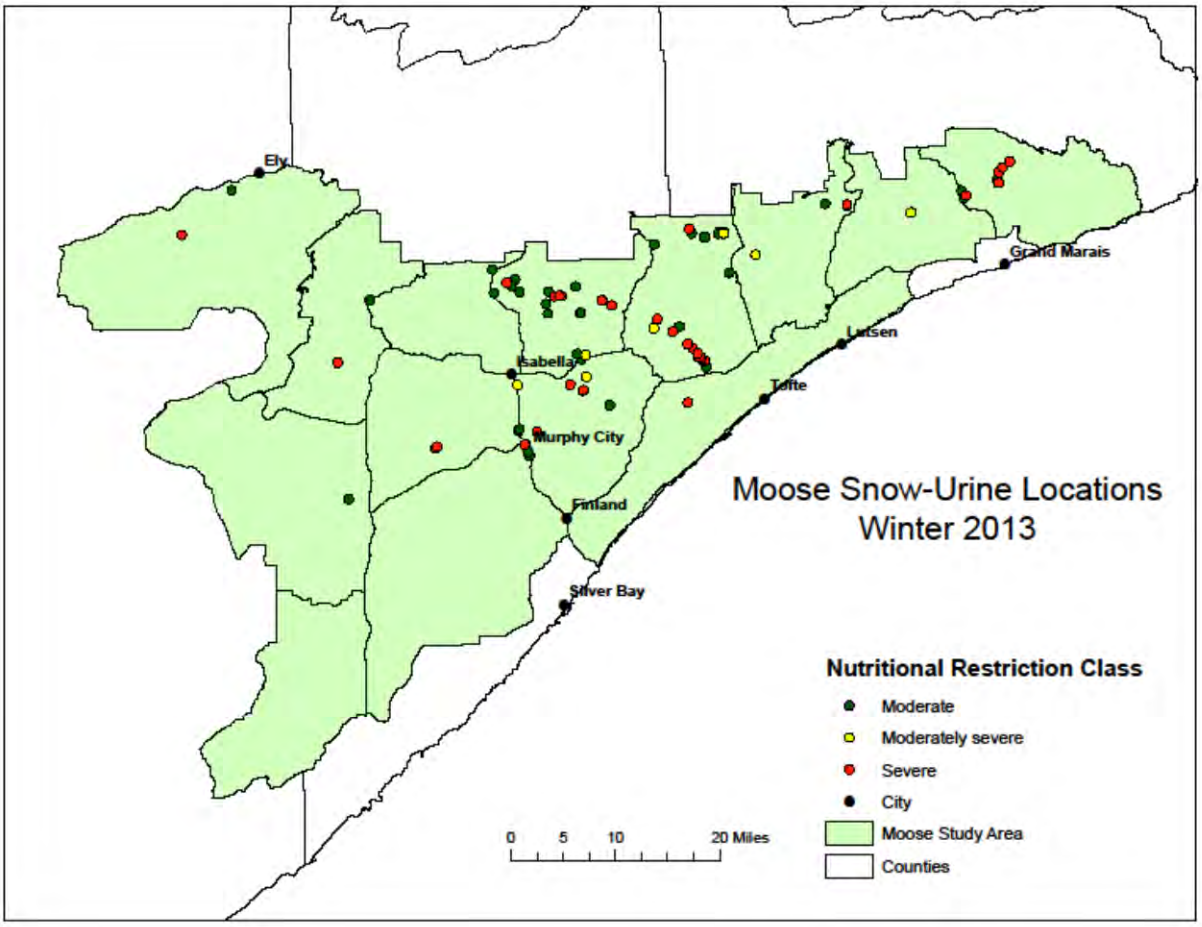

Figure 8. Spatial distribution of fresh urine samples of moose, serially collected for chemical analysis to assess the severity of winter nutritional restriction. Urinary urea nitrogen:creatinine (UN:C) ratios of <3.0, 3.0–3.4, and ≥3.5 mg:mg are indicative of moderate/normal (green), moderately severe (yellow), and severe (red) nutritional restriction, northeastern Minnesota, 23 January–25 March 2013.

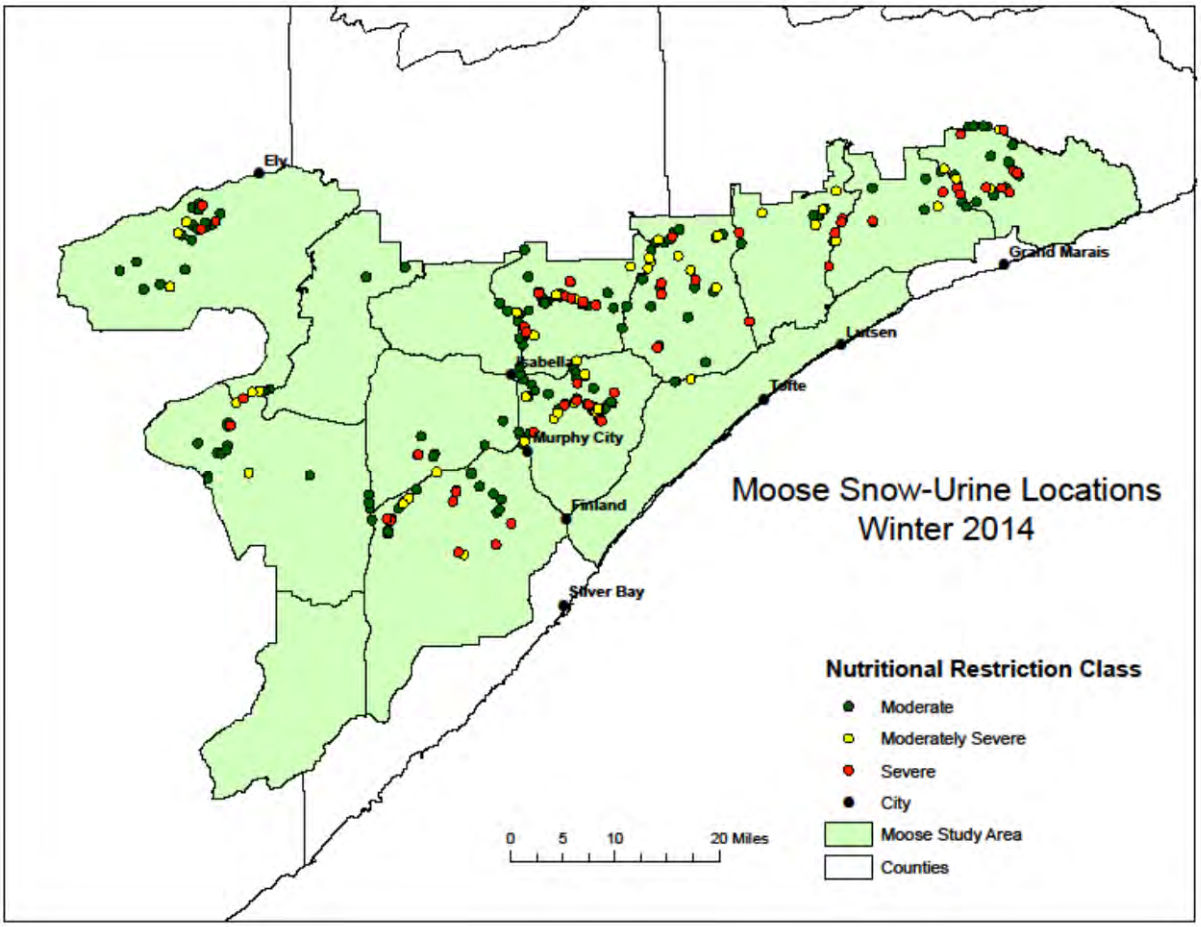

Figure 9. Spatial distribution of fresh urine samples of moose, serially collected for chemical analysis to assess the severity of winter nutritional restriction. Urinary urea nitrogen:creatinine (UN:C) ratios of <3.0, 3.0–3.4, and ≥3.5 mg:mg are indicative of moderate/normal (green), moderately severe (yellow), and severe (red) nutritional restriction, northeastern Minnesota, 9 January–26 March 2014.

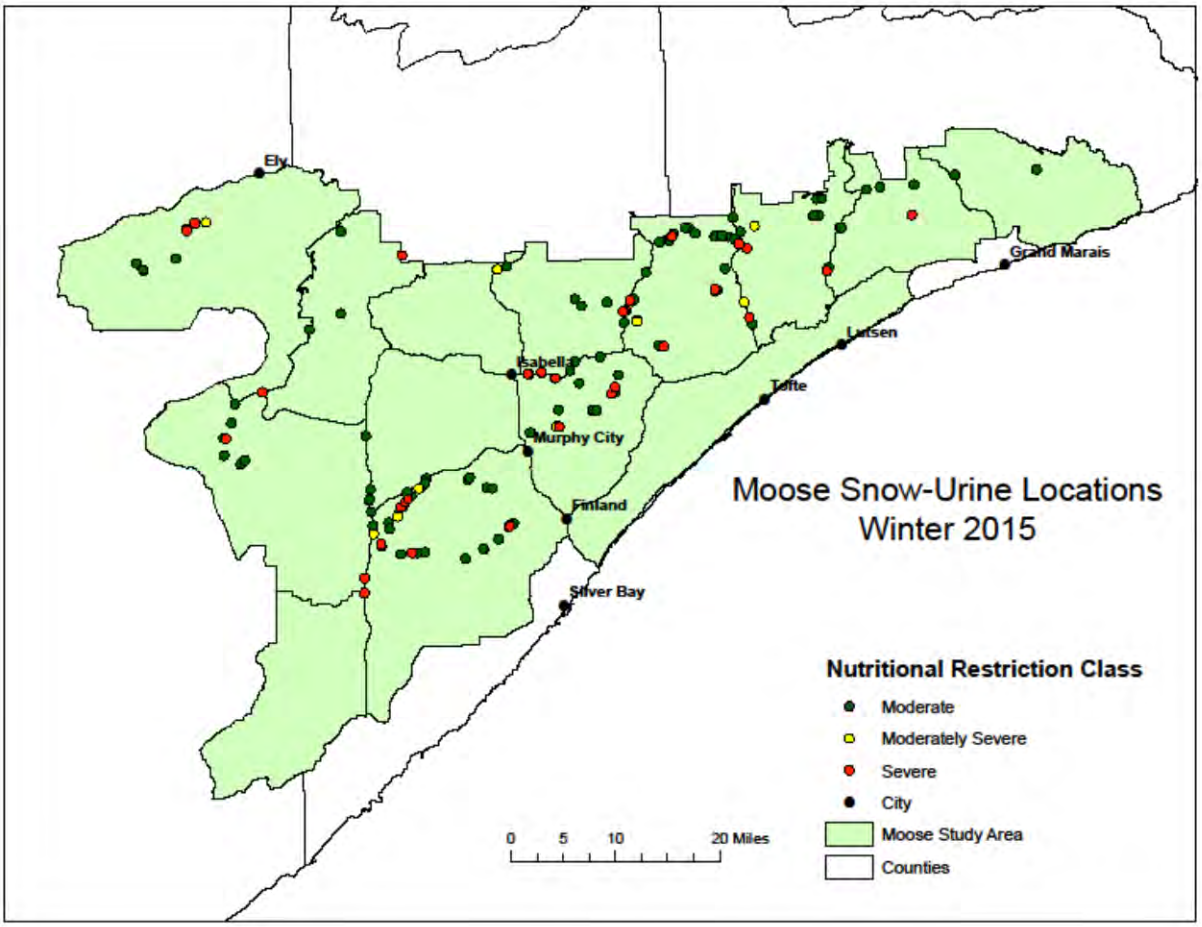

Figure 10. Spatial distribution of fresh urine samples of moose, serially collected for chemical analysis to assess the severity of winter nutritional restriction. Urinary urea nitrogen:creatinine (UN:C) ratios of <3.0, 3.0-3.4, and ≥3.5 mg:mg are indicative of moderate/normal (green), moderately severe (yellow), and severe (red) nutritional restriction, northeastern Minnesota, 12 January–19 March 2015.

MONITORING MOVEMENT BEHAVIOR ENHANCES RECOGNITION AND UNDERSTANDING OF CAPTURE-INDUCED ABANDONMENT OF MOOSE NEONATES[1]

Glenn D. DelGiudice, William J. Severud[2], Tyler R. Obermoller, Robert G. Wright[3], Thomas A. Enright, and Veronique St-Louis

ABSTRACT

Capturing and collaring mammalian newborns is a valued technique in studies focused on survival, cause-specific mortality, maternal investment, and other aspects of animal behavior and ecology. Abandonment of ungulate neonates is a primary cause of capture-related mortality, has been highly variable, and often may be sorely underestimated due to limited understanding of this maternal behavior. In a study of survival and cause-specific mortality of global positioning system (GPS)-collared moose (*Alces americanus*) calves in a declining population in northeastern Minnesota, 9 of 49 (18.4%) neonates (25 females, 24 males) were abandoned postcapture (8–17 May 2013) by 7 of 31 (22.6%) mothers. During the 1- to 6-h interval postcapture, nonabandoning and abandoning mothers were similar distances from their calves. However, for nonabandoning mothers, from 13 to 48 h postcapture mean 6-h-interval distances to their calves steadily approached 0 m, whereas for abandoning mothers mean distances to their calves continued to increase from 7 to 48 h. Five of the 7 abandoning mothers stayed with their calves immediately after capture for up to 11 h before leaving. Additionally, 5 abandoning mothers and 5 that did not abandon returned a mean 1.4 and 1.3 times, respectively, but abandoning mothers were notably farther from their calves just 1 h prior to returning than the nonabandoning mothers. There were no differences in birth date, capture date, bonding or handling times, metrics of body size or rectal temperature of neonates abandoned versus not abandoned, or in mean age of their mothers. Our study improves understanding of capture-induced abandonment and postcapture behavior of mothers that abandoned and mothers that did not. Employment of GPS collars and associated monitoring technology will continue to enhance our recognition and understanding of human-induced abandonment as it occurs for many species, allow rapid mortality investigations, limiting introduction of biases into analyses due to inaccurate data, and should help to minimize the occurrence of human-induced abandonment.

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[1] Abstract from published paper: DelGiudice, G. D., W. J. Severud, T. R. Obermoller, R. G. Wright, T. A. Enright, and V. St-Louis. 2015. Monitoring movement behavior enhances recognition and understanding of capture-induced abandonment of moose neonates. Journal of Mammalogy 96:1005-1016.

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USING GPS COLLARS TO DETERMINE PARTURITION AND CAUSE-SPECIFIC MORTALITY OF MOOSE CALVES[1]

William J. Severud[2], Glenn D. DelGiudice, Tyler R. Obermoller, Thomas A. Enright, Robert G. Wright[3], and James D. Forester[2]

ABSTRACT

Global positioning system (GPS) collars have been deployed on adult moose (*Alces americanus*) and other ungulates to study various aspects of their ecology, but until the current study they have not been fitted to moose neonates. The moose population in northeastern Minnesota, USA, has been declining since 2006, and information on neonatal survival and cause-specific mortality are needed. We monitored hourly movements of GPS-collared females for indications of calving. During 2 May–2 June 2013 we observed 47 of 73 collared females (50 known pregnant, 17 not pregnant, 6 unknown pregnancy status) make 'calving movements' followed by a clustering of locations. After allowing a mean bonding time of 40.2 hours, we approached their calving sites and captured and GPS-collared 49 neonates from 31 dams. We closely monitored dam–calf movements and launched rapid investigative responses to calf mortality notifications to determine cause of mortality. Mean response time was 53.3 hours, but ranged from 0.3 hour to 579 hours, depending on collar accessibility and proper functioning of the GPS component. We censored capture-related mortalities and slipped collars. Twenty-five of 34 calves (74%) died of natural causes as of 31 December 2013, including 1 after natural abandonment, 1 after abandonment of unknown cause, 1 drowning, 1 unknown predator kill, 1 lethal infection from wolf (*Canis lupus*) bites, 4 black bear (*Ursus americanus*) kills, 12 wolf kills, and 4 'probable wolf kills.' As this technology develops, the quantity and quality of survival, cause-specific mortality, movement, and habitat use data generated from intense monitoring of GPS-collared adults and offspring will have unprecedented value associated with management at the population and landscape scales.

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[1] Abstract from published paper: Severud, W. J., G. D. DelGiudice, T. R. Obermoller, T. A. Enright, R. G. Wright, and J. D. Forester. 2015. Using GPS collars to determine parturition and cause-specific mortality of moose calves. Wildlife Society Bulletin 39:616-625.

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POTENTIAL VERTICAL TRANSMISSION OF WINTER TICKS (*DERMACENTOR ALBIPICTUS*) FROM MOOSE (*ALCES AMERICANUS*) DAMS TO NEONATES[1]

William J. Severud[2] and Glenn D. DelGiudice

ABSTRACT

North American moose (*Alces americanus*) frequently become infested with winter ticks (*Dermacentor albipictus*). During capture of neonatal moose in northeastern Minnesota in May–June 2013 and 2014, we recovered adult ticks from neonates, presumably vertically transferred from dams, heretofore not documented. Infestations on neonates may have population-level implications.

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SURVEILLANCE FOR HIGHLY PATHOGENIC AVIAN INFLUENZA IN MINNESOTA'S WILD WATERFOWL IN 2015

Chris Jennelle[1], Michelle Carstensen, Erik Hildebrand, and Lou Cornicelli

SUMMARY OF FINDINGS

Since detection of highly pathogenic avian influenza (HPAI) strain H5N2 in a poultry facility in Pope County MN on February 27 2015, the Minnesota Department of Natural Resources (MNDNR) partnered with the United States Department of Agriculture's Wildlife Services (USDA-WS), the United States Geological Survey's National Wildlife Health Center (USGS), the United States Fish and Wildlife Service (USFWS), and the University of Georgia – Southeast Cooperative Wildlife Disease Study (SCWDS) to conduct surveillance for the virus in Minnesota waterfowl. From March through June 2015, 108 poultry facilities in MN tested positive for HPAI, resulting in severe economic losses to producers and local economic disruption. From March through August 2015, the MNDNR collected and tested 3,138 waterfowl fecal samples, 104 wild bird morbidity or mortality events, and 84 spring hunter-harvested wild turkeys (*Meleagris gallopavo*) for detectable HPAI. Only 1 HPAI positive case was confirmed on April 29 2015 from a wild bird mortality event; a Cooper's hawk (*Accipiter cooperii*). In addition, 100 waterfowl fecal and 2 wild bird mortality samples tested positive for low pathogenic avian influenza (LPAI). On July 10 2015, a black-capped chickadee (*Poecile atricapillus*) from Ramsey County, MN was submitted to the MN Wildlife Rehabilitation Center and also tested positive for HPAI. These two cases are the only verified HPAI positive wild birds detected in Minnesota through August 2015. In June and July 2015 the MNDNR collected 619 swab and blood samples of resident Canada geese (*Branta canadensis*) in central Minnesota. Only two swab samples indicated active shedding of LPAI, and results on serological analysis are pending at SCWDS. As part of our collaboration with SCWDS, the MNDNR is in the process of finishing collection of approximately 600 paired swab and blood samples from dabbling ducks in MN. There is concern from the poultry industry that fall migratory waterfowl movements will place poultry facilities in MN at greater risk of infection (or reinfection) and MNDNR is preparing additional HPAI surveillance efforts to test a sample of migratory waterfowl both in counties with poultry facilities that were infected in Spring 2015 and uninfected facilities. In addition to a MNDNR goal of collecting 800 tracheal and cloacal swab samples from fall hunter-harvested dabbling ducks, we will be collecting an additional 385 swab samples from dabbling ducks in fall and winter 2015 as part of a National USDA HPAI surveillance plan. Aside from these efforts, MNDNR will continue to monitor the health of wild birds by testing wild bird morbidity and mortality events, and screening for HPAI when appropriate.

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INTRODUCTION

Avian Influenza (AI) is a viral infection that occurs naturally in wild birds, especially waterfowl, gulls, and shorebirds. It is caused by type A influenza viruses that have 2 important surface antigens, hemagglutinin (H) and neuraminidase (N), that give rise to 144 possible virus subtypes. Influenza viruses vary widely in pathogenicity and ability to spread among birds. The emergence of an Asian strain HPAI H5N1 virus in 1996 and subsequent spread of the virus in Asia, Africa, and Europe killed thousands of wild birds and millions of domestic poultry. In 1997, HPAI H5N1 became zoonotic in Hong Kong and to-date has infected at least 844 humans around the world, resulting in 449 deaths (World Health Organization 2015). Since there is a risk of worldwide pandemic due to quickly evolving strains of HPAI, there is an urgent need to understand transmission dynamics, host-species susceptibility, and role of the environment in AI dynamics.

From 2006 to 2010, the MNDNR tested over 12,000 wild birds in MN for HPAI and none were detected to be actively shedding virus (Hildebrand et al. 2010). The migratory movements of waterfowl and other shorebirds and subsequent mixing of birds from Asia and North America in the northern latitude breeding grounds facilitated the mixing of LPAI and HPAI strains. Such mixing has resulted in recent discovery of three reassortant highly pathogenic strains including H5N1 (World Organisation for Animal Health 2014), H5N2 (World Organisation for Animal Health 2014, Pasick et al. 2015), and H5N8 (Ip et al. 2015) in British Columbia and the western United States. On March 3 2015, a poultry facility in MN was confirmed infected with HPAI H5N2; the first time any HPAI strain has been detected in Minnesota poultry. The MNDNR subsequently conducted aerial surveillance for waterfowl in a 700 mi^2 surveillance zone centered on the infected farm. Only 100 mallards (*Anas platyrhynchos*) and 18 trumpeter swans (*Cygnus buccinator*) were detected, and staff collected 148 fresh feces samples from these individuals for AI testing. No HPAI was detected, but 6 LPAI positive samples were identified from the mallards, which demonstrated that the sampling technique was effective in detecting shed virus.

In late March, additional poultry facilities became infected with H5N2 and the MNDNR rapidly initiated surveillance efforts to detect the virus in wild birds and waterfowl feces. As such, our objectives were to conduct waterfowl feces, morbidity/mortality event, and hunter-harvested turkey sampling to detect H5N2 in wild birds and the environment (via feces) they inhabit. These efforts permit the estimation of HPAI distribution and magnitude on the Minnesota landscape, which leads to development of specific hypotheses that can help us understand and manage HPAI in wild birds. The scope of the outbreak in MN poultry facilities was unprecedented and by June 2015, 23 counties contained 108 infected farms (102 turkey, 5 chicken, and 1 backyard flock) and 9.3 million birds were euthanized (20% of MN's annual poultry population) causing an economic impact of 650 million dollars.

METHODS

Fecal, morbidity and mortality, and hunter-harvested surveillance

We collected three types of samples: waterfowl feces, public-reported morbid or dead wild birds (i.e., morbidity and mortality events), and hunter-harvested wild turkeys (*Meleagris gallopavo*). The sampling design differed for each data type and required different protocols for collecting samples. Given the rapid onset of infected poultry facilities, we focused primarily on collecting waterfowl feces for three reasons: (1) this sample type afforded us the most control over sampling design elements, (2) a large sample size could be collected relatively quickly (on the order of weeks), and (3) the timing of the outbreak coincided with breeding activities when birds do not congregate and available live capture methods are inefficient. The other two data types (sampling dead wild birds) depended on opportunistic circumstances and public willingness to report or submit dead birds.

We designed fecal sampling efforts to compare potential differences in H5N2 prevalence spatially. We choose five counties with infected facilities (Lac Qui Parle, Kandiyohi, Pope, Meeker, and Stearns) and five wildlife management areas (WMA)/National Wildlife Refuges (NWR) without infected facilities that typically attract large numbers of waterfowl (Carlos Avery WMA, Minnesota Valley NWR, Swan Lake WMA, Thief Lake WMA, and Whitewater WMA) (Figure 1). Since this was designed early in the Minnesota outbreak, we chose 5 of the initial counties that reported infected facilities as our treatment areas. Within the ten selected counties, we collated a list of available wetlands and lakes from which we scouted for waterfowl activity and sampled waterfowl feces deposited at 17 types of locations (Table 1) associated with waterfowl activity. We sampled only what we perceived to be fresh feces (<24 hrs old) that were at least two meters apart. We used polyester-tipped swabs to sample fecal material, placed samples in brain-heart infusion (BHI) medium, and stored media in a cooler with ice packs or a refrigerator. For any given site, swabs could be pooled in groups of 3 in the same media vial. For each county, our goal was to collect at least 300 fecal samples, and we

prioritized collecting no more than approximately 20 samples from a given location to obtain spatial representation within a target county. Assuming fecal samples represented individual waterfowl independently with test sensitivity and specificity of 100%, our lower bound on detection permitted at least a 95% probability of detecting H5N2 viral DNA if at least 1% of the population was actively shedding virus.

Through outreach on the MNDNR and Minnesota Board of Animal Health websites and official press releases, we solicited the public to report any wild birds exhibiting neurological symptoms consistent with AIV infection, dead raptors or wild turkeys, and groups of 5 or more dead birds of any species aggregate. We did not investigate reports of dead ducks as HPAI infected ducks are generally asymptomatic, and ducks that were confirmed with HPAI infection had died from other causes. However, we did investigate reports of dead Canada geese because recent evidence in Wyoming and Michigan documented HPAI H5N2 positive geese with clinical signs of illness. We emphasized the need to report dead birds as soon as possible to ensure collection of viable tissue samples; generally we only collected samples from birds that were deceased for <24 hours. Depending on the resources available for staff (e.g., BHI media), we either collected whole carcasses (double-bagged and frozen) or used swabs to sample tissues from the tracheal and cloacal cavities. Each swab sample was placed in the same BHI media, and kept cool in a portable cooler with ice packs or a refrigerator. Whole carcasses were sent overnight to the USGS for necropsy and AIV testing using RT-PCR. Swab samples were submitted to the USDA National Wildlife Disease Laboratory (Fort Collins, CO) for AIV testing using RT-PCR. If samples tested AIV positive initially at either lab, they were forwarded to the National Veterinary Services Laboratories (Ames, Iowa) for confirmation and strain-typing. We made no fixed sample goals for this sample type due to the opportunistic nature of public discovery and reporting. We used this data as an auxiliary source of information in our surveillance efforts, and obtained samples from all parts of Minnesota.

The H5N2 outbreak coincided with the spring harvest season for wild turkeys, and it is unclear whether this species is susceptible to infection. This afforded an opportunity for a pilot project, and we set a goal to collect swab samples from the tracheal and cloacal cavities of 300 hunter-harvested wild turkeys in the heart of affected poultry areas including Kandiyohi, Meeker, Pope, and Stearns counties. Hunter provision of harvested turkeys was voluntary and at sampling, swabs were placed in BHI media, and stored in a cooler with ice packs or a refrigerator. These samples were submitted to the National Wildlife Health Center (Madison, WI) for AIV testing using RT-PCR.

Paired serology and virus shedding surveillance: A SCWDS collaboration

The MNDNR is partnering with SCWDS in order to evaluate serological results of blood samples obtained from Canada geese and susceptible duck species (primarily mallard and blue-winged teal). Serology, although not yet established as a stand-alone surveillance tool, provides information about whether an animal has mounted an immune response to AIV and has circulating levels of antibodies present in its blood. There are several major limitations; one cannot discern when or how an animal was initially exposed to AIV, and subtyping HPAI and LPAI is an active area of research with unestablished standard protocols.

MNDNR established sampling sites for Canada geese in five area of Minnesota in counties that contained poultry facilities that experienced spring HPAI infection and counties with poultry facilities that were not affected (Figure 2). Sampling occurred during scheduled goose banding efforts with a special Bird Banding Laboratory code applied to records of geese with blood drawn. A goal of 3mL of blood was drawn from each sampled goose and was later centrifuged for serum, which was decanted into 2mL cryovials and stored frozen until shipment to SCWDS. We also collected oropharyngeal and cloacal swab tissue samples from each goose, pooled each pair of swabs in the same BHI media vial, and placed vials in a chilled cooler with ice packs for later transfer to a refrigerator for storage. These samples were submitted to the National Wildlife Health Center (Madison, WI) for AIV testing using RT-PCR.

Goose serum samples were shipped to SCWDS in late July, and are in the process of serological analysis.

Similarly, we selected six work areas for collecting paired samples (i.e., swabs and serology) from ducks (primarily mallards and teal) in counties that contained poultry facilities that experienced spring HPAI infection and counties with poultry facilities that were not affected (Figure 3). Sampling is currently underway with a goal of collecting 625 samples from all works areas. Each captured duck will receive a uniquely numbered aluminum leg band and ascribed a special Bird Banding Laboratory code indicating blood was drawn. A goal of 2mL of blood is drawn from each sampled goose and will be later centrifuged for serum, to be decanted into 2mL cryovials and stored frozen until shipment to SCWDS. We will also collect oropharyngeal and cloacal swab tissue samples from each duck, pool each pair of swabs in the same BHI media vial, and place vials in a chilled cooler with ice packs for later transfer to a refrigerator for storage. These samples will be submitted to the National Wildlife Health Center (Madison, WI) for AIV testing using RT-PCR. Duck serum samples will be shipped to SCWDS in late September of early October.

RESULTS AND DISCUSSION

From March 1 through August 31 2015, the MNDNR collected a combined total of 3,327 samples from the waterfowl feces (*n=3,138*), wild birds mortalities (*n=104*) (Table 2), and spring hunter-harvested wild turkeys (*n=84*) (Figure 4). Only one HPAI positive case was confirmed from a Cooper's hawk mortality sample in Yellow Medicine County on 29 April 2015; it was approximately 12.5 miles from the nearest infected poultry facility. This predatory bird is typically found in woodlands and has a diet consisting mainly of small birds and mammals, and we suspect the infected hawk was exposed to HPAI through a food item. Although not part of MNDNR surveillance, a black-capped chickadee submitted for testing from the MN Wildlife Rehabilitation Center was also confirmed positive on 10 July 2015. These two birds were the only confirmed HPAI infected wild bird samples in MN through 31 August 2015. A total of 100 fecal samples, 1 mortality sample (non-H5 or H7), and 0 wild turkey samples were determined to be LPAI positive. The testing protocol limited the screening for H5, H7, and N1 subtypes only; however, in some cases other subtypes were identified and reported elsewhere.

From the 619 Canada goose swabs samples collected, there were 0 HPAI positive and 2 LPAI positive cases based on RT-PCR analysis (Figure 5). Serology analysis of these samples is pending completion. The collection of paired swab-serology samples for dabbling ducks is currently underway with 196 paired samples collected on dabbling ducks. Data collection is expected to be complete by the end of September.

Future Surveillance

As part of the 2015 USDA National Surveillance Plan, MN is asked to submit 545 oropharyngeal and cloacal cavity swab samples of dabbling ducks for AIV testing to the USGS by the end of winter 2015 (Figure 6). The samples requests are broken down by watershed (Mississippi Headwaters, Red River, St.Croix, Upper Mississippi – Black Root, and Western Lake Superior) and season; summer, fall, and winter. The summer quota of 30 samples for the St. Croix and 130 samples for the Red River watersheds was achieved and is in the process of analysis at the USGS. The fall quota of 40 samples for the Red River, 140 samples for the Mississippi Headwaters, 50 samples for the St. Croix, 40 samples for the Upper Mississippi – Black Root, and 60 samples for the Western Lake Superior watersheds will be forthcoming. Similarly, the MNDNR expects to collect a winter quota of 55 samples from the Upper Mississippi – Black Root watershed.

MNDNR is continuing the collaboration with SCWDS to capture approximately 600 known HPAI susceptible ducks from August through September 2015. Paired tissue samples will be obtained from each duck captured; swab samples from the oropharyngeal and cloacal cavities and approximately 1.5ml of blood for serological testing. Again there are two types of

study areas at the county scale designated by whether poultry facilities experienced HPAI infection or not (Figure 3).

During the fall duck hunting season starting September 26 2015, MNDNR is planning to sample the tracheal and cloacal cavities of 800 hunter-harvested dabbling ducks. The sampling will take place in two types of study areas at the county scale designated by whether poultry facilities experienced HPAI infection or not (Figure 7). The sample size will be approximately split between the two types study areas for comparison. The sample size is chosen to detect HPAI viral shedding at a 1% prevalence level with at least 95% confidence assuming an 80% diagnostic test sensitivity and 100% specificity.

ACKNOWLEDGMENTS

These efforts would not have been possible without the valuable contribution of the Wetland Wildlife Population and Research Group, including J. Lawrence, B. Davis, P. Hagen, K. Young, AI techs M. Kuzel and S. McDowell, and banding interns. MNDNR management and research staff were invaluable in providing guidance for identifying sampling locations and capture/sampling assistance; they include K. Arola, C. August, R. Baden, F. Bengtson, T. Dale, M. Deters, M. Dexter, S. Gibbs, J. Guidice, J. Huener, J. Jaeger, A. Knutson, K. Kotts, W. Krueger, J. Labarre, B. Liddell, J. Markl, R. Markl, J. Miller, D. Nelson, C. Netland, M. Oehler, R. Olsen, B. Olson, K. Pharis, T. Pharis, S. Piepgras, B. Schuna, N. Snavely, J. Stangel, V. St-Louis, J. Strege, E. Thorson, T. Tonsager, N. Trauba, C. Vacek, J. Vorland, and A. Westmark. We recognize our USDA-WS partners P. Wolf, B. Welinski, and assistants; USFWS partners T. Cooper, F. Oslund, N. Williams, and A. Strzelczyk; and USGS partners B. Bodenstein, D. Grear, and H. Ip for their assistance in meeting our sample goals and diagnostic testing needs. We thank the MN.IT GIS experts B. Anderson and S. Benson for their GIS assistance. We thank P. Taskash, D. Schueller, and D. Rose for their help with information dissemination and media contacts. We also thank A. Fojtik and D. Stallknecht from UGA SCWDS. We thank the UMN College of Veterinary Medicine faculty L. Minicucci and veterinary residents J. Evanson and J. Lee for their assistance in field sampling. We are certain we missed some people and for that we apologize. We also thank all of the turkey hunters willing to allow us to sample their harvested animals and the citizens willing to report sick or dead birds that we screened for sampling.

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Table 1. Description of 17 types of locations that Minnesota Department of Natural Resources staff sampled for waterfowl feces, which was subsequently submitted for avian influenza testing in spring 2015.

Description of Sampling Sites
Foam baiting stations in ditches, ponds, and marshes
Mowed grass and gravel around ponds or along dikes
Top of gravel or grass dikes
Waste water ponds
Lake sand bars
Mud flats
Vegetation mats
Golf courses
Mowed road ditches along roads
Gravel or glass shore along lakes
Upland hay meadow
Sandy lake beaches
Loafing rocks in open water and logs along shoreline
Cleared areas along lakes
Softball fields
Residence yards
Park grounds

Table 2. Species of wild bird morbidity and mortality samples (n = 104) submitted to the Minnesota Department of Natural Resources for avian influenza testing from March to August 31 2015. One Cooper's hawk tested positive for highly pathogenic avian influenza and one American coot (*Fulica americana*) tested positive for low pathogenic avian influenza.

Agency	Species sampled	*n*
MNDNR	American coot	4
	American crow	1
	Bald eagle	5
	Belted kingfisher	1
	Blackbird	2
	Broad-winged hawk	1
	Canada goose	11
	Cedar waxwing	1
	Cooper's hawk	7
	Finch	1
	Great horned owl	3
	Hawk	3
	Herring gull	1
	Mallard	3
	Mourning dove	1
	Common nighthawk	1
	Osprey	1
	Pelican	1
	Ring-necked pheasant	7
	Rail, warbler, and coot	1
	Red-tailed hawk	3
	Ring-billed gull	2
	Rock dove	1
	Rose-breasted grosbeak	1
	Sandhill crane	2
	Sharp-shinned hawk	9
	Starling	3
	Swainson's thrush	1
	Trumpeter swan	4
	Unknown passerine	1
	Wild turkey	19
	Wood duck	2
Total		104

* Note that multiple birds may have been submitted for a given location and time and samples submitted represent one batch

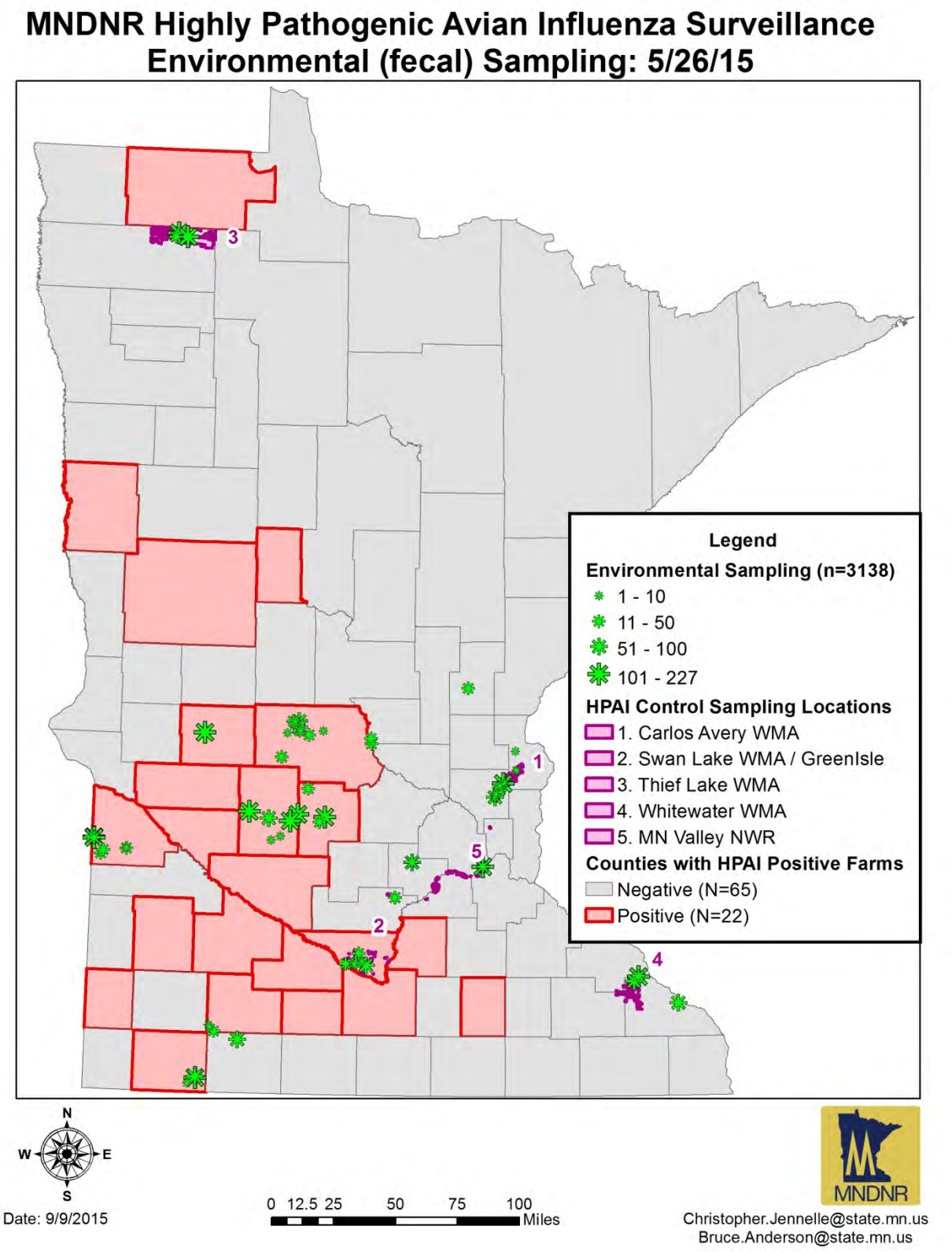

Figure 1. Sites in Minnesota where the Minnesota Department of Natural Resources collected waterfowl feces samples that were subsequently tested for highly pathogenic avian influenza by the United States Department of Agriculture in 2015.

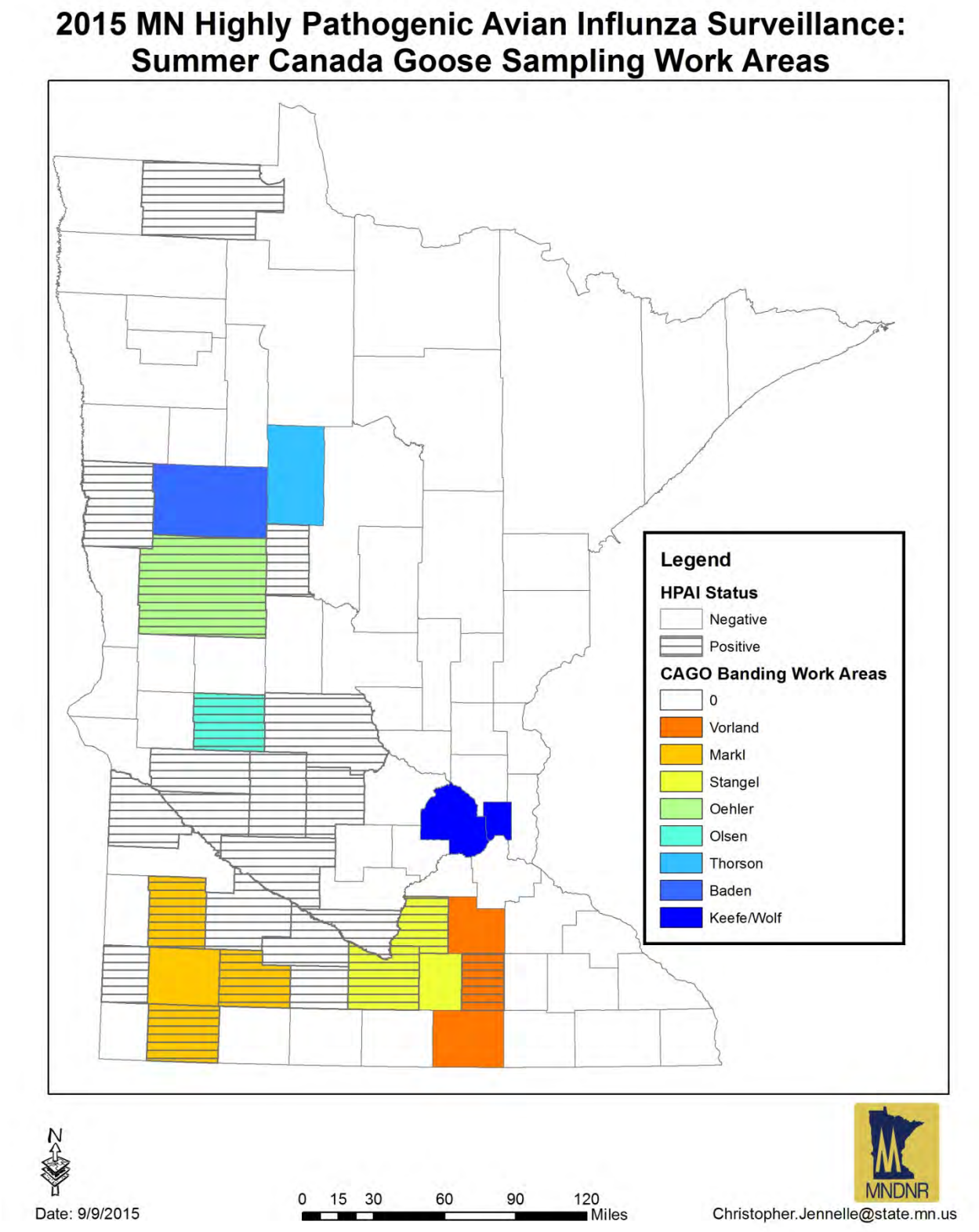

Figure 2. Study area design for summer 2015 paired collection and testing of Canada goose (*Branta Canadensis*) swab and blood samples in collaboration with SCWDS. A total of 619 goose samples were subsequently collected with 0 HPAI positive cases, 2 LPAI positive cases, and pending serology results.

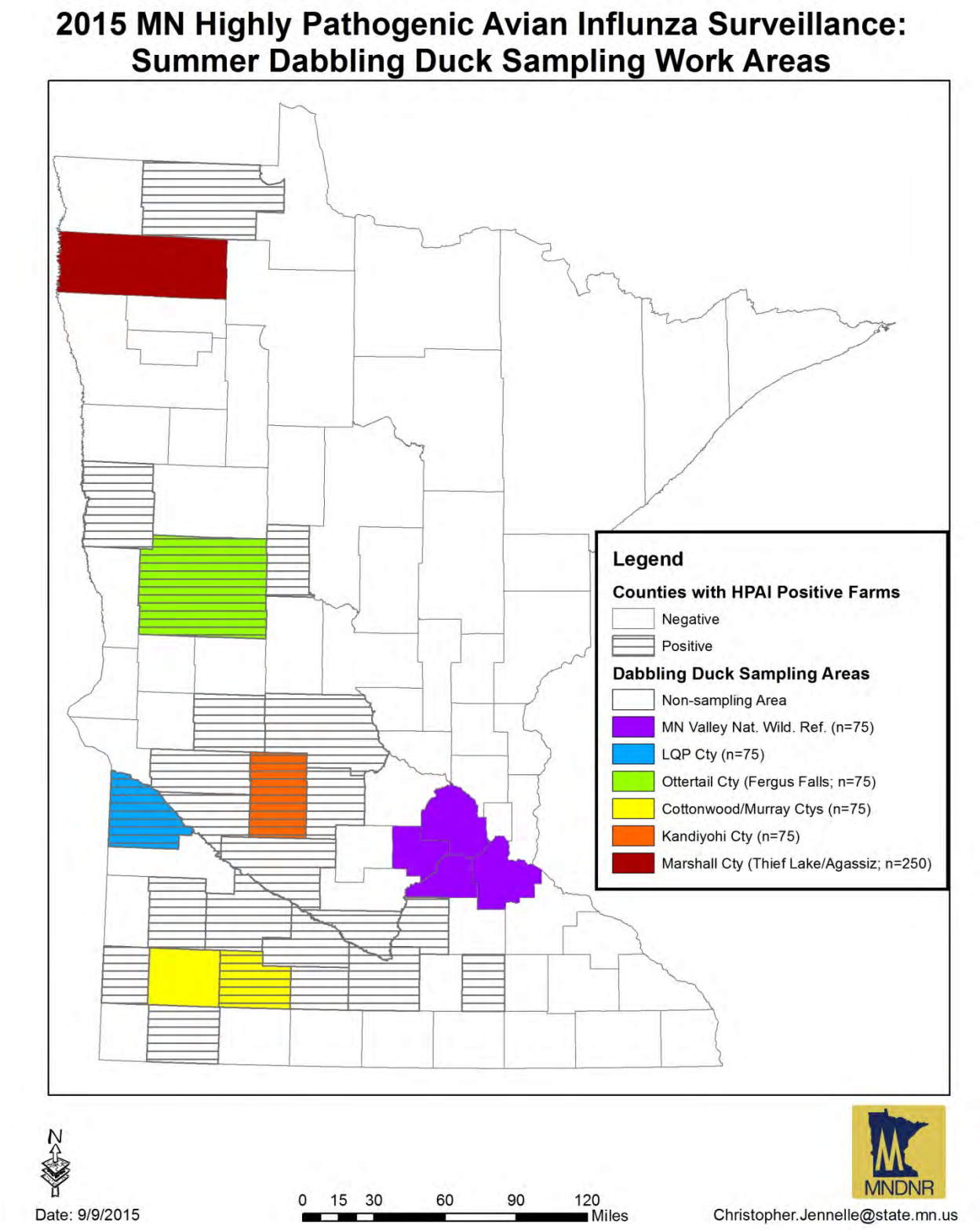

Figure 3. Study area design for summer 2015 paired collection and testing of dabbing duck swab and blood samples in collaboration with SCWDS. A goal of 625 paired samples is established in six work areas throughout Minnesota, and sampling is currently underway.

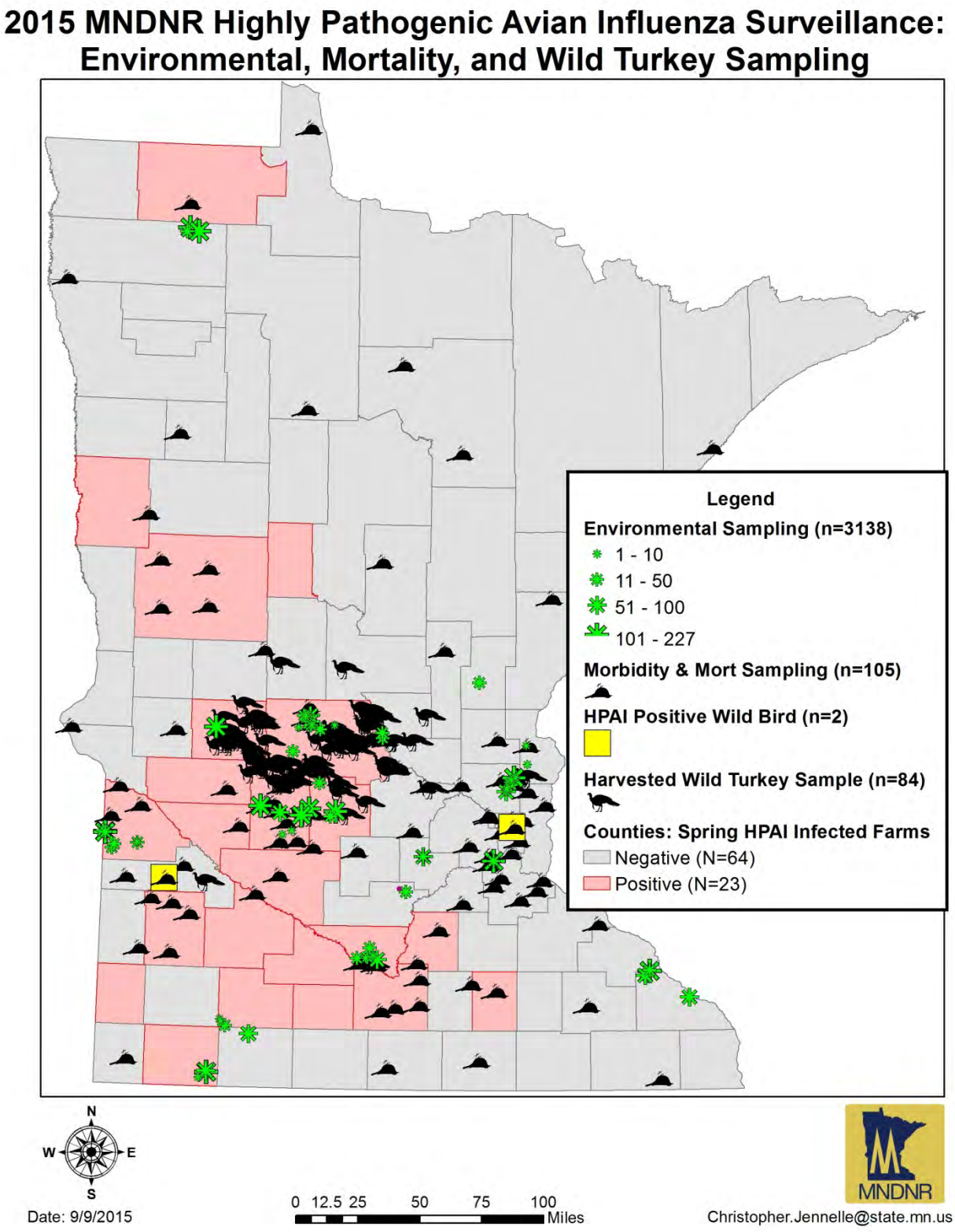

Figure 4. The distribution of waterfowl fecal, wild bird mortality, and hunter-harvested wild turkeys samples collected and tested in MN by the MNDNR through 8/31/15. Note that one of the HPAI positive wild bird mortalities (black-capped chickadee) in Ramsey County was obtained by the MN Wildlife Rehabilitation Center.

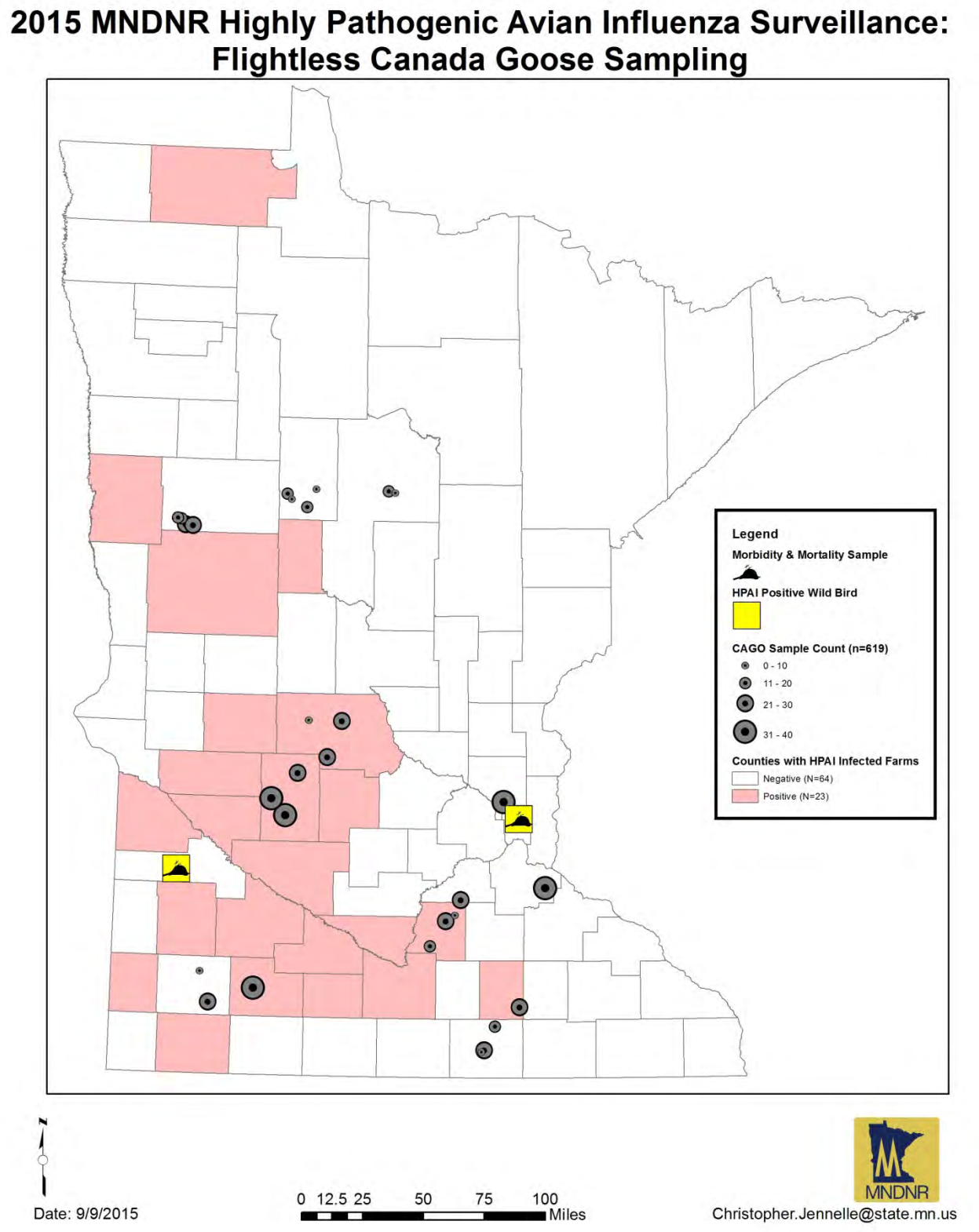

Figure 5. The distribution of 619 Canada goose paired swab and serology samples collected by the MNDNR in collaboration with SCWDS. All samples were collected between June and July 2015, and serology results are pending.

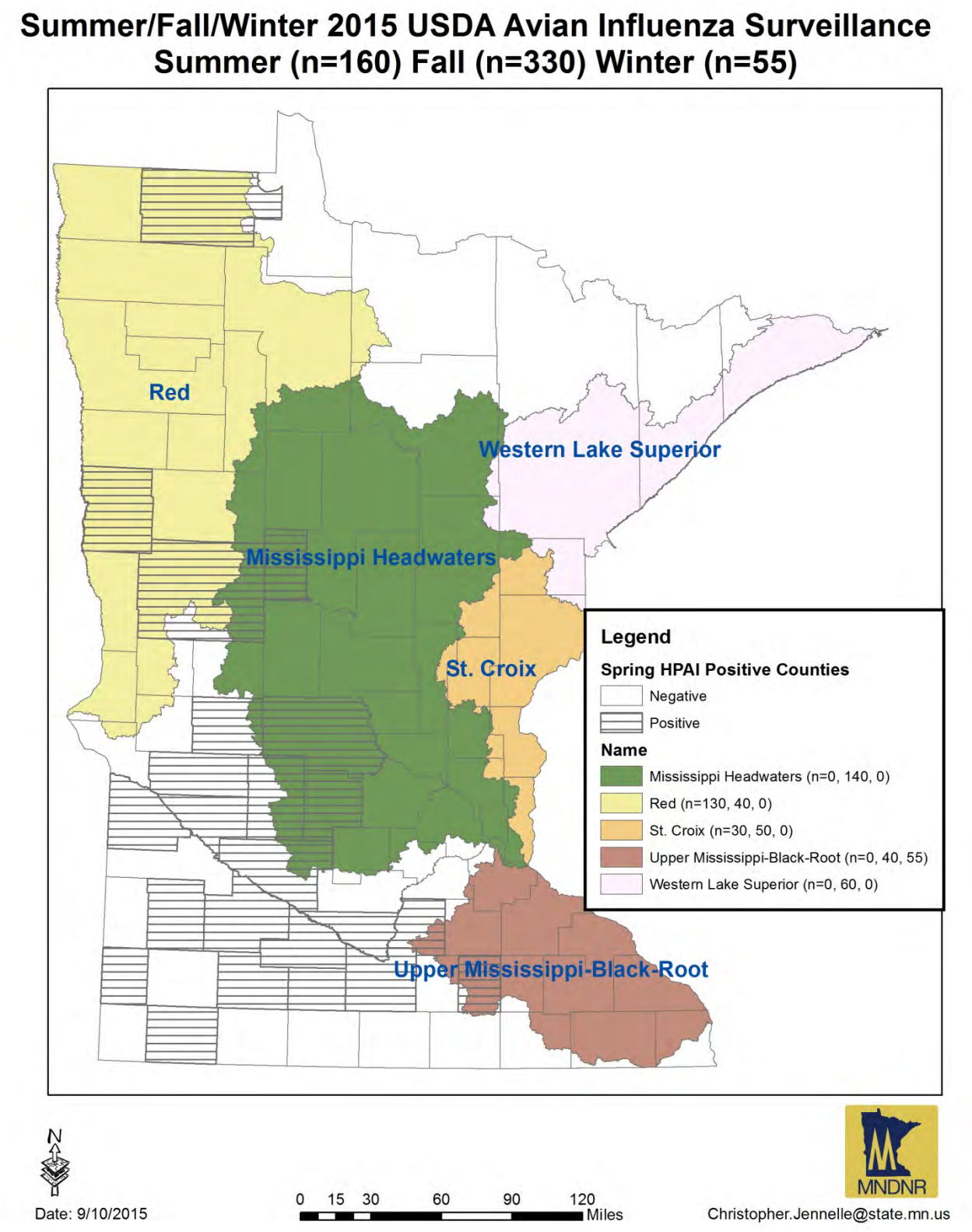

Figure 6. The distribution of USDA targeted MN watersheds for avian influenza sampling in summer, fall, and winter 2015. The three sample sizes noted beside watersheds in the legend are the quotas requested by USDA for summer, fall, and winter sampling, respectively.

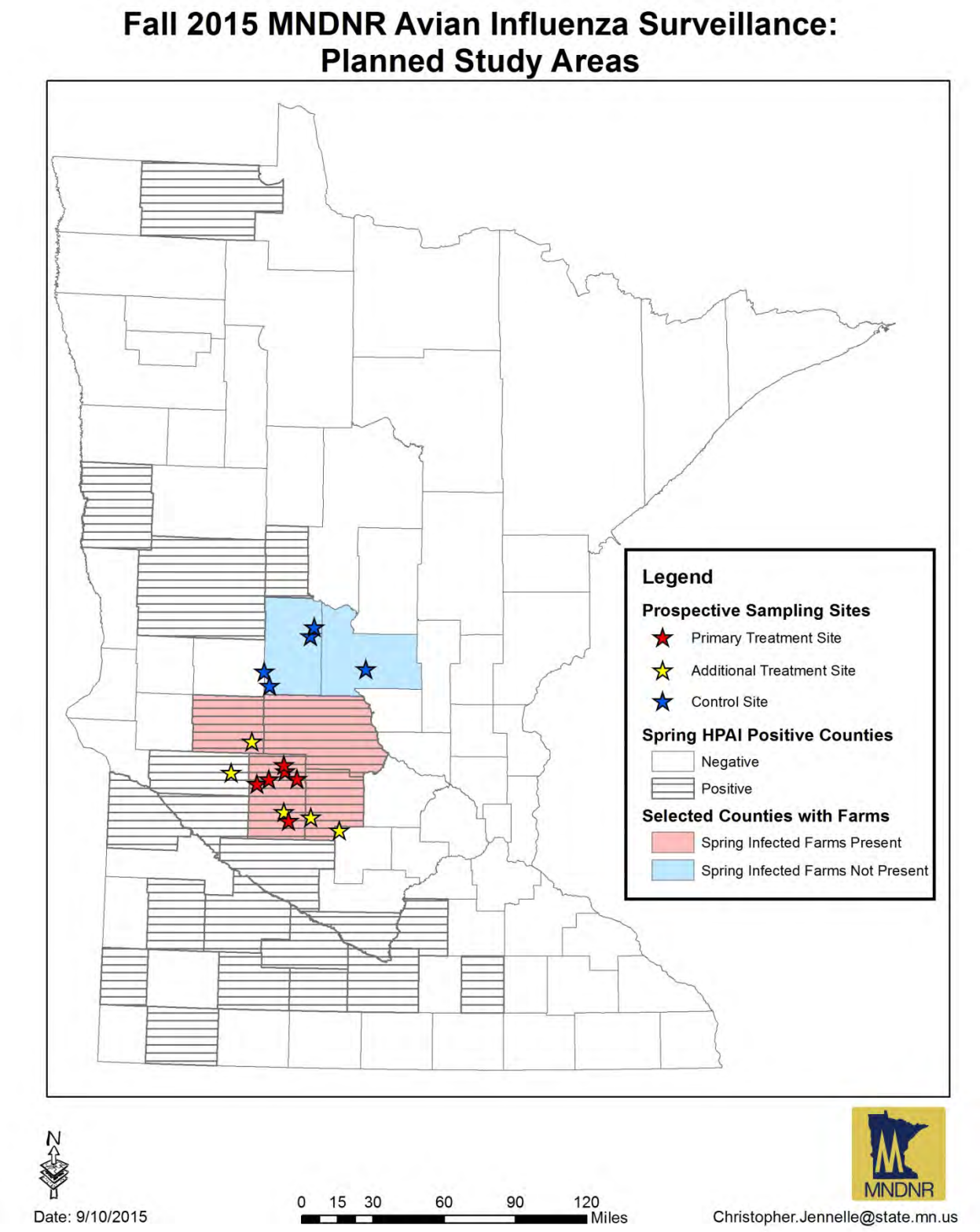

Figure 7. The distribution of MNDNR targeted counties and sampling locations for planned MN fall duck hunter-harvest sampling for avian influenza. Approximately 400 tracheal and cloacal swab samples will be collected per bird in each of the two study area types.

CHRONIC WASTING DISEASE SURVEILLANCE IN MINNESOTA'S SOUTHEASTERN WILD DEER HERD

Erik Hildebrand[1], Michelle Carstensen, Margaret Dexter, and Chris Jennelle

SUMMARY OF FINDINGS

In fall 2014, the Minnesota Department of Natural Resources (MNDNR) sampled 411 hunter-harvested white-tailed deer (*Odocoileus virginianus*) for chronic wasting disease (CWD) in southeastern Minnesota. The surveillance effort focused on testing deer within deer permit areas (DPA) 348 and 349, in response to the first detection of CWD in a free-ranging deer by the Iowa Department of Natural Resources in Allamakee County. All deer were negative for the disease. MNDNR also submitted samples from 69 deer from within DPA's 236 and part of 601 (north metro surveillance area) where a captive European red deer *(Cervus elaphus)* farm was found positive for CWD in summer 2012. These deer were collected through vehicle kills, special hunts, and depredation permits; all deer were negative for CWD. In addition, MNDNR submitted samples from 18 cervids through targeted surveillance, which included sick animals, escaped captive cervids, and vehicle-kills; these were also all negative for CWD. Currently, MNDNR has suspended efforts to test for CWD through hunter-harvested surveillance in the state, but will continue with targeted surveillance efforts.

INTRODUCTION

Chronic wasting disease is a transmissible spongiform encephalopathy (TSE) that affects elk *(Cervus elaphus)*, mule deer *(Odocoileus hemionus)*, white-tailed deer, and moose *(Alces alces)*. TSEs are infectious diseases that alter the morphology of the central nervous system, resulting in a "sponge-like" appearance of this tissue. The etiological agent of CWD is an infectious protein, called a prion. Incubation time of the disease can range from 1.5 to nearly 3 years, although infected animals have been shown to shed prions in their feces up to a year before showing signs of illness (Tamguney et al. 2009, Haley et al. 2011). Clinical signs are non-specific and may include a loss of body condition and weight, excessive salivation, ataxia, and behavioral changes. There is no known treatment or vaccine for the disease and it is always fatal. Experimental and circumstantial evidence suggest that transmission of the disease is primarily through direct contact with infected animals or their infective saliva or excrement (Mathiason et al. 2006, Safar et al. 2008). However, persistence of prions in the environment and resulting indirect transmission has been shown to occur (Miller et al. 2004, Johnson et al. 2007, and Maluquer de Motes et al. 2008).

The Center for Disease Control (CDC) and other public health agencies have concluded there is no known link between CWD and any neurological disease in humans (MaWhinney et al. 2006, Sandberg et al. 2010). However, both the CDC and the World Health Organization (WHO) recommend that no part of a known positive animal should be consumed by humans. Additionally, there is no evidence that CWD can be naturally transmitted to species other than deer, elk, or moose.

To date, CWD has been diagnosed in 3 captive elk *(Cervus canadensis)* herds, 1 captive white-tailed deer herd, and 1 captive European red deer (*Cervus elaphus)* herd in Minnesota. Two of the elk herds (Stearns and Aitkin counties) were discovered in 2002 and depopulated; no additional CWD-positive animals were found. In 2006, a captive white-tailed deer from a mixed deer/elk herd in Lac Qui Parle County was infected with CWD and depopulated without additional infection being detected. In 2009, another captive elk herd (Olmsted County) was found infected

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with CWD and, following depopulation of >600 animals, a total of 4 elk were confirmed with the disease. The United States Department of Agriculture's (USDA) indemnification document noted there was an apparent longstanding infection within this captive elk facility. In 2012, a captive European red deer was found infected with CWD in a herd of approximately 400 animals in North Oaks, MN. This marked the first time CWD was discovered in this species. This red deer herd was depopulated in 2014; no additional infected animals were found. According to the indemnity agreement, perimeter fences must remain intact at this property until 2019, in an effort to keep wild deer from entering the property to reduce disease transmission risks.

Currently, Minnesota has approximately 500 captive cervid facilities. As the current statewide population estimate of wild deer approaches one million, there is an element of inherent disease transmission risk between captive and wild cervids. Overall, risk is difficult to quantify because deer populations are unevenly distributed over the landscape ranging in densities from < 1-15 deer/km^2 (i.e., 1–40 deer/mi^2), facility fences vary in construction quality, and direct/indirect contact rates between captive and wild cervids are unknown. In addition, captive cervid facilities are sporadically distributed on the landscape and are independent of wild deer densities.

In November 2010, MNDNR sampled 564 hunter-harvested deer focused on a 32.2-km (20-mi) radius around a CWD-positive captive elk facility near Pine Island (Olmstead county), discovered in 2009. One free-ranging deer tested positive for CWD, marking the first detection of the disease in Minnesota's wild deer population. In response to this disease detection, MNDNR conducted a fixed-wing aerial deer survey in a 16.0-km (10-mi) radius of the index case in late January 2011 and estimated 6,200 deer (7.3 deer/km^2 or 19 deer/mi^2). A supplemental surveillance effort was conducted in February–March 2011; 752 adult deer were sampled and all tested negative. To prevent further disease spread, MNDNR banned recreational feeding of deer in a 4-county area in southeastern Minnesota and created a CWD Management Zone DPA 602. From 2011–2013, a total of 4,050 (n = 1,125, 1,195, and 978 for 2011, 2012, and 2013, respectively) deer were sampled for CWD within DPA 602 with no further infection detected.

These data, in combination with historical data from 2002-2009 indicated >99% probability that disease prevalence was no greater than 0.5% assuming independence between years and animals within year. These results provide strong evidence that Minnesota was on the front end of a CWD outbreak in wild deer. Our inability to detect any additional infected deer in the immediate vicinity of the index case or in surrounding DPA's or in DPA's bordering neighboring infected counties is encouraging. The data suggests CWD was recently introduced on the southeastern MN landscape, with a high likelihood that widespread wild cervid exposure has been minimal.

METHODS

Hunter-harvested surveillance during 2014 was conducted at deer registration stations during the first two weekends of the regular firearm hunting season in southeastern Minnesota. Selected stations were staffed with MNDNR personnel and students (veterinary medicine and natural resources) trained in lymph node collection. Stations were selected based on deer volume and distribution throughout the surveillance zone to meet a sampling goal of 450 between DPAs 348 and 349 combined. Hunters were asked to voluntarily submit medial retropharyngeal lymph node samples from deer ≥1.5 years of age to be tested for CWD, and a front incisor was extracted from all deer visually assessed to be ≥2.5 years old for aging by cementum annuli. To obtain access to deer from the north metro surveillance area MNDNR worked with local contractors and the Wildlife Science Center to collect vehicle-killed deer within a 10-mile radius of the CWD-infected red deer farm in North Oaks. Additional deer were obtained through special hunts in Ramsey and Anoka counties, as well as both private and city depredation permits. All deer samples were inventoried, entered into a database, and sent to Colorado State University (Fort Collins, CO) for enzyme-linked immunosorbent assay (ELISA) testing. Any presumptive positive deer from ELISA testing would be confirmed using immunohistochemistry (IHC) testing at the National Veterinary Services Laboratory in Ames, Iowa.

At the time when deer were sampled, hunter information was recorded, including the hunter's name, a telephone number, MNDNR number, and location of harvest. Maps were provided to assist the hunters in identifying the location (Township, Range, and Section) of the harvest site. Cooperating hunters were given a cooperator's patch.

Across MN, MNDNR consistently samples any cervid exhibiting clinical symptoms of CWD

infection (targeted surveillance). We have disseminated information to wildlife staff regarding clinical signs of infection for symptomatic deer. We also provided staff with the necessary equipment and training for lymph node removal and data recording. The number of samples expected through targeted statewide surveillance is estimated to be less than 100 animals annually, as few reports of deer with clinical signs are received.

RESULTS AND DISCUSSION

MNDNR collected a total of 411 samples in southeastern Minnesota from hunter-harvested deer during fall 2014 (Figure 1). All samples were negative for CWD. The sampling goal was 450 samples between DPA's 348 and 349 combined, and we achieved 91% of our surveillance goal in southeastern MN.

From July 2014 to June 2015, MNDNR collected a total of 18 samples from targeted surveillance efforts. This included samples from 2 escaped captive deer, and 16 free-ranging deer with clinical signs; all samples were negative for CWD.

In the north metro surveillance area, 69 deer were tested in fall 2014. From 2012 – 2014, a total of 350 (160, 121, and 69, respectively) deer were tested for CWD through vehicle-kills (*n*=48), special hunts (*n*=163), and from a city-contracted sharpshooting effort within the city of North Oaks (*n*=139), with no detection of the disease (Figure 2).

Hunter-harvested deer was and remains the primary source for obtaining adequate samples for continued monitoring and management of this disease since the first discovery of CWD in MN in 2002. MNDNR remains concerned about CWD spread in wild cervids, and has increased surveillance focus in southeastern Minnesota with evidence of increasing CWD detections in wild deer in southwestern Wisconsin and northeastern Iowa.

Future Surveillance Plans

Given there have been no CWD detections in hunter-harvested wild deer since 2010, and no detections via targeted surveillance efforts, MNDNR will not conduct hunter-harvest surveillance in 2015. Targeted CWD surveillance of deer exhibiting clinical signs of illness will continue statewide.

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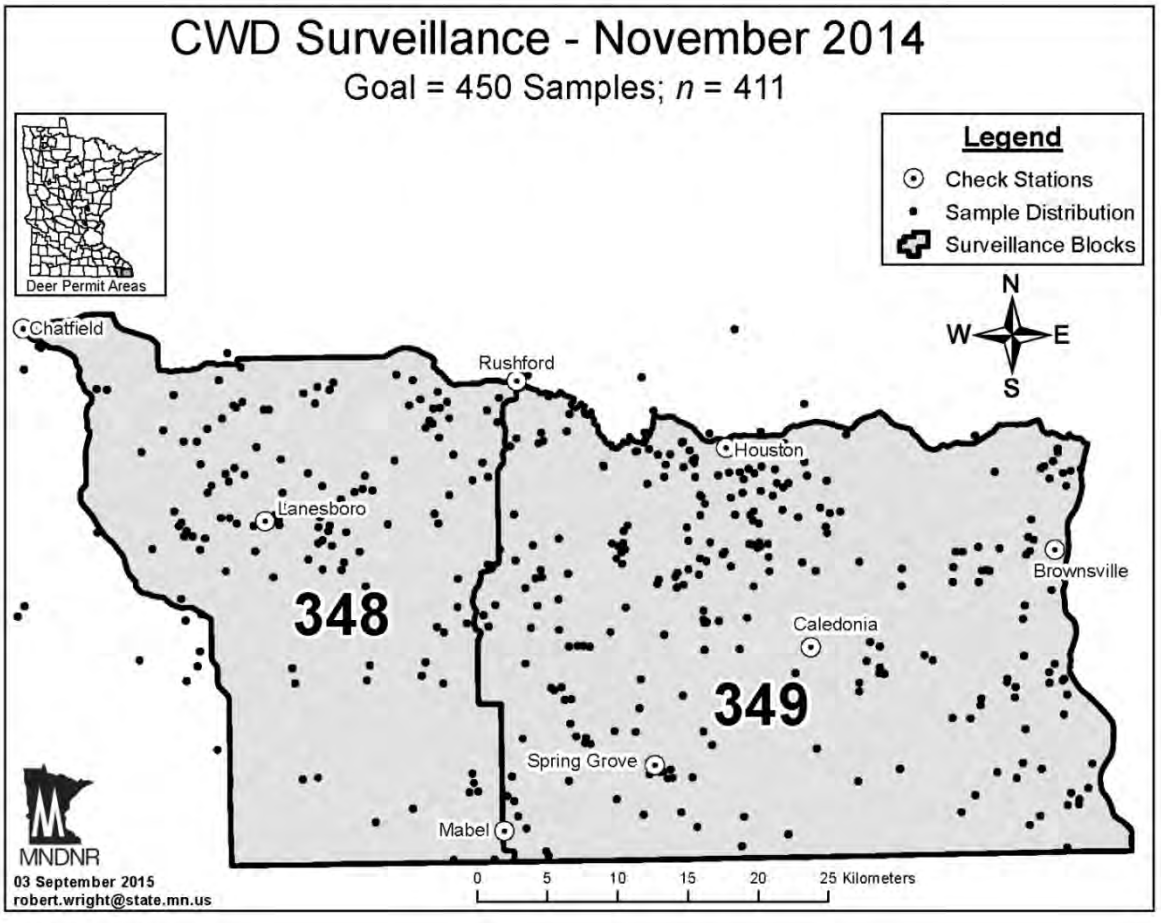

Figure 1. Samples collected from deer *(n*=411*)* for chronic wasting disease (CWD) testing in southeastern Minnesota during fall 2014.

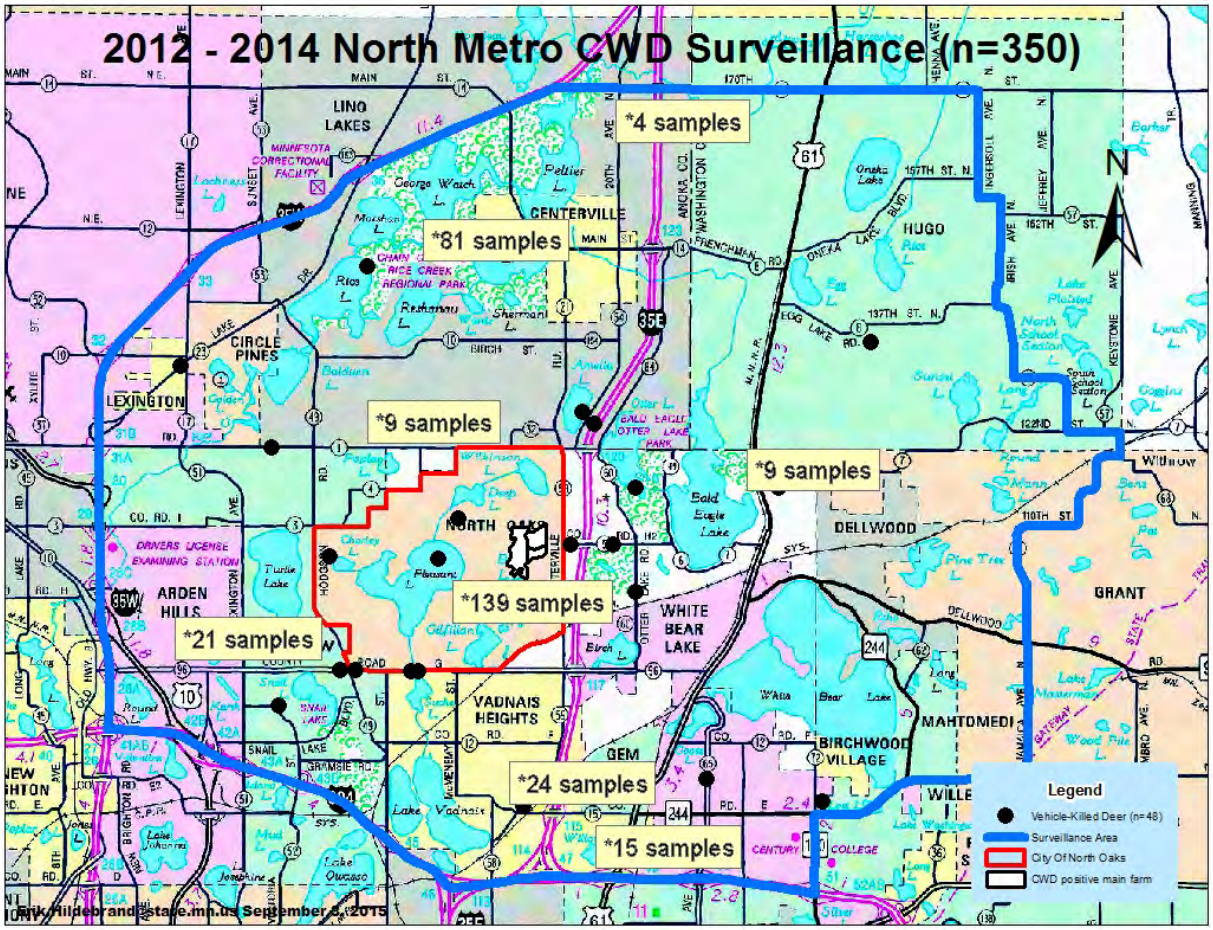

Figure 2. Samples collected from deer *(n*=350*)* for chronic wasting disease (CWD) testing in the north metro surveillance area, in relation to the location of CWD-positive European red deer farm, 2012 through fall 2014.

MNDNR

DETERMINING CAUSE- SPECIFIC MORTALITY OF ADULT MOOSE IN NORTHEAST MINNESOTA

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SUMMARY OF FINDINGS

The primary goal of this study is to improve our understanding of the causes of non-hunting mortality in northeastern Minnesota's declining moose (*Alces alces*) population. Our goal is to respond to potential mortalities within 24 hours of death, prior to decomposition of tissues. In the first 2.5 years of this multiyear study, we've captured and radio collared 173 adult moose (123 females, 50 males), and mean age at capture (n = 120, some ages still pending) was 5.7 years (±0.3 yrs; range = 1 to 14). A total of 41 collared moose have died since this study began, excluding 12 capture-related mortalities that will be censored from subsequent survival analyses. Annual mortality varied from 19% in 2013 to 12% in 2014; the 2015 mortality rate to date is 9%. Overall proximate causes of death included: 16 confirmed and likely wolf kills (39%), 8 bacterial infections (20%), 5 confirmed and likely *Parelaphostrongylus tenuis* infections (12%), 4 multiple, chronic health issues (10%), 3 winter tick infestations (7%), 1 accident (2%), and 4 undetermined health issues (10%). Whole carcasses were retrieved for 13 (32%) of mortalities, with field necropsies performed on the remaining 28 (68%) moose. Response times from initial mortality notification (e.g., text message or email) to a team in the field at the death site were ≤24 hours in 25 cases (61%), between 24 and 48 hours in 11 cases (27%), and >48 hours in 5 cases (12%). Delays in mortality responses > 24 hours have been due to collar failures and wolves actively feeding on the moose carcass and preventing the collar from sending a mortality alert. There are currently 115 remaining moose in the study, but 30 of these have collars that are experiencing significant transmission failures and we are not certain of their status; thus, 85 moose are actively transmitting data.

INTRODUCTION

Historically, moose were found throughout the forested zone of north central Minnesota. By the 1960's there were two distinct populations, the northwest (NW) population of the aspen parklands and northeast (NE) population of the boreal forest (Fuller 1986). In the mid-1980's the NW population began a precipitous decline, falling from 4,000 to <100 animals by the early 2000's (Murray et al. 2006, Lenarz 2007). Murray et al. (2006) identified pathogens, including liver flukes (*Fascioloides magna*) and brainworm (*P. tenius*), as the principal cause of death for 37-62% of radio-collared animals; 25% of additional mortalities were likely pathogen-induced, but limited necropsy evidence was inconclusive. They also observed that many moose in NW MN dying of natural causes were malnourished, as evidenced by 51% of carcasses having bone marrow fat (BMF) contents below a critical threshold (< 30%) and trace mineral deficiencies (i.e., copper and selenium).

Subsequently, in NE MN, Lenarz et al. (2009) reported a 21% average non-hunting mortality rate for radiocollared moose, which was much higher than the 8-12% reported for moose elsewhere in North America (Larsen et al. 1989, Ballard 1991, Kufeld and Bowden 1996). Specific causes of most of the non-anthropogenic mortality (89%) could not be determined, as assessing cause-specific mortality was not the primary objective of the study

(Lenarz et al. 2009). Many of the deaths appeared health-related, with prime age animals dying during unusual times of the year or carcasses found intact with little evidence of scavenging.

Aerial surveys also indicate the NE population is declining. Since the estimated peak at 8,840 moose in 2006, the 2015 estimated moose population (3,450) is 61% lower and time series analysis of estimates since 2006 indicate a significant downward trend (DelGiudice 2015). Butler et al. (2013) documented evidence of exposure of NE MN moose to a variety of disease agents (e.g., West Nile Virus, eastern equine encephalitis, malignant catarrhal fever), which could be potential mortality factors. Additionally, a recent study of sick and vehicle-killed moose (*n*=62) from 2003-2013 had found 85% of animals were undernourished and infected with a variety of disease agents, including brainworm (45%), liver flukes (60%), and winter ticks (*Dermacentor albipictus*) (22%) (Wuenschmann et al., 2014). Researchers have hypothesized that brainworm was responsible for historic declines in moose populations (Karns 1967, Prescott 1974, Lankester 1987), but it is questionable whether brainworm currently represents a major threat to the NE MN population; clinical signs consistent with brainworm infection were first reported in MN moose in 1912 (Fenstermacher and Olson 1942). Lenarz et al. (unpublished data) found that brainworm may have caused an average 19% (0-32%) of the population's total annual mortality. Recently, Mech and Fieberg (2014) have suggested that wolves (*Canis lupus*) had a stronger role in the northeast moose decline than previously reported.

Climate change has been implicated as an underlying factor in both population declines. There were inverse relationships between warming ambient temperatures and decreasing survival of adult moose or negative rates of population change (Murray et al. 2006; Lenarz et al. 2009, 2010). Trends in temperature and precipitation patterns are likely to increase in intensity over the next century (Houghton et al. 2001). If moose are unable to sufficiently thermoregulate above certain ambient temperature thresholds (Renecker and Hudson 1986, 1990; McCann et al., 2013) we might expect to see increased body temperatures and energy expenditures required to stay cool, which over time could have negative consequences for body condition, reproduction, and survival. Currently, no data exist to support the direct adverse effects of ambient temperature on the physiology, survival, or reproduction of free-ranging moose. Recently, a minimally invasive telemetry system for ruminants, called a mortality implant transmitter (MIT), has been developed to allow nearly continuous monitoring of body temperature with a battery lifetime of ≥2 years. Using these MITs and global positioning system (GPS) collars on adult moose in this study will allow us to correlate ambient temperature with physiology, behavior (habitat use and activity), and fitness (survival and reproduction). This study will be the first to examine these relationships in a way that includes monitoring body temperature. The results of this study will be critical to determine if moose modify their activity and use available habitat in response to ambient temperatures, and to evaluate population performance.

METHODS

Moose were captured within the study area (Figure 1) by aerial darting (Quicksilver Air Inc., Alaska) with carfentanil (4.0, 4.5mg or 6.0mg), or thiafentanil (16mg) and xylazine (150mg or 30mg) from a helicopter; immobilizations were reversed with naltrexone (425-575mg) and tolazoline (400mg). Blood (serum and whole blood) was collected at capture by venipuncture of the jugular vein. Serum was screened for evidence of exposure to 10 disease agents following the same protocol as described by Carstensen et al. (2014). Additionally, serum was submitted for a large animal serum chemistry profile and reproductive hormones to assess physiological status, overall health, and pregnancy status (Franzmann and LeResche 1978, Haig et al. 1982, Duncan et al. 1994). Serum progesterone levels were determined by the Smithsonian Institute; levels >2.0 ng/mL were considered pregnant. Whole blood in Ethylenediamine tetraacetic acid (EDTA) was used to make blood smears and complete differential blood cell counts were performed, which may be indicative of condition and health status (Duncan et al. 1994), presence of tick-borne illnesses, and evaluation for the presence of microfilaria. An incisor (I4) was removed for aging by cementum annuli (Sergeant and Pimlott 1959). A general fecal

floatation examination for parasites was performed. A thorough physical examination was performed, including assessment of body condition score (very thin, thin, normal, fat), winter tick load, and hair loss. Rump fat measurements (Maxfat, cm) were measured by portable ultrasound to further assess body condition and nutritional status (Cook et al. 2010, DelGiudice et al. 2011). Total body length and girth (cm) were measured to estimate body weight of moose (Hundertmark and Schwartz, 1998) and hair samples were collected from the withers. Any mortalities that occurred within two weeks of capture were censored from the study.

Moose were fitted with mortality-sensing collars utilizing GPS and Iridium two-way communication technologies (Vectronic Aerospace GmbH; Berlin, Germany). Collars transmit location and status data to a base station (Forest Lake, MN) at user-defined intervals. The base station also analyzes location data to identify animals that have "localized" (e.g., remained within a 20m radius for >24 hours), to assist with detecting sick animals that are potentially moribund. When a mortality or localization event occurred, the mortality response team was notified via text and email messages. Mortality implant transmitters (Vectronic Aerospace GmbH) were placed orally into a subset of the captured moose. These devices are similar to a cow magnet in size, log internal temperatures every 15 minutes, and transmit a subset of this data through the collar. Additionally, MITs are meant to provide immediate notification of mortality via detection of minimal internal activity (e.g., lack of a heart beat) and this notification is also sent via text and email message to the moose mortality response team. External temperature loggers (Hobo TibdbitV2; Onset Corporation, Bourne, MA) were affixed to the GPS collar and were programmed to collect ambient temperature every 60 minutes.

Moose mortality response teams have 8 primary team leaders that have undergone extensive necropsy training, and they are supported by about 20 secondary and tertiary team members (including MNDNR, tribal, academic, US Forest Service, and other personnel) available upon request. Every effort is made to remove carcasses intact from the field and deliver them to the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL) for a complete necropsy by a board-certified pathologist. Teams are able to utilize special equipment, including trucks with 2000lb winches, an amphibious ARGO, chainsaw winch (e.g. Lewis winch), heavy duty snow machines with long tracks, all-terrain vehicles, and specialized rubber mats with built-in hitches for dragging carcasses. Primary members have also taken specialized training with DNR forestry and fire units to be able to sling out a moose carcass via helicopter. If a moose was found to be alive, but obviously ill, it was euthanized (via gunshot). If carcass extraction was not possible, a thorough and complete field necropsy was performed, guided by an established protocol. Samples were submitted to the UMN VDL for diagnostic evaluation (Carstensen et al. 2014).

RESULTS AND DISCUSSION

Annual survival and timing of mortalities

A total of 41 collared moose (33 females, 8 males) have died since this study began; which excludes 12 capture-related mortalities that are censored from subsequent survival analyses. Overall proximate causes of death were as follows: 16 confirmed and likely wolf kills (39%), 8 bacterial infections (20%), 5 confirmed and likely *P. tenuis* infections (12%), 4 multiple, chronic health issues (10%), 3 winter tick infestations (7%), 1 accident (2%), and 4 undetermined health issues (10%; Figure 2). A third of the wolf-killed moose had significant health conditions that likely predisposed them to predation, including encephalitis and meningitis in the brain*, P. tenuis* infections, and pneumonia in the lungs. Health-related causes were attributed to 61% of total deaths, with the remaining 39% being predator-related. Timing of these mortalities suggest that most deaths occur in spring (54%, March–May); however, moose died in all seasons (Winter 17%, Summer 22%, and Fall 7%; Figure 3). Annual (January–December) survival rate was 81% and 89% in 2013 and 2014, respectively; 91% of moose have survived from January–August 2015 (Figure 4). There are currently 115 remaining moose in the study, but 30 of these have collars that are experiencing significant transmission failures and we are not certain of their status; thus, 85 moose are actively transmitting data.

A total of 12 collared moose were censored due to capture-related mortalities, 4 (3.6%), 3 (8.1%), and 5 (15.6%) in 2013, 2014, and 2015, respectively. Unfortunately, the elevated number of captured-related deaths experienced in 2015 resulted in our decision to discontinue captures and not deploy 9 additional collars for Dr. Ron Moen's study as we had intended. Capture myopathy was the primary cause of these deaths; however, the exact mechanism involved that led to these mortalities has not been identified. Immobilizing drugs and dosages were consistent with previous years' captures in MN and comparable to other moose capture efforts in North America and Scandinavia. Pursuit and handling times averaged 6 and 55 minutes, respectively, which was similar to capture efforts in 2013 with 12% less capture-related mortalities.

Overall age of moose (*n*=35) at death was 7.8 years (±0.7 year), with ages still pending from the remaining 6 moose that died during this study. Mean age of moose that died from health-related causes (*n*=17) was 8.1 years (±1.0 year), similar to those (*n*=18) that died of wolf-related causes (7.5 ±1.1 years). Interestingly, both health and predator-related causes of death impacted nearly every age cohort in this study (Figure 5), which suggests that wolves are not selecting for just young (<3 years of age) or old (>8 years of age) moose and are able to prey upon prime aged (4-8 years old) individuals as well.

Thus far in 2015, the moose mortality rate is similar to 2014 and only half of what occurred in 2013. It appears that winter survival was enhanced by the prolonged winter 2013, which may have suppressed winter tick numbers, as we have not reported any winter tick mortalities during winter 2014 or 2015. Also, the 2 consecutive, historically severe, winters of 2014 and 2015 likely reduced deer numbers in our study area, which would lessen disease exposure risks of moose to *P. tenuis* and liver flukes.

Health Screening at Capture

Pregnancy rate was 89% in 2015; higher than 2013 (83%) and 2014 (77%). Moose at capture in 2015 (*n*=32) were generally in good body condition (44% normal, 53% thin, and 3% very thin). Ultrasonic rump fat measurements were obtained from 25 moose, and maximum rump fat averaged 1.05cm (SE = 0.17cm). There was minimal hair loss noted from winter ticks. Overall, nutritional condition in moose at capture was the poorest in 2013, but average to good in 2014 and 2015, as evidenced by the combination of body condition scores, ultrasonic rump fat measurements, and snow urine chemistries.

Overall exposure to West Nile Virus (29/158, 18%), Eastern Equine Encephalitis (0/158, 0%), various serovars of *Leptospira interrogans(*23/158, 15%), malignant catarrhal fever (51/158, 32%), and *Borreilia* (27/158, 17%) were reported. While blood evidence indicated exposure to these various diseases (with the exception of EEE), clinical evidence of infection was not observed either during capture or at death in applicable cases. Little is still known about how these diseases may impact moose or contribute to reduced survival or productivity. Further analyses of serum chemistries are pending.

Mortality Response Times

Whole carcasses were retrieved for 13 (32%) of the study cohort, with field necropsies preformed on the remaining 28 (68%) moose. Response times from initial mortality notification (e.g. text message or email) to a team in the field at the death site were ≤24 hours in 25 cases (61%), between 24 and 48 hours in 11 cases (27%), and >48 hours in 5 cases (12%). Delays in mortality responses > 24 hours have been due to collar failures and wolves actively feeding on the moose carcass and preventing the collar from sending a mortality alert.

Mortality Implant Transmitters

We successfully deployed 61 MITs in moose during this study. An additional 20 MITs were spit out by moose shortly after oral application. This rate of MIT rejection was markedly reduced from 40% (12 of 20) in 2014 to 13% (3 of 23) in 2015 due to improved application methods that included a specialized bolus applicator and properly timed reversal of xylazine to ensure the swallowing reflex was intact.

In December 2014, we began a MIT calibration project with the Moose Research Center within Alaska's Department of Game and Fish. Thus far, the study has shown the MIT to be a highly accurate measurement of internal body temperature in moose. On average, the MIT was only 0.25°C higher than body temperature determined by vaginal implant transmitters. Further, preliminary analyses of data from MITs recovered from moose that have died in Minnesota (*n*=8) indicated prolonged elevated temperatures (>102°F) for 10-30% of readings during the summer months.

Management Implications

This aggressive study has demonstrated that it is possible to respond to moose mortalities in a timely manner and obtain valuable diagnostic information to help illuminate the many causes of death for this species. The use of satellite-GPS technology was instrumental in allowing us to identify mortality events; however, we had to overcome some significant challenges in collar functionality. Previous studies in MN have pointed to health impacts as a potential driver in population declines; yet, many of those deaths lacked diagnostic data to assign causation. In this study, we not only obtained diagnostic evidence of parasites and pathogens, we also identified predisposing conditions that may be contributing to proximate causes of mortality (e.g., a brainworm infection moose is then killed by wolves). Another example is the documentation of 4 cases of initial wolf-induced injuries that did not result in immediate death, rather the moose lived for several days to weeks before succumbing to bacterial infection from that initial attack. It is likely that this type of mortality has been largely undiscovered or underestimated in previous studies, given the often lengthy time delays in getting to carcasses to obtain needed evidence. Further, 61% of all proximate mortalities in this study were health-related and 39% were predator-related. Teasing apart some of the ultimate causes (e.g. toxicities, pathogens, parasites, and climate change) will require more data over a longer time-span, as we are only 2.5 years into this current study.

Funding and Future Project Direction

This 2 million dollar project initially began through funding from the Legislative Citizen Commission on Minnesota's Resources (LCCMR; $600,000) and in-kind contributions from the MNDNR, University of Minnesota, tribal partners, and nonprofit organizations (e.g. MDHA, Northstar Museum). This project was also funded in part by the Wildlife Restoration Program (Pittman-Robertson). Additional funding through LCCMR (ENRTF project "Moose Decline and Air Temperatures in Northeastern Minnesota", M.L. 2014, Chp. 226, Sec. 2, Subd. 5m) was recently secured for $600,000 to continue the study and expand on the use of MITs to gain insight into the potential role ambient temperature may play in moose survival and productivity. Sample size of at least 100 collared adult moose has been maintained over 3 years (2013-2015) and no additional capture operations are planned at this time. Collars deployed in 2015 should last 4-5 years, meaning this study could continue until 2020

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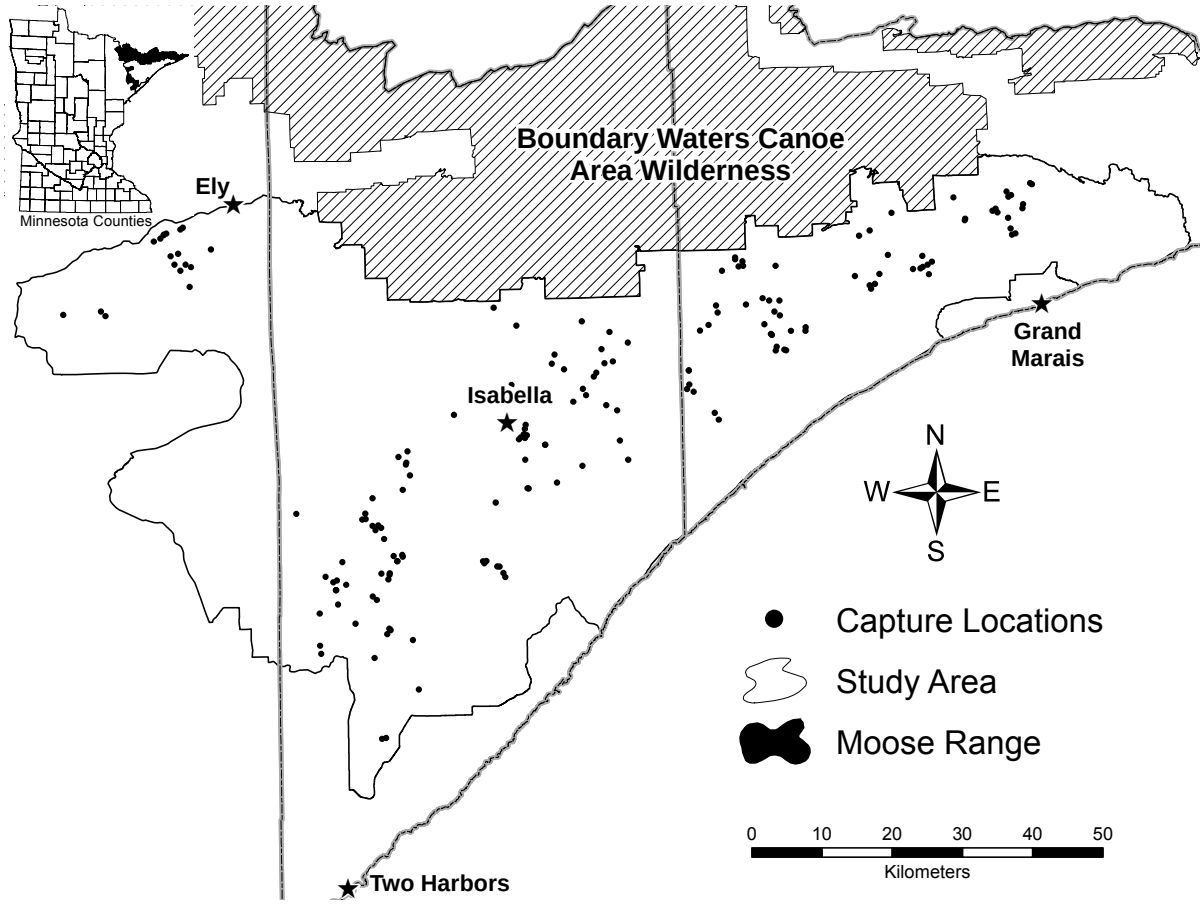

Figure 1. Study area in northeast Minnesota where 179 moose (included 6 recaptures) have been captured and radiocollared (2013–2015) to study cause-specific mortality.

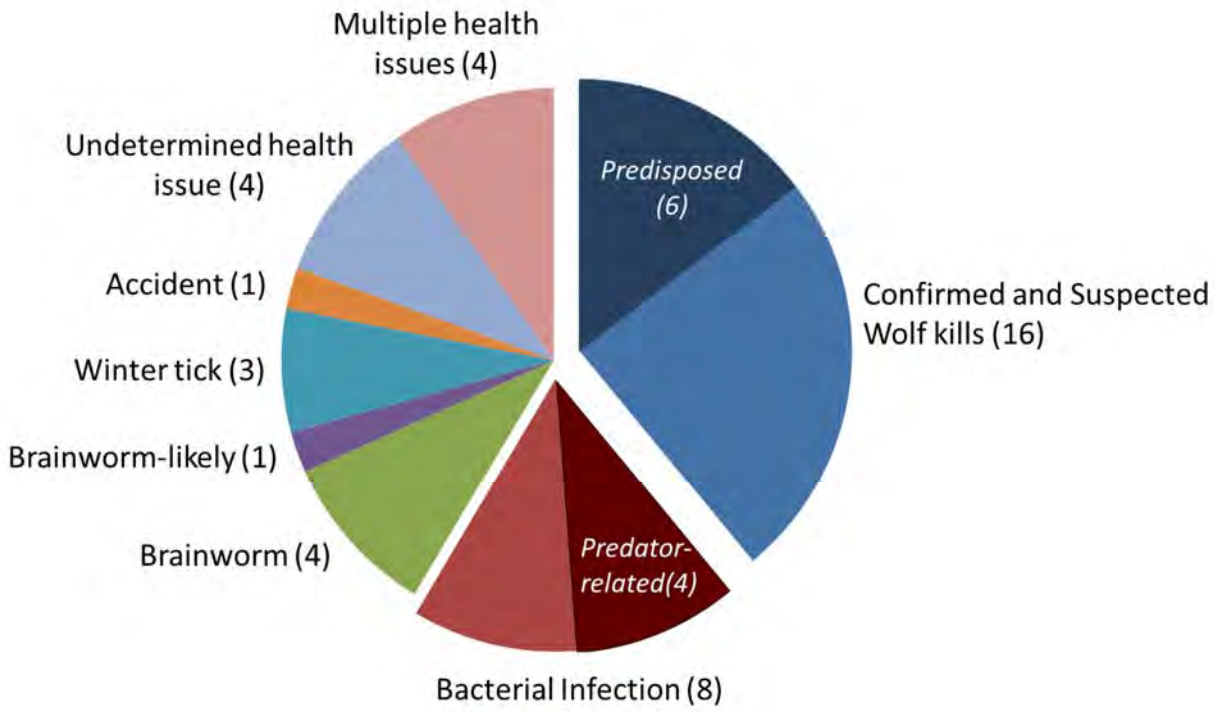

Figure 2. Cause-specific mortality of radiocollared, adult moose (*n*=41) from February 2013 to August 2015, northeast Minnesota. Predisposed wolf killed moose indicated that a significant health issue was identified that may have contributed to its death.

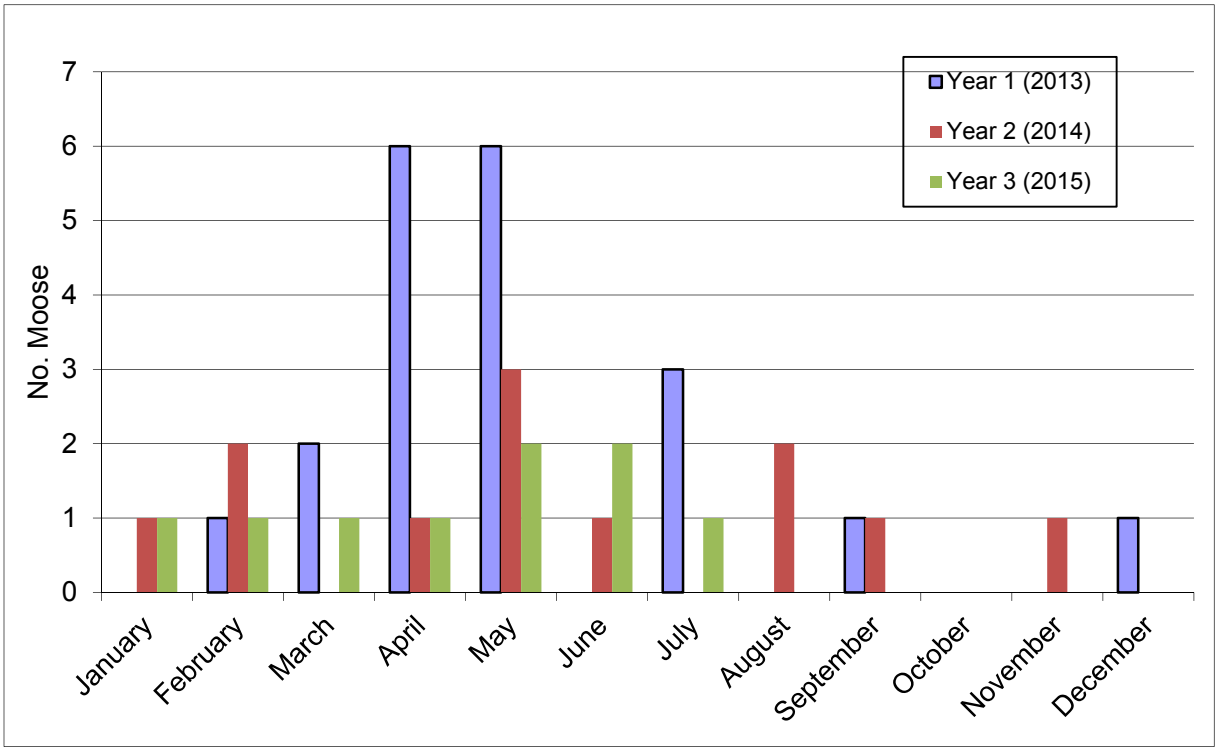

Figure 3. Timing of mortalities for radiocollared, adult moose (*n*=41) from January 2013 through August 2015, northeast Minnesota.

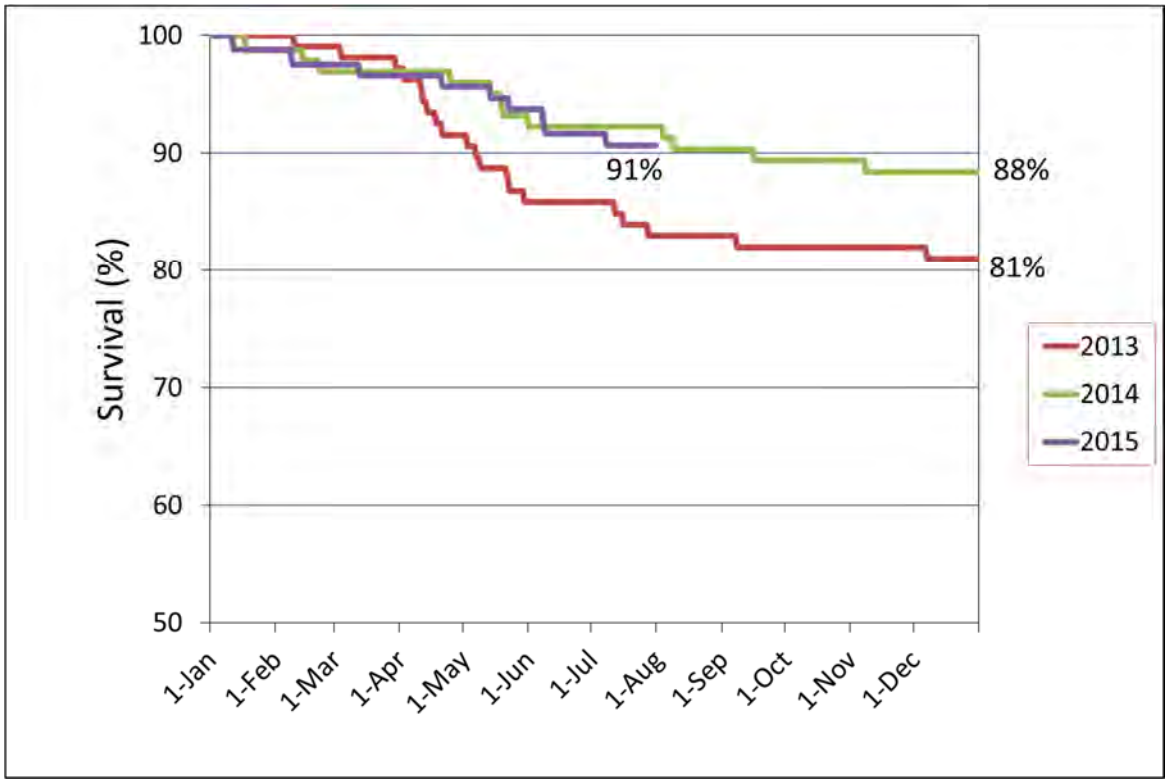

Figure 4. Annual survival of radio-collared, adult moose (*n*=173) captured in 2013, 2014, and 2015 in northeast Minnesota.

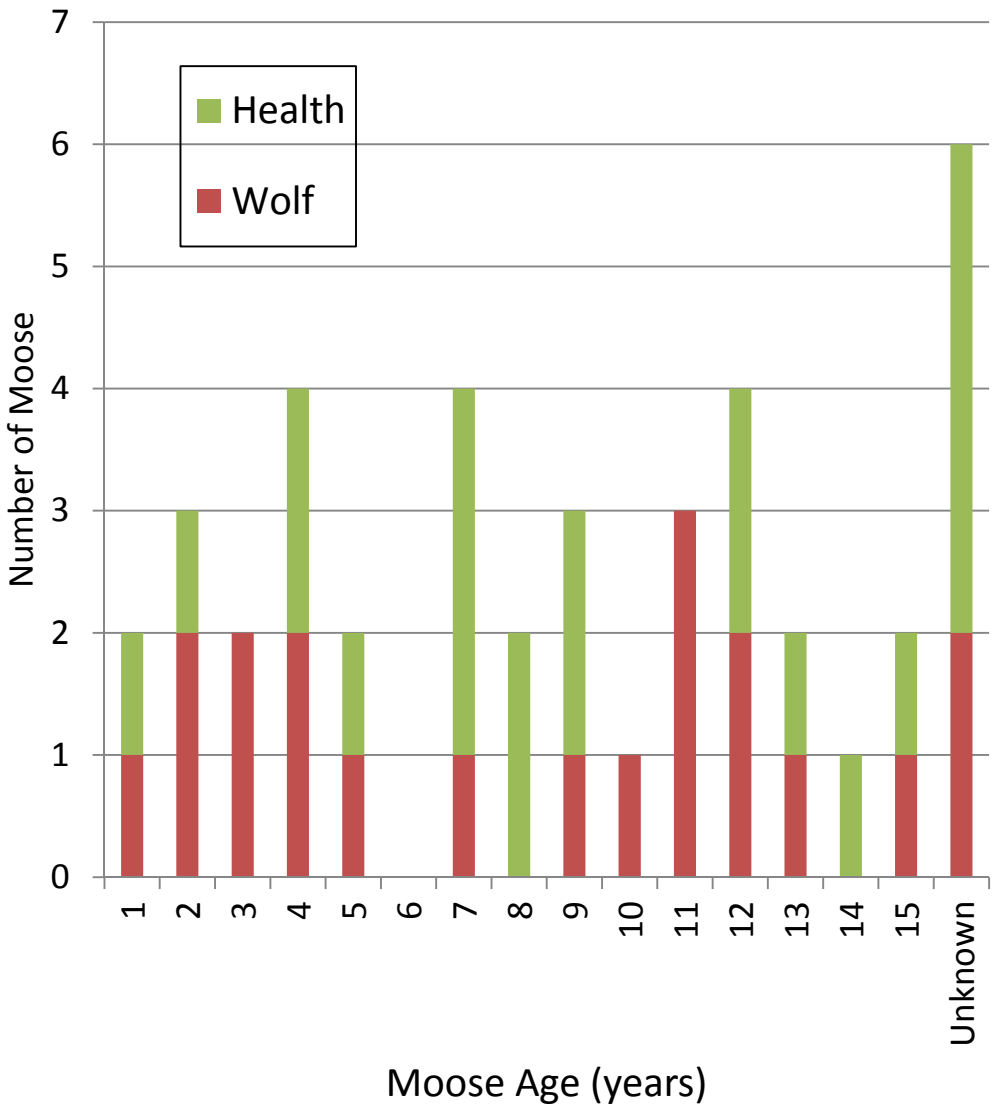

Figure 5. Age of radiocollared, adult moose (*n*=35, 6 moose have ages pending) that died from health-related (green) or wolf-related (red) causes (2013-2015), northeast Minnesota.

MNDNR

HEALTH ASSESSMENT FOR FREE-RANGING ELK IN NORTHWEST MINNESOTA, FROM 2004-2014

Michelle Carstensen, Erik Hildebrand, and Lou Cornicelli

SUMMARY OF FINDINGS

The goal of this project was to assess the health of free-ranging elk (*Cervus elaphus)* from northwestern Minnesota (NW MN) by screening animals for a variety of diseases and parasites. Results indicate which diseases the NW MN elk were exposed to, though not necessarily clinically ill. From the elk (*n*=146) included in this study, our results indicated exposure to eastern equine encephalitis (14%), West Nile virus (64%), malignant catarrhal fever (38%), anaplasmosis (4%), borreliosis (59%), bovine viral diarrhea virus 1 and 2 (10%), bovine herpes virus (5%), *Leptospira sp*., (13%) and parainfluenza virus 3 (30%). A variety of fecal parasites were also identified (*Coccidia, Strongyle-type ova,* and *Moniezia*) in 22% of elk examined. Lung and liver tissue were cultured for bacterial infection; *Streptococcus sp.* was isolated from the lung of one individual and no isolations were found in liver samples. All elk were negative for *Mycobacterium paratuberculosis,* blue tongue virus, neospora, epizootic hemorrhagic disease, brucellosis, chronic wasting disease, and bovine tuberculosis. Hepatic mineral levels were also evaluated.

INTRODUCTION

Elk in Minnesota

Elk (*Cervus elaphus*) are native to Minnesota and were originally distributed across most of the state. They were formally protected from hunting in 1893 and by the early 1900s, over-hunting and prairie conversion to agriculture led to a functional extirpation (Hazard 1982). Reintroduction efforts were initiated in 1914 and 1915 using elk from Yellowstone National Park and Jackson, Wyoming that were translocated to Itasca State Park in north central Minnesota. The herd expanded and in 1935, 27 elk were moved from Itasca State Park to the Red Lake Game Preserve in northwest Minnesota. By the 1940s, the northwest elk population was estimated at nearly 100 animals (MNDNR 2015). Currently, this population (herein referred to as the "Grygla herd") occupies a 45 mi^2 area north of Grygla, Minnesota (Figure 1). Existence of these animals has been controversial and in 1987, the Minnesota Legislature mandated the pre-calving population range between 20–30 animals. Consequently, the Minnesota Department of Natural Resources (MNDNR) instituted elk hunts in 1987, 1996, 1997, and 1998; however, few animals were taken each year (MNDNR 2015). From 2004-2013, hunts have been held annually to keep elk numbers between 30 and 38 animals**.** The 2013, 2014, and 2015 surveys counted 28, 20, and 18 elk, respectively. A second herd of elk occurs in Kittson and Roseau Counties (Figure 2), and is termed the Kittson County herd. These animals were first observed along the Manitoba border in the early 1980s and are loosely segregated into 3 subgroups based on distinctive areas of use (Figure 2). These three subgroups are the Water Tower subgroup (north of Lancaster), the Lancaster subgroup (east of Lancaster) and the Caribou/Vita subgroup (located between Caribou, MN and Vita, Manitoba). The Caribou/Vita herd is known to occupy either side of the international border at any time of year. The extent to which the other two subgroups cross into Canada is unknown. Little is also known regarding the extent of

animal interchange between the Caribou/Vita subgroup and the other two subgroups (MNDNR 2015). Due to crop depredation issues, a hunting season was first held in 2008. The most recent elk survey counted 34 animals in the Kittson County herd. The current Elk Management Plan set a pre-calving population goal for the Watertower and Lancaster subgroups at 20-30 each. The population goal for the Caribou-Vita subgroup is still under discussion with MNDNR and Manitoba Conservation.

Research background

Infectious diseases can reduce reproductive rates and increase mortality and are thus known to regulate wildlife populations (Delahay et al., 2009). For example, meningeal worm (*Parelaphostrongylus tenuis*) has been implicated in both failures to reestablish eastern elk populations and elk population declines in sympatric white-tailed deer range (Raskevitz et al., 1991; McIntosh et al., 2007). Other research has shown that meningeal worm has a minimal long-term impact on elk population growth in Michigan (Bender et al., 2005) or Kentucky (Bolling, 2009). Conversely, research on northwestern Minnesota moose indicated parasites and disease as reasons that population collapsed (Murray et al., 2006).

The objective of our study was to conduct a health assessment of NW MN elk that included: 1) serological survey of pathogens that are known to cause mortality in other mammalian species or are important from a human health perspective; 2) analysis of fecal material for parasites; and 3) examination of tissues to ascertain presence of bacterial infection. Results of this testing could be used to make inferences regarding the potential limiting factors related to elk herd expansion, explanation of disease transfer risk (both zoonotic and wild-domestic), and explanation of potential disease risks to this population.

Further, the discovery of bovine tuberculosis (TB) in cattle and free-ranging deer from 2004-2009 has brought increased scrutiny as to the health status of the NW MN elk, particularly the Grygla Herd. While overlap in range between elk and known TB-infected deer or cattle farms is known to occur, there has been no evidence of TB-infection in MN's elk herd. TB-infected cattle and deer in MN shared the same strain, which is considered of Mexican or southwest US origin, and was not related to the strain of bovine TB found in elk in Manitoba's Riding Mountain National Park. Although Minnesota is now declared 'bovine TB' free, interest remains regarding the role elk may play in future disease maintenance and/or transmission.

METHODS

For this report, all elk sampled from NW MN were grouped as either *harvested* animals (including hunter-harvested, removed under shooting permit, and illegally poached) or *other* (including vehicle kills, sick, and found dead elk). All elk within the harvested category were assumed to be representative of healthy individuals within the population.

Hunters were asked to collect samples of lung, liver, feces, blood, hair, ticks, and an incisor for aging from hunter-harvested elk. MNDNR provided a project overview, instructions of sample collection, and sampling kits at the mandatory elk hunter orientation sessions. Elk shot through depredation permits or other methods were sampled by trained MNDNR. For sick animals, displaying clinical signs of illness, every effort was made to obtain intact carcasses for full necropsy at the University of Minnesota Veterinary Diagnostics Laboratory (VDL), St. Paul, MN.

All equipment needed for hunter-harvested sampling was included in the sampling kit: soft-sided cooler; 1-60cc syringe for blood collection; 6-15cc serum tubes for blood storage; 3 whirlpaks for a sample of liver, lung, and feces; 2 specimen jars with formalin for liver and lung samples; 2 coin envelopes for hair and tooth; datasheet; protocol; Sharpie marker; 1 pair of large vinyl gloves; and 1 ice pack. Successful hunters dropped off their sampling kits when they registered their animal and also provided information on the location of their kill.

Hunters collected blood from the chest cavity as soon after death as possible, using a 60 cc syringe. The blood was placed in serum tubes and kept cool until they were delivered to official MNDNR registration station. Liver and lung samples were collected and split, with half

placed in a formalin jar, while the other half was frozen in whirlpak bags. If the hunter found anything unusual, such as a large abscess or tumor, those samples were also collected and split between the preservation methods (formalin fixation and freezing). Complete sets of samples were not collected from all elk included in this project, as field conditions and sample quality varied; however, there were very few errors in tissue identification or insufficient sample quantities in those submitted. Blood was centrifuged at the registration stations and serum was extracted and frozen. Cranial lymph nodes and obexes were removed by trained MNDNR staff at the registration stations to allow for chronic wasting and bovine tuberculosis testing. Where appropriate, MNDNR made arrangements with taxidermists to collect samples from trophy animals. All samples were submitted to the VDL, where the majority of the testing occurred; some tests were outsourced to the National Veterinary Services Laboratories (NVSL) in Ames, IA. Teeth were sent to Matson's Laboratory (Milltown, MT) for aging by cementum annuli.

RESULTS AND DISCUSSION

A total of 146 elk (83 females, 63 males) were included in this health assessment project (Figure 3). Harvested elk accounted for 138 of the animals (106 hunter-harvested, 29 shooting permits, and 3 poached). In addition, 8 other animals were sampled (2 roadkill, 3 found dead, 3 clinically ill (observed with neurological symptoms) elk that were euthanized by gunshot. Necropsy results from 2 of the clinically ill elk confirmed migrations tracks from *P. tenuis* infection; insufficient samples were collected from the third sick elk to diagnose any illness. Exact age was determined for 130 elk (*M* = 4.4 years; *SE*= 0.3 years; range 0.5 to 16 years old (Figure 4).

Serologic results from harvested elk indicate exposure to eastern equine encephalitis, West Nile Virus, malignant catarrhal fever, anaplasmosis, borreliosis, bovine viral diarrhea virus 1 and 2, bovine herpes virus 1, *Leptospira* spp., and parainfluenza virus 3 (Table 1). Liver samples from harvested elk were evaluated for heavy metal and mineral status (Table 2). Fecal samples from 114 elk were screened for evidence of parasites. Parasites were identified in 25 samples (21.9%), including *Fascioloides magna (n=10), Coccidia sp. (n*=2*), Strongyle-type ova (n*=8*), Moniezia sp (n*=2*), Capillaria sp. (n*=1*),* and mite ova *(n*=2*).* Negative results do not necessarily mean the animal was parasite-free, only that it was not actively shedding at the time the feces were collected.

Our testing indicated that elk in NW MN were not exposed to brucellosis, blue tongue virus, epizootic hemorrhagic disease, neospora, mycobacterium paratuberculosis, bovine tuberculosis, or chronic wasting disease (Table 1).

Mosquito-borne diseases

Positive results were reported for 14 (14.4%) and 36 (37.5%) elk tested for eastern equine encephalitis (EEE) and West Nile Virus (WNV), respectively (Table 1). Both of these arboviruses are spread by mosquitoes, with EEE typically posing a greater mortality treat to most species. Clinical signs of EEE in horses and sheep involve neurologic signs and often death (Bauer et al. 2005, Rutledge 2008). It is also a zoonotic disease and human infections are reported to the Center for Disease Control. Schmitt et al. (2007) reported clinical infection of EEE in free-ranging white-tailed deer (*Odocoileus virginianus*) in Michigan, suggesting this disease can cause mortality in wild cervids and maybe often be overlooked if biologists are only seeking to rule-out chronic wasting disease (CWD).

Little is known about the effects of WNV in elk. Palmer at al. (2004) reported WNV infection in 2 reindeer (*Rangifer tarandus)*, which was the first confirmed cases of this disease in cervids. A wild white-tailed deer in Georgia was reported to die from a WNV infection (Miller et al., 2005). As with EEE, clinical signs of WNV include ataxia, tremors, head tilt, and depression; which are commonly reported neurological signs in wild cervids with numerous causes (e.g., brain abscess, CWD, blunt trauma, etc.); thus, true WNV infection may be under-reported.

Tick-borne diseases

Positive results were reported for 58 (59.1%) and 4 (3.8%) elk tested for *borrelia burgdorferi* (Lyme disease) and anaplasmosis, respectively (Table 1). Borreliosis is a tick-borne (*Ixodes sp*.) bacterial disease that is maintained through sylvatic cycles involving a variety of species, including mammals (primarily wild rodents as the reservoir hosts) and birds. Clinical disease typically includes arthritis and neurologic or cardia dysfunction. While evidence of natural infection exists in wild cervids through serosurveys, there has been no documentation of clinical disease in elk.

Anaplasmosis (*Anaplasma phagocytopila*, formerly *Ehrlichia phagocytophila*) infection in sheep and cattle produces significant effects on the immunological defense system, increasing their susceptibility to disease and secondary infections (Larsen et al., 1994). Experimental studies have shown that elk can harbor asymptomatic infections with *A. marginale* and *A. ovis,* the causes of anaplasmosis in cattle and sheep, respectively. Renshaw et al. (1979) experimentally inoculated elk with *A. marginale* from infected cattle and these elk produced disease in spenectomized bovine calves. This study suggested that free-ranging elk could become infected and act as a reservoir for this disease, but wouldn't likely compromised their survival. This implication of elk as a disease reservoir for anaplasmosis could undermine elk population expansion in NW MN, as cattle producers in the area have been concerned with disease transmission risks between at the wildlife-cattle interface. However, efforts to recover *Anaplasma* spp. from free-ranging elk populations have been unsuccessful, suggesting that even though these species are susceptible, they are probably not responsible for maintaining infections or acting as a source of infection for cattle (Corn et al., 2001). Clinical anaplasmosis has not been reported in elk.

Malignant Catarrhal Fever

A total of 36 (37.5%) elk were positive for peroxidase-linked assay (PLA) testing for malignant catarrhal fever (MCF) in this study (Table 1). Virus neutralization (VN) testing is performed on all PLA-positive samples; however, all elk were negative on VN. The PLA test is more sensitive than the virus isolation, meaning it is much better at identifying true positives. Whereas, VN is more specific, which means it is better at identifying true negatives. There are a couple of concerns with this testing. First, the PLA reacts with multiple gammaherpes viruses (including strains from wildebeest, sheep and deer). A PLA-positive test does not indicate which strain has been found, only indicated that one of the various strains was detected. The higher the positive value with the PLA test, the stronger the positive in the sample. Second, the VN test only screens for the wildebeest strain (which is exotic to the U.S. and a reportable foreign animal disease) and would be negative if other strains are present. This means a sample that was positive on PLA and negative on VN was likely exposed to a gammaherpes virus, but not the wildebeest strain. We do not know for certain what stain of MCF elk are being exposed to in NW MN.

Gammaherpes viruses have been documented to cause serious illness and death in elk and other ruminants. The clinical symptoms can mimic *Parelaphostrongylus tenuis* infection as the animals often exhibit neurological deficits, blindness, high fever, and salivation. While infection with MCF frequently results in death, carrier status can occur and is identified with serology. Li et al. (1996) found small numbers of United States free-ranging elk were seropositive; these animals were once exposed to MCF viruses but whether they had recovered from a non-lethal disease is unknown. A serosurvey to MCF in Alaskan wildlife reported high antibody prevalences for several wildlife species including 96% in muskox (*Ovibos moschatus*), 95% in Dall sheep (*Ovis dalli*), and 27% in elk; impact on survival is unknown (Zarnke et al. 2002).

Cattle-borne Diseases: Bovine Viral Diarrhea Virus (BVD) 1 and 2, Bovine Herpes Virus 1 (BHV), and Parainfluenza Virus 3 (PI)

Positive results were reported for 11 (10.1%) and 5 (4.5%) of elk tested for BVD and BHV, respectively (Table 1). BVD is considered a major disease of cattle and is thought to be

the most common infectious cause of reproductive failure in beef herds in the western U.S. BVD also causes enteritis, mucosal disease, infections, and respiratory disorders in cattle, though experimentally inoculated non-pregnant elk showed no clinical signs and remained healthy for >50 days post inoculation (Barber-Meyer et al, 2007). Tessaro et al. (1999) demonstrated that while experimentally inoculated elk do not show sign of the disease, they can shed and transmit BVD once exposed. Natural exposure of BVD to wild ungulates suggests a spillover from cattle or maintenance within wildlife populations (Duncan et al. 2008).

Bovine herpes virus type 1 is a disease that can lead to respiratory tract disorders, conjunctivitis, genital disorders and immune suppression. It is believed to infect all ruminant species and has been isolated from a large number of wild species. It is most commonly isolated in feedlot cattle. As with BVD, exposure of elk in NW MN to BHV, both cattle-borne diseases, demonstrates contact between these species (direct or indirect) is sufficient to promote exchange of pathogens.

A total of 31 elk (29.5%) were positive for exposure to parainfluenza virus 3 (Table 1). Domestic ruminants are considered the main source of infection for free-ranging ruminants. PI causes mild respiratory disorders in domestic cattle and sheep that serve as initiators for secondary infections of *Pasteurella* spp., which can result in bacterial pneumonia, but clinical symptoms in wild elk remains unknown (Barber-Meyer et al, 2007).

Leptospirosis

Leptospirosis is a bacterial disease that can infect a wide variety of mammals, both domestic and wild. In ungulates, it causes abortion (Fraser and Mayes, 1986). Exposure usually occurs through direct contact with urine from carrier animals or indirectly by contact with a urine- contaminated environment (Bender and Hall, 1996). Much of the landscape of NW MN contains environments where moist alkaline soils are present to house the bacteria, and it may survive for several weeks (Thorne 1982).

A total of 111 elk were screened for 6 species of *Leptospira,* using a microscopic agglutination test (MAT); 12 elk 10.8%) were exposed to at least one strain, 1 elk was co-infected with 2 strains of *Letospira*.(Table 1). Free-ranging elk in Washington had high seroprevalence to *Leptospira interrogans* and high local productivity, suggesting clinical affects may be more dramatic in cattle (Bender and Hall, 1996).

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Table 1. Serological results from harvested elk in northwestern Minnesota, 2004 2014.

Disease	*n*	Apparent prevalence %
Eastern Equine Encephalitis	97	14.4 (*n*=14)
Malignant Catarrhal Fever	96	37.5 (*n*=36)
West Nile Virus	89	64.1 (*n*=57)
Anaplasmosis	104	3.8 *(n=4)*
Borreliosis	98	59.1 (*n*=58)
Brucellosis	112	0
Bovine Viral Diarrhea Virus 1 and 2	109	10.1 (*n*=11)
Bovine Herpes Virus	111	4.5 (*n*=5)
Blue Tongue Virus	112	0
Epizootic Hemorrhagic Disease	112	0
Leptospira Bratislava	111	0.9 (*n=1)*
Leptospira Canicola	111	0
Leptospira Grippothyphosa	111	0
Leptospira Hardjo	111	0.9 (*n*=1)
Leptospira Interrogans Serovar Icterohaemorrhagicae	111	9.1 (*n*=10)
Leptospira Pomona	111	1.8 (*n*=2)
Neospora	104	0
Parainfluenza Virus 3	105	29.5 (*n*=31)
Mycobacterium paratuberculosis	99	0
Bovine Tuberculosis	113	0
Chronic Wasting Disease	115	0

Table 2. Hepatic mineral values of harvested elk in northwestern Minnesota, 2004-2014.

Element (units)	*n*	Mean	Standard deviation	Minimum	Maximum
Arsenic (ppm)[1]	122	0	0	0	0
Boron (ppm)	72	0.148	0.04	0.12	0.21
Barium (ppm)	72	0.14	0.03	0.12	0.17
Calcium (ppm)	72	51.73	16.49	27.6	111
Cadmium (ppm)	122	0.23	0.16	0.06	0.82
Cobalt (ppm)	122	0.07	0.02	0.03	0.13
Chromium (ppm)	72	0.32	0.04	0.32	0.32
Copper (ppm)	122	15.01	14.68	1.3	87.1
Iron (ppm)	122	173	135	37.9	946.3
Mercury (ppm)[2]	81	0	0	0	0
Potassium (ppm)	72	2631.86	226.48	1934	3031
Magnesium (ppm)	113	164.25	20.33	86	214
Manganese (ppm)	116	2.62	0.99	0.28	5.60
Molybdenum (ppm)	122	1.10	0.34	0.39	1.77
Sodium (ppm)	72	897.51	190.27	579	1490
Phosphorous (ppm)	72	4190.17	579.40	1650	5354
Lead (ppm)	122	1.42	0.37	0.04	3.49
Antimony (ppm)[3]	72	0	0	0	0
Selenium (ppm)	122	0.94	0.42	0.25	1.90
Thallium (ppm)[4]	81	0	0	0	0
Zinc (ppm)	122	25.35	9.57	13	89

[1]Cut-off values for arsenic ranged from <0.03 to <0.50 (ppm); all elk were below these thresholds.

[2]Cut-off values for mercury ranged from <0.13 to <.2.0 (ppm); all elk were below these thresholds.

[3]Cut-off values for antimony were <1.0 (ppm); all elk were below these thresholds.

[4]Cut-off values for thallium ranged from <0.03 to <2.50 (ppm); all elk were below these thresholds.

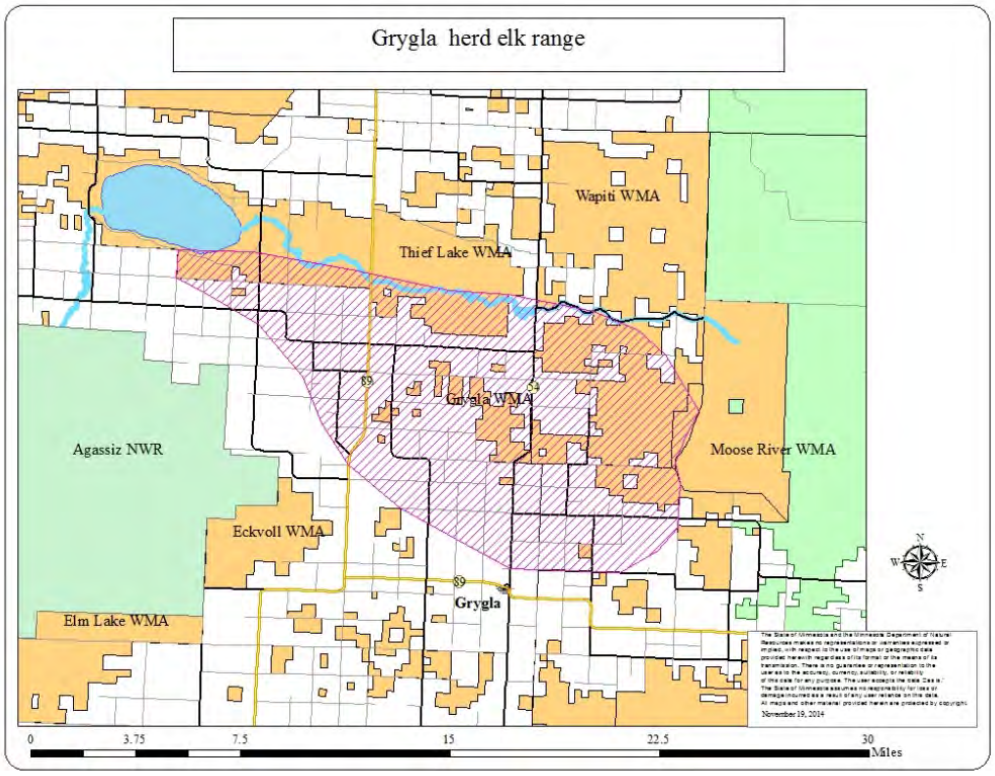

Figure 1. The Grygla elk herd, a remnant from a 1935 reintroduction effort, primarily occupies a 45 mi^2 area north of Grygla, Minnesota.

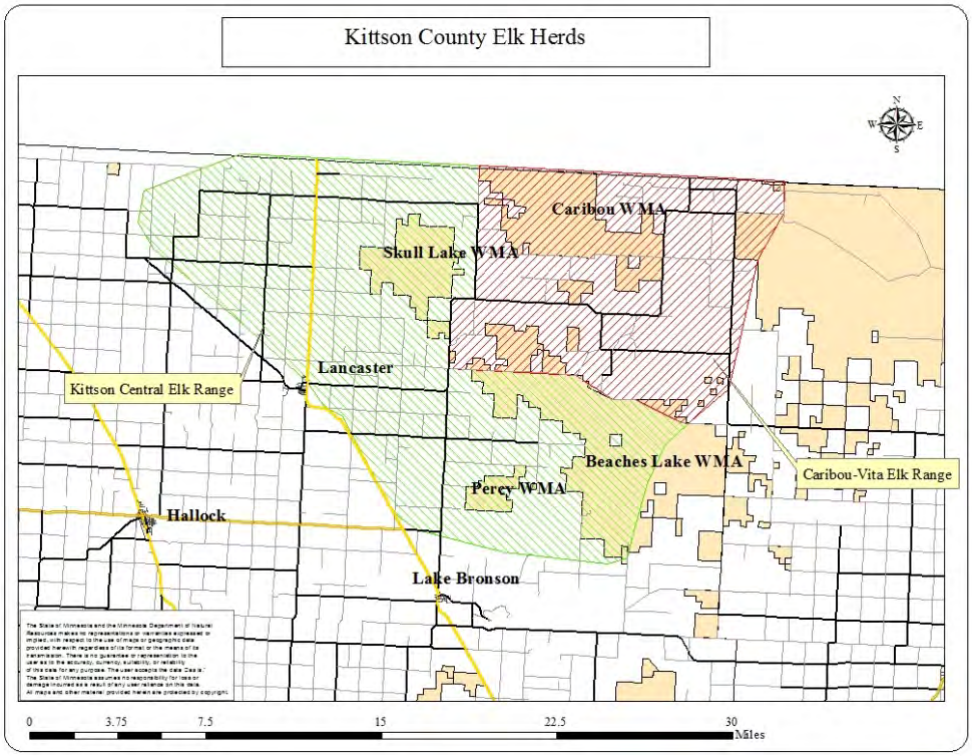

Figure 2. Kittson County elk range.

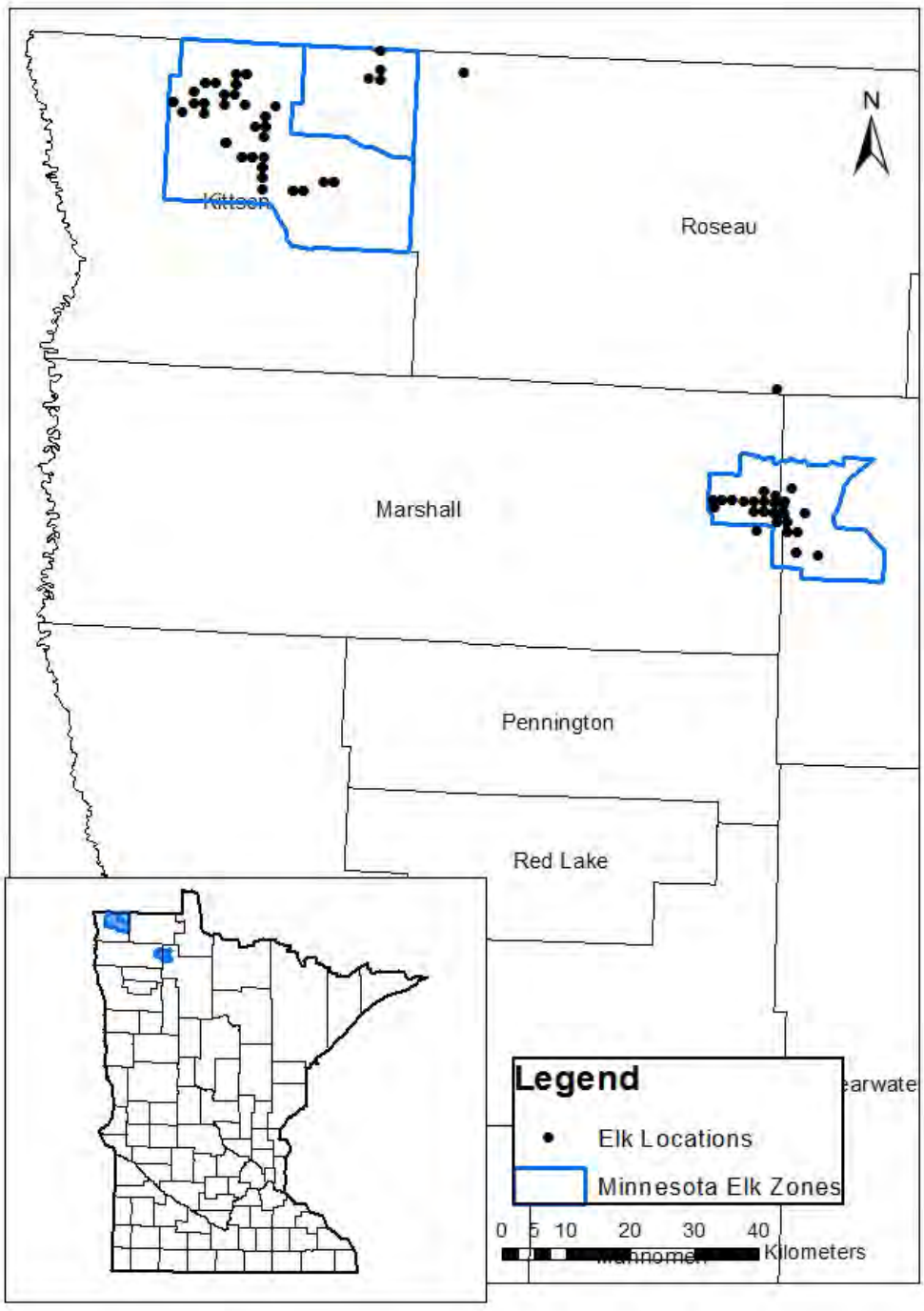

Figure 3. Locations were elk (*n*=146) were sampled for health status in northwest Minnesota, 2004-2014.

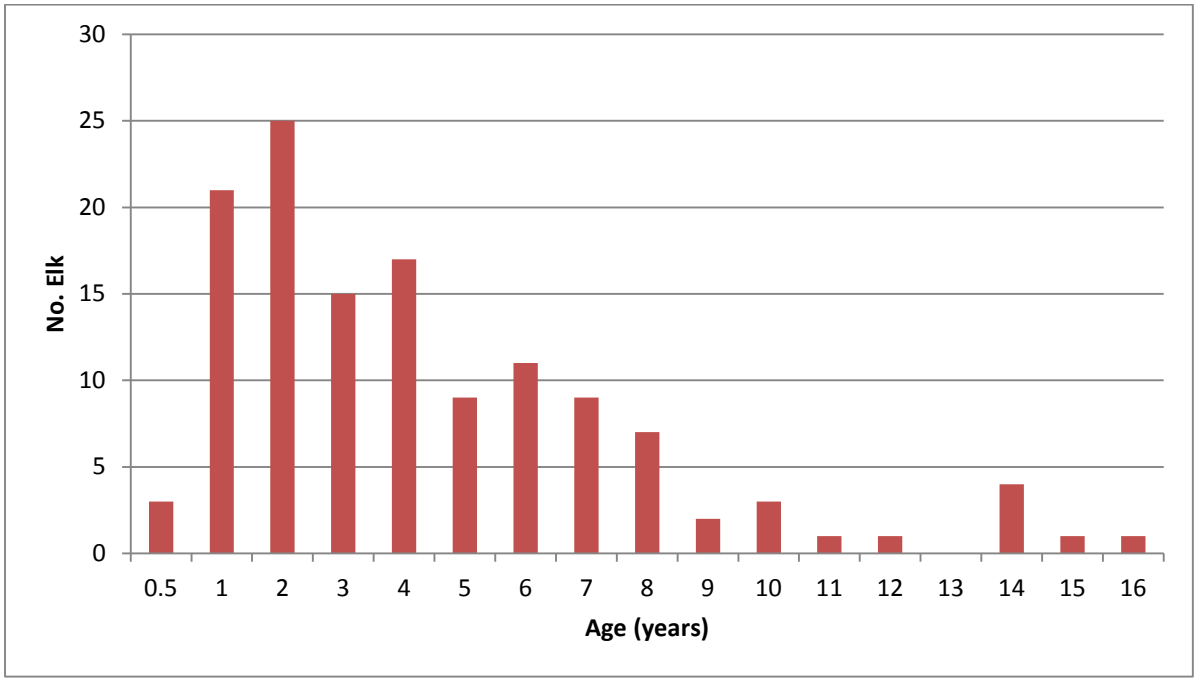

Figure 4. Age distribution of elk (*n*=130) included in the 2004-2014 health assessment project, northwestern Minnesota.

SEROPREVALENCE, ISOLATION, FIRST GENETIC CHARACTERIZATION OF *TOXOPLASMA GONDII*, AND CONGENITAL TRANSMISSION IN WILD MOOSE FROM MINNESOTA, USA[1]

Shiv K. Verma[2], Michelle Carstensen[3], Rafael Calero-Bernal[2], Seth Moore[4], Tiantian Jiang[5], Chunlei Su[5], Jitender P. Dubey[2]

ABSTRACT

Toxoplasma gondii infections are widespread in white tailed deer (*Odocoileus virginianus*) but little is known of its prevalence in other cervids in the USA. Moose (*Alces alces*) is a popular large game animal, hunted for its meat and trophy antlers. Here, we report seroprevalence, isolation and genetic characterization of *T. gondii* from moose from Minnesota. Antibodies against *T. gondii* were detected in 8 of 79 (10%) moose tested by the modified agglutination test (MAT 1:25 or higher). The myocardium of 68 moose was bioassayed individually in mice, irrespective of serological status. *T. gondii* was detected in 3 moose (2 adults, 1 three-week old). The parasite from 2 adults was further propagated in cell culture. PCR-RFLP genotyping of cell culture derived tachyzoites using 10 genetic markers, SAG1, SAG2 (5'and 3' SAG2, and alt.SAG2) SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico revealed two different ToxoDB PCR-RFLP genotypes (#5, designated TgMooseUS1, and #7, TgMooseUS2). The mice inoculated with myocardium of the juvenile moose developed antibodies against *T. gondii* and DNA extracted from infected mouse brain was only partially characterized by PCR-RFLP genotyping, which suggests a potential new genotype. Result documented prevalence of *T. gondii* in moose, and its possible transplacental/transmammary transmission of *T. gondii* in moose.

[1] Parasitology Research, 2015, *In Press*

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HABITAT FUNCTIONAL RESPONSE MITIGATES REDUCED FORAGING OPPORTUNITY: IMPLICATIONS FOR ANIMAL FITNESS AND SPACE USE[1]

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ABSTRACT

Animals must selectively use landscapes to meet their energetic needs. Depending on an animal's state, it may use a critical resource more or less frequently, and trade-offs involving the use of different habitats may depend on their availability and environmental conditions (e.g., temperature). For example, habitat selection at high temperatures may favor areas that provide thermal cover at the cost of reduced foraging efficiency under consistently thermally stressful conditions. We estimated individual step selection functions (SSFs) with shrinkage using telemetry data from 134 adult female moose (*Alces alces*) in Minnesota, U.S.A., and 64 in Ontario, Canada, to assess the consistency of habitat selection with variation in temperature, time of day, and habitat availability. We averaged model coefficients across all animals within a site to quantify selection strength for five habitat types differing in forage availability and thermal cover. Moose in Ontario favored areas dominated by deciduous and mixedwood forest, consistent with selection for foraging habitat across both the diurnal cycle and a wide range of temperatures. Space-use and habitat-selection patterns exhibited by moose in Minnesota were more dynamic and were indicative of time- and temperature-dependent trade-offs between use of critical foraging habitat (i.e., deciduous) and thermal cover (i.e., conifer, treed wetlands). The amount of deciduous forest associated with both used and available locations declined during mid-day and also with increasing temperatures. Yet, the rate of decline was higher for available than used points, indicating a scale-dependent functional response in habitat selection driven by the trade-off between selection for foraging habitat and thermal cover. These trends suggest that variation in landscape composition and quality interact to produce complex patterns of space use and habitat selection, and foraging animals exposed to increasingly high temperatures may mitigate fitness losses due to reduced foraging efficiency by increasing their selection for foraging habitat in sub-prime foraging landscapes.

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DESCRIPTIVE EPIDEMIOLOGY AND WHOLE GENOME SEQUENCING ANALYSIS FOR AN OUTBREAK OF BOVINE TUBERCULOSIS IN BEEF CATTLE AND WHITE-TAILED DEER IN NORTHWESTERN MINNESOTA[1]

Linda Glaser[2], Michelle Carstensen[3], Sherry Shaw[4], Suelee Robbe-Austerman[5], Arno Wunschmann[6], Dan Grear[7], Tod Stuber[7]

ABSTRACT

Bovine tuberculosis (bTB) was discovered in Minnesota through routine slaughter surveillance in 2005 and the resulting epidemiological investigation led to the discovery of infection in both cattle and white-tailed deer in the state. From 2005 through 2009, a total of 12 beef cattle herds and 27 free-ranging white-tailed deer (*Odocoileus virginianus*) were found infected in a small geographic region of northwestern Minnesota. Genotyping of isolates determined both cattle and deer shared the same strain of bTB, and it was similar to types found in southwest United States and Mexico. Whole genomic sequencing confirmed the introduction of this bTB into Minnesota was recent, with little genetic divergence. Aggressive surveillance and management efforts in both cattle and deer continued from 2010-2012; no additional infections were discovered. Over 10,000 deer were tested and 705 whole herd cattle tests performed in the investigation of this outbreak.

[1] PLOS ONE, 2015, *In Review*

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MOSQUITOES IN MOOSE COUNTRY: POTENTIAL ARBOVIRUS VECTORS IN NORTHERN MINNESOTA[1]

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ABSTRACT

A mosquito surveillance study was conducted following the discovery of serologic evidence of eastern equine encephalitis virus (EEEV) and West Nile virus (WNV) in moose and elk in northern Minnesota. Adult mosquitoes were collected at twelve sites using carbon dioxide traps throughout the summer of 2012. Specimens were counted, identified to species, sorted into pools, and tested for EEEV and WNV. None of the pools were positive for either virus. Low numbers of *Culiseta melanura* (Coquillet) (Diptera: Culicidae) and greater numbers of previously identified eastern equine encephalitis virus and West Nile virus vectors were present in both study regions. Mosquito vectors for arboviruses historically present in Minnesota, such as La Crosse virus and western equine encephalitis virus, were also collected in the study locations. Our findings extend the known range of *Culiseta melanura, Anopheles barberi* (Coquillet) (Diptera: Culicidae), and *Anopheles quadrimaculatus* (Say) (Diptera: Culicidae) into regions of Minnesota with evidence of wild ungulate exposure to eastern equine encephalitis virus, and document the presence and abundance of twenty seven other mosquito taxa in the region.

[1] Journal of the Mosquito Control Association, 2015, *In Review*

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SHALLOW LAKES IN MINNESOTA: CAN WE PREDICT THE GOOD, THE BAD, AND THE VULNERABLE?

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SUMMARY OF FINDINGS

Our initial efforts focused on (1) using linear mixed-effects models to predict total phosphorus (TP) levels in shallow MN lakes from watershed and in-lake features and (2) developing an integrated framework to classify lake states (clear or turbid) and estimate state-dependent relationships between key system variables in shallow lakes. Our best TP model to date includes relative abundance of benthivorous fish, average lake depth, and proportion of forest and shrublands within lake watersheds. Higher TP levels were observed in lakes with high benthivore mass, relatively shallow depth, and low proportions of forest and shrublands in adjacent watersheds. Chlorophyll *a* (Chla) concentration, TP, and relative abundance of submerged aquatic vegetation (SAV) were incorporated into a Bayesian latent variable regression framework to classify lake states and identify relationships between nutrients and turbidity. The model produced reasonable classifications and regression relationships, though modifications to incorporate nutrient thresholds and improve coefficient estimates are ongoing. These two analyses will eventually allow us to use Chla, SAV, and TP to assess the relative state transition risk of shallow lakes in MN to aid in management decisions.

INTRODUCTION

Shallow lake ecology

Shallow lakes generally conform to one of two alternative stable states: a clear state with primary production dominated by submerged aquatic vegetation (SAV) and a turbid state with phytoplankton dominating over SAV. Excessive nutrient inputs from current and historical land use, food web-mediated influences and sediment disturbance caused by planktivorous and benthivorous fish, and wind all drive transitions to, and impact the resilience of, turbid states. Shallow lakes with high nutrient levels are especially prone to explosive phytoplankton “blooms” when phosphorus (P) is readily available. Submerged aquatic vegetation, which sustains the diverse invertebrate communities that provide important food sources for waterfowl, is reduced in this turbid, algae-dominated state. Parasites associated with amphibian malformations likely have higher prevalence in turbid lakes (Johnson and Chase 2004), and nitrogen may accumulate at higher rates (Zimmer et al. 2003). It is not surprising that key goals for shallow lake management are to prevent shifts from clear to turbid states, to induce shifts from turbid to clear states, and to maintain the natural resilience of clear-water shallow lakes.

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Alternative states have been described from field studies of shallow lakes in the US (Hanson and Butler 1994, Hansel-Welch et al. 2003, Zimmer et al. 2009), Great Britain (Moss et al. 1996), the Netherlands (Gulati and Van Donk 2002, Scheffer 2004), Denmark (Søndergaard et al. 2007), Sweden (Hargeby et al. 1994), New Zealand (Mitchell et al. 1988), and China (Wang et al. 2014). Complex ecological and physical mechanisms are responsible for maintaining the stability of each alternative state, such as competition between primary producers. When SAV declines, phytoplankton abundance typically increases, limiting light reaching the lake bottom and further restricting SAV in a positive-feedback fashion. Additionally, when SAV is sparse, sediments are easily disturbed by benthivorous fish and waves. Suspended sediments further increase turbidity, and mobilized P stimulates even higher phytoplankton growth rates. In contrast, in clear-state lakes, SAV remains widely distributed and helps maintain water clarity by stabilizing sediments and taking up nutrients. Charophytes (*Chara* spp.) often accompany clear-water conditions in Minnesota lakes and are believed to release algal toxins (Berger and Schageri 2004) and provide refuge for zooplankton, which may further reduce the phytoplankton population and help stabilize clear-water conditions.

Theory of regime shifts

Shallow lakes are notoriously difficult to restore after shifting from clear to turbid states, with turbid conditions frequently returning within 5-10 years following lake management (Søndergaard et al. 2007). Theoretical models are useful for understanding why reducing nutrient input to previous levels does not always induce the reverse state shift. For example, Figure 1 shows a bifurcation diagram derived from a model describing shallow lake dynamics similar to those in Scheffer and Carpenter (2003) and Scheffer (2004). At low nutrient levels (left of “flip down!” threshold in Figure 1), lakes can only exist in the clear stable state. At high nutrient levels (right of the “flip up!” threshold in Figure 1), lakes only exist in the turbid state. In between these two thresholds, the system exhibits hysteresis in which two different steady states are possible under the same nutrient conditions, depending on whether the initial turbidity levels lie above or below the unstable state in this region of bistability (dotted line in Figure 1).

The bifurcation diagram is also useful for understanding temporal dynamics and shifts between stable states. If a lake is in the clear state with high SAV (lower solid line) and nutrient input increases beyond the “flip up” bifurcation point, the lake will likely transition quickly to the turbid state with low SAV (upper solid line). Once SAV is lost, the internal loading of nutrients becomes hard to control, and nutrient input must be substantially reduced to the lower “flip down” bifurcation point to reverse the state shift (Scheffer and Carpenter 2003). In practice, such a drastic nutrient reduction may not be possible. Alternatively, managers may attempt to induce a state shift by forcing the system across the unstable point, e.g., by decreasing the planktivore and benthivore populations with rotenone (if nutrients can at least be reduced to the region of bistability). These transitions may be short-lived, however, since perturbations to the system (e.g., fish colonization) can force the lake back to the turbid state.

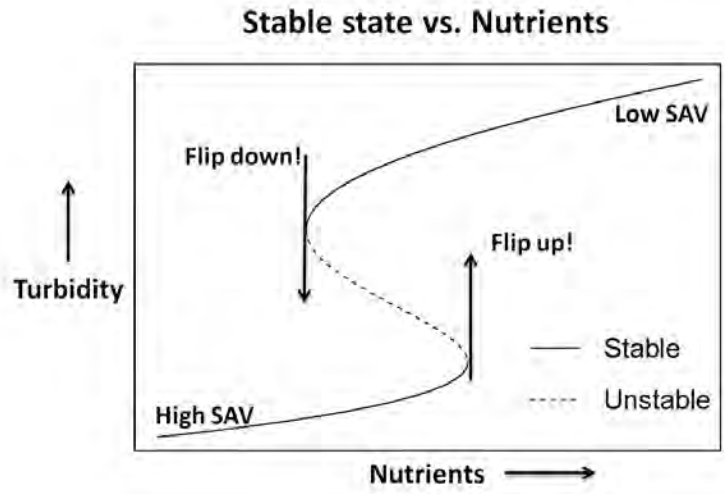

Figure 1. Bifurcation diagram from a theoretical model for state shifts in shallow lakes.

Earlier research provides strong evidence that shallow lakes in Minnesota generally conform to conceptual models like those summarized by Scheffer and Carpenter (2003) and in Figure 1 here. One notable example is Lake Christina, a large shallow lake near Fergus Falls, Minnesota. To improve habitat quality for migrating waterfowl, the lake has been rehabilitated (using fish toxicants) three times during the past five decades. In each case, improved water quality and clear-state characteristics followed lake management, but the lake persistently transitioned back to turbid conditions during 5-10 years after treatment (Hanson and Butler 1994, Hansel-Welch et al. 2003, Hobbs et al. 2012). Zimmer et al. (2009) surveyed 75 shallow Minnesota lakes and showed that a large proportion of these sites had characteristics of turbid-water conditions. More recently, our research team monitored eight rehabilitated shallow lakes, and data indicated that stable clear-state conditions had not become established in these sites during 2-4 years following management (Hanson et al. unpublished data).

These studies illustrate the fact that managers need better tools to evaluate relative transition risks of shallow lakes in Minnesota. Theoretical models suggest that to prevent undesirable state shifts in shallow lakes, we need to more accurately predict implications of nutrient levels, land use practices, lake depth, geographic location, and biological community features to identify the current attracting states and to assess the likelihood that lakes will flip to turbid states. A better understanding of transition risks will also help lake managers identify lakes that are good candidates for rehabilitation and will inform future conservation strategies for both lakes and adjacent watershed areas.

OBJECTIVES

The overarching objectives of this study are stated below. However, the Methods and Discussion sections here concern only the first two objectives because approaches for Objectives 3 and 4 have not been formalized and may depend upon results of analyses supporting Objectives 1 and 2.

1. Model Total Phosphorus (TP) in shallow Minnesota lakes using depth, benthivore mass, upstream watershed land cover variables, and geographic location of lakes.

2. Develop a modeling framework that allows us to classify lake states and estimate state-dependent relationships between measures of turbidity (Chla) and nutrients (TP) (similar to Figure 1).
3. Extend the model in Objective 2 to allow for temporal dynamics, with state transitions modeled as a function of varying nutrient levels and biological variables (e.g., zooplankton size, fish community types and densities).
4. Using results from Objectives 1-3, develop a tool to compare the relative risk of state transitions for different lakes. Conceptually, this objective can be viewed as attempting to determine where lakes "sit" in Figure 1, and for lakes falling within the region of bistability, the likelihood of the lake transitioning as the result of (possibly management-induced) perturbations (e.g., fish colonization or extirpation).

In summary, Objective 1 will help place lakes along the *x*-axis of Figure 1. Objective 2 will attempt to capture the salient features of Figure 1 using statistical models that can be applied to data from lakes in MN. Objective 3 will determine how far lakes may shift both horizontally and vertically in Figure 1 as a result of various perturbations. Lastly, Objective 4 aims to translate, as necessary, the results of more complex mathematical and statistical models into a simpler quantitative tool that can be used by wildlife managers to make informed decisions regarding shallow lakes and their management potential.

METHODS

Data

Analyses described here are based on data from two sources. First, we compiled a "research lakes dataset" (hearafter, research lakes), based on a sampling of 132 lakes surveyed by our research team once in July during each of three consecutive years, 2009-2011. Measures of TP, Total Nitrogen, turbidity (nephelometric turbidity units or NTUs), depth, chlorophyll *a* concentration (hereafter, Chla), as well as relative abundances of SAV, fish (planktivores, benthivores, piscivores), and invertebrates (cladocera and copepods) were obtained in each year (details of data gathering and project logistics are summarized in Hanson 2012). Land cover data in the upstream watershed of each research lake were derived by summarizing manually-delineated cover type polygons that were created using on-screen digitizing procedures in ArcGIS. Color air photos from 2008 were used as the primary interpretive reference for distinguishing cover types, with 2001 National Land Cover Database and 1991 GAP land-cover used to corroborate air photo interpretations as needed. A second similar set of water quality and land cover data was developed from 330 additional lakes using data provided by the MNDNR Shallow Lakes Program (hereafter program lakes). Preliminary modeling has focused on data from research lakes, but program lake data will also be incorporated into the analyses.

TP Model

We fit a series of linear mixed effects models, using the 'nlme' package in R (Pinheiro and Bates 2006), to describe within- and between-lake variability in the natural logarithm of TP. We included a random intercept for each lake to account for correlation among repeated measurements. The fixed explanatory variables we considered were depth, total benthivore abundance/presence, common carp abundance/presence, bullhead abundance/presence, watershed area to lake area ratio, proportion of different upstream watershed land cover types, and ECS Province. We also considered aggregate land cover variables, such as Total Agriculture (row crops, pasture, other ag), Disturbance (Total Ag, residential, roads, other

impervious surfaces) and Filters (woodlands, shrubs), in addition to specific land cover types. We determined the most parsimonious model by comparing AIC values of candidate models, and model assumptions were checked with residual and Q-Q plots. Twelve lakes in the Red Lake Region were removed due to difficulties in distinguishing unique watershed boundaries. Additionally, we investigated the effects of removing two lakes with an average depth of five meters or greater on model choice and parameter estimates.

State classification and estimation of Chla/TP relationships

Following Zimmer et al. (2009), we used TP as a surrogate for P input (*x*-axis) and Chla as a metric for turbidity (*y*-axis) to create a diagram similar to Figure 1. In contrast to Zimmer et al. (2009), however, we used a Bayesian latent variable regression framework to both classify discrete steady states (clear/turbid) and to estimate state-dependent relationships between TP and Chla together in one model. In this framework, we treated the state of each lake (clear or turbid) as a latent variable, used logistic regression to model the probability of being in the turbid state as a function of SAV mass and TP, and estimated state-dependent relationships between TP and Chla using linear regression after taking the natural logarithm of both variables. We chose informative priors to ensure that the probability of being turbid decreased with SAV mass, increased with TP, and that the slopes describing the relationships between Chla and TP were positive.

We ran separate models in JAGS (Plummer 2003) for each of the three years, and examined convergence using trace plots and the Gelman-Rubin convergence statistic (Gelman and Rubin 1992). We classified a lake as turbid (clear) if over half of the sampled states from the MCMC chains were turbid (clear) for that lake. We estimated regression coefficients using means of the posterior distributions.

PRELIMINARY RESULTS

TP Model

The most parsimonious of the candidate models, as determined by AIC, included three continuous explanatory variables: percent filters (woodlands/shrubs) in the upstream watershed, log(benthivore kg +1), and depth (see Table 1 for parameter estimates and standard errors). TP decreased as the percentage of woodlands and shrubs in the upstream watershed increased, as depth increased, and as the relative mass of benthivores decreased (Table 1, Figure 2). Percent filters had the lowest AIC of all single predictor models, and total benthivore mass was a better predictor than benthivore presence, bullhead mass/presence, and carp mass/presence. ECS Province was not a significant predictor of TP after the inclusion of percent filters, and the ratio of watershed area to lake area was not significant after the inclusion of percent filters, benthivores, or depth. Replacing depth with the natural logarithm of depth resulted in a model with a similar AIC as the best-fit model (see Table 1 for coefficient estimates). We also observed a significant positive interaction between percent filters and depth (but not for the logarithm of depth). Finally, the removal of two very deep lakes did not affect model choice or estimation (Table 1).

The joint impacts of benthivores and woodlands on TP are better seen by considering lakes within a restricted range of depths. Figure 3 shows that for lakes with average depths between 2.0-2.3 meters, TP decreases as the percentage of filters in the watershed rises, and TP tends to increase with benthivore mass regardless of filter percentage. It is worth noting that while percent filters is a better predictor than ECS Province, provinces also differ by filter percentage,

with the Prairie Parkland having the lowest percentage of filters in the lake watersheds and the Laurentian Mixed Forest having the greatest percentage of filters (Figures 3, 4).

State classification and estimation of Chla/TP relationships

We ran the Bayesian latent regression model separately for each year. The model produced reasonable state classifications and linear relationships between log(Chla) and log(TP) (results from 2009 are shown in Figure 5). Lakes classified as clear have higher SAV mass compared to lakes classified as turbid, and both the probability of being turbid and the expected Chla value in a lake increase with TP.

For year 2009, lake classifications match those produced using the methods described in Zimmer et al. (2009) for 92% of the lakes, and the linear regression slope estimates are also similar, though standard errors are larger for the Bayesian analysis (results not shown). Results for year 2010 are similar. However, classifications for year 2011 matched Zimmer et al. (2009) for only 55% of lakes, and coefficient estimates also differed. Further investigation revealed outliers and probable misclassifications of clear, deep lakes with low Chla, low TP, and low SAV mass by the Bayesian latent variable regression model.

Table 1. Regression coefficients for linear mixed effects model for log(TP). The first column of coefficient estimates and standard errors includes all lakes with depth measured in meters. The second column of estimates is the same model but with two lakes with an average depth of five or more meters removed. The third column of estimates includes all lakes but with depth measured in log(m).

Parameter	Estimate (SE) (All lakes)	Estimate (SE) (Deep lakes removed)	Estimate (SE) (All lakes, log Depth)
Intercept	4.74 (0.11)	4.74 (0.12)	4.53 (0.092)
Percent filters (Woods/Shrubs)	-1.41 (0.17)	-1.43 (0.17)	-1.39 (0.17)
log(benthivore kg + 1)	0.17 (0.035)	0.17 (.036)	0.18 (0.035)
Depth	-0.19 (0.057)	-0.18 (.064)	-0.33 (0.097)
Lake intercept SD	0.48	0.49	0.48
Within-lake residual SD	0.51	0.52	0.51

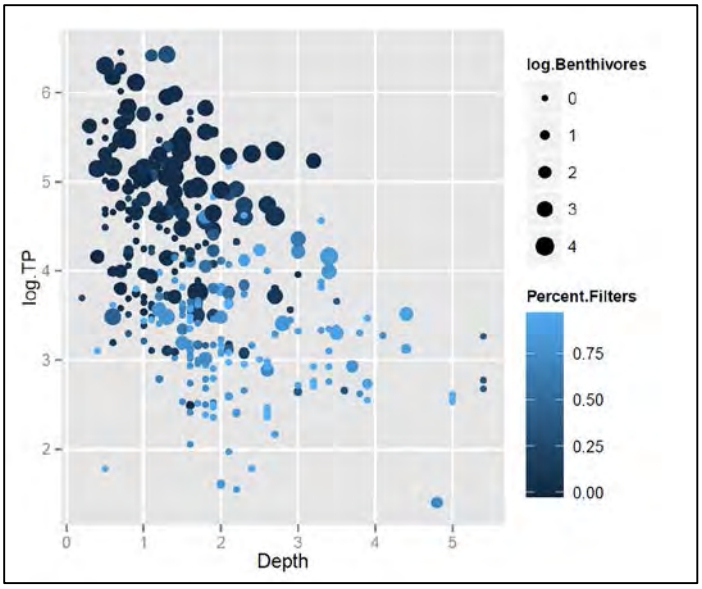

Figure 2. Log(TP) with three best predictors: percentage of filters (woodlands/shrubs) in the upstream watershed, log(benthivore kg +1), and depth.

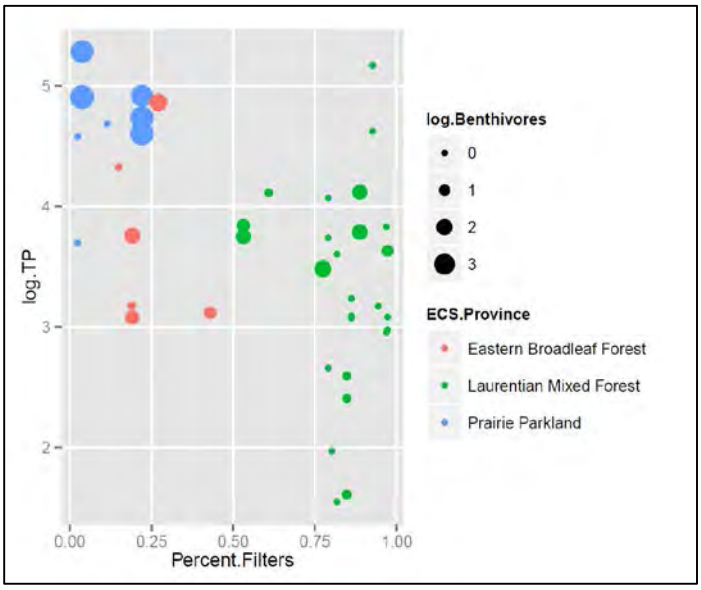

Figure 3. Log(TP) vs. percent filters for lakes with depth between 2.0-2.3 meters. Point size is proportional to log(benthivore mass +1), and color corresponds to the ECS Province of the lake.

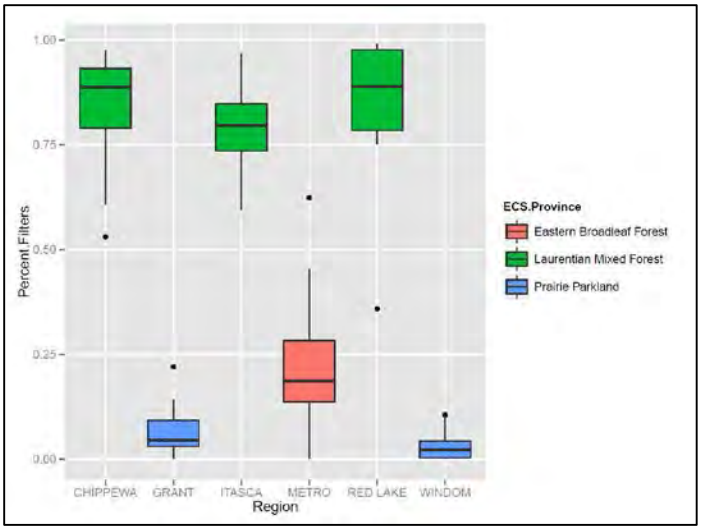

Figure 4. Boxplots for percent filters by ECS Province and Region.

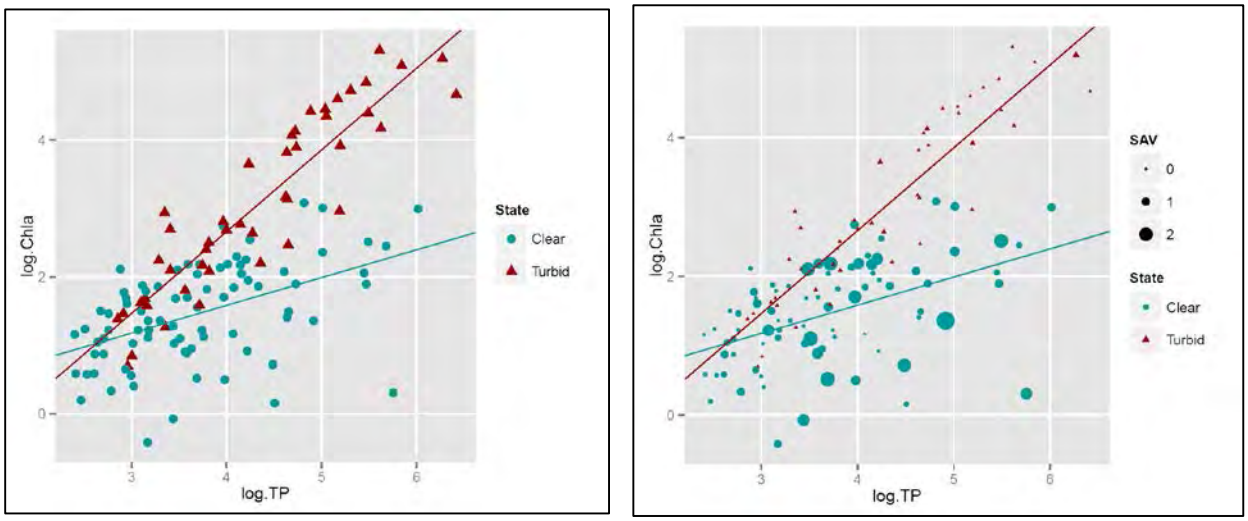

Figure 5. Log(Chla) vs. log(TP) for year 2009. Lake classifications are distinguished by color and shape and state-dependent regression lines are distinguished by color. The right figure is the same as the left but with point size proportional to SAV mass.

DISCUSSION

The three best predictors of TP included the percentage of filters in the upstream watershed of each lake, benthivore mass, and lake depth. While the significance and influence of these three predictors is not surprising, it is perhaps unexpected that percent woodlands/ shrubs was a better predictor than percent disturbance or agriculture. Filter percentage was strongly correlated with disturbance percentage (r=-0.84), and thus these predictors explain similar patterns in the TP data. However, the percentage of woodlands/shrubs may be the best land cover predictor because in addition to being correlated with disturbance, woodlands and shrubs

filter out P, reduce overland flow, and may be better indicators of historical land use (or lack thereof) compared to current presence of agriculture or human disturbance.

Results were not shown, but a significant positive interaction was found between depth and filter percentage. That is, the negative influences of depth and woodlands/shrubs on TP are mitigated as both depth and percent filters increase together. Perhaps this result is spurious or perhaps certain deep lakes in forested areas have unique characteristics that lead to higher TP levels, such as differences in soil or bedrock type. This relationship probably deserves further attention but is a good example of the difficulty of accounting for the multiple sources of P in lakes and the complex relationships that exist among driving variables.

For 2009 and 2010, lake classifications using the Bayesian latent variable regression framework were similar to those produced using the methods of Zimmer et al. (2009). An advantage of the Bayesian approach, however, is that it can capture uncertainty in the estimated state classifications. Additionally, because lake classification and regression estimation are done in an integrated framework, uncertainty in the classifications is propagated to the error of the regression coefficient estimates. Thus, we should obtain more realistic confidence bounds for the relationship between Chla and TP using the integrated Bayesian framework.

The Bayesian analysis may not have worked as well for year 2011 due to the presence of outliers and deep lakes with low TP, low Chla, and low SAV mass. Record precipitation was observed in Minnesota in the twelve months prior to July 2011 (NOAA National Climatic Data Center 2011), and lakes may therefore have been perturbed far from their steady states or have been in the process of transitioning. Additionally, SAV may not be useful for classifying very deep or very shallow lakes because low Chla levels and low SAV mass may be observed in deep lakes, and high SAV mass and high Chla levels can occur in very shallow lakes. Furthermore, others have demonstrated that depth affects the susceptibility of lakes to state shifts (Genkai-Kato and Carpenter 2005). Therefore, depth may be an important variable to consider in future analyses.

The two preliminary analyses presented here could eventually be incorporated into a management tool to assess the relative transition risks of different lakes. The TP model could be used to predict TP levels on the *x*-axis of Figure 5 depending on land use, depth, and benthivore mass. Once thresholds are identified in Figure 5, lakes can be compared based on their relative proximity to thresholds to aid in management decisions.

FUTURE WORK

Goodness-of-fit of the TP model needs to be evaluated, as well as its predictive ability. Additionally, we plan to explore possibilities for refining data inputs that would allow for variations in soil and bedrock types among the lakes to account for this additional source of P input. Such data may also help explain the significance of the interaction between depth and filter percentage.

To test the usefulness of the Bayesian latent variable regression approach to classify attracting states and estimate steady state relationships between Chla and TP, we will apply the method to data simulated from an ordinary differential equation (ODE) model that produces dynamics similar to those observed in shallow lakes (see Beisner et al. (2003) for a candidate model). We will also use the ODE model to better understand the implications of using TP as a surrogate for P input, especially in terms of the differences between the bifurcation diagram for Chla and P input (Figure 1) versus a phase plane for Chla and TP, and to help us determine an appropriate

method for estimating nutrient thresholds. We plan to incorporate TP thresholds into the model to more easily obtain standard errors for threshold estimates, rather than attempt to estimate thresholds post-hoc. We also hope to explore using other measures of turbidity (NTUs for research lakes, Secchi depth for program lakes) in conjunction with Chla to separate out the non-algal components of turbidity, possibly similar to Wang et al. (2014).

Finally, the last two objectives, modeling state transitions between years and developing a tool to assess relative transition risk, will be addressed after further progress is made on the preliminary work presented here. Scientists and managers urgently need better means of predicting ecological transitions in shallow lakes, along with more formal strategies for assessing water-quality consequences of watershed conversions. We think the current research is an important step toward improving management of shallow lakes and toward more productive conservation planning efforts for both shallow lakes and adjacent upland watersheds.

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EVALUATING THE SUCCESS OF THE MINNESOTA PRAIRIE CONSERVATION PLAN: CHALLENGES AND LESSONS LEARNED USING WATERFOWL AS A CASE STUDY

James B. Berdeen

SUMMARY OF FINDINGS

The Minnesota Prairie Conservation Plan (MPCP) established 36 Core Areas to protect, restore, and enhance grasslands, wetlands, and other habitats in the Prairie and Prairie – Forest Transition areas. One measure of success outlined in the plan is a stable or increasing breeding population of mallards (*Anas platyrhynchos*) in Minnesota. I conducted a pilot study during 2014 in which the primary goals were to measure habitat use in Core Areas by mallards and other waterfowl and waterbirds, ascertain which metrics that could be used to measure success in the MPCP and the limitations and challenges associated with interpreting those metrics over space and time, and evaluate the feasibility of using ground-based wetland surveys to quantify the success of the MPCP in terms of populations of indicator species. Feasibility includes the ability to collect sufficient baseline data to precisely estimate population parameters for pairs and broods of waterfowl and waterbird species within Core Areas. A secondary goal was to use these estimates to rank Core Areas with respect to mallard habitat suitability to identify appropriate Core Areas for habitat enhancement projects.

I initially ranked Core Areas based on habitat characteristics (i.e., wetlands, grasslands) important to mallard pairs and broods. Personnel then conducted surveys at 403 wetlands within 11 Core Areas, and observed breeding pairs of 10 species of waterfowl and 1 waterbird during the pair period (4–25 June). Field crews also surveyed 265 wetlands within 8 Core Areas and observed broods of 12 waterfowl and 3 waterbird species during the brood period (25 June – 23 July). The most commonly observed species were mallards and blue-winged teal during the pair period, and Canada geese and hooded mergansers during the brood period. However, count data for all waterfowl and waterbird species were sparse, and there were zero counts at many wetlands. These data characteristics limited the opportunity to model and precisely estimate the population metrics, compare these estimates among Core Areas, and evaluate the covariate effects on the state and detection processes. Nevertheless, the modeling exercise was performed to gain an understanding of how models performed with the available data and how precisely parameters could be estimated.

Hierarchical models were built to examine how survey-, habitat-, and weather-related covariates influenced the detectability and wetland occupancy of mallard pairs and waterfowl broods, and generate estimates of these parameters. The top-ranked mallard-pair model suggested that occupancy varied among Core Areas, but these differences were not significant based on overlapping 95% confidence intervals. One strongly supported occupancy model for broods of all species aggregated suggested that this parameter was not influenced by any of the predictive covariates examined (i.e., a null model). The estimated wetland occupancy rates by mallard and blue-winged teal pairs were 8.9% and 5.5%, respectively. The estimated occupancy by broods of all waterfowl species aggregated was 29%. Unfortunately, differences in the occupancy of either mallard pairs or waterfowl broods of all species could not be discerned among Core Areas, in-part because of sparse count data and relatively few wetlands available to be surveyed from roadsides. It may be that anomalous weather (late spring and ice-out, followed by

above average monthly precipitation and heavy precipitation events) influenced the habitat condition to the extent that the spatial settling pattern of pairs and production of young also were affected.

After converting a wetland-level abundance metrics for both mallard pairs and waterfowl broods of all species to Core Area-level density using several approaches, these sites were ranked based on the latter parameter. Empirical ranks of Core Areas often were similar, but there were substantial differences between the predicted and empirical ranks of these sites.

My results suggest that it may be difficult to observe a sufficient number of mallard pairs or broods in Core Areas to assess whether the MPCP is achieving 1 indicator of success. Therefore, I highlight some of the challenges associated with evaluating the success of large-scale conservation and management projects (e.g., MPCP) and ways to improve the assessment of such projects. Such projects will require the establishment of realistic well-defined metrics of "success" that can be measured at the appropriate spatial and temporal scales, and at a reasonable cost. Further, more accurate spatial habitat data than was available for this pilot study is needed to increase both the efficiency of surveys and the accuracy of the calculations of the areal extent of important habitats.

Background and Justification

The Minnesota Prairie Conservation Plan (MPCP) was established in 2010 to protect, restore, and enhance grasslands, wetlands, and other habitats in the Prairie and Prairie – Forest Transition areas via joint efforts among government agencies, private organizations, and individuals. As part of this plan, 36 Core Areas interconnected by corridors were established. Several characteristics of prairie ecosystems are considered to be representative of properly functioning landscapes, and were chosen as measures of success for the MPCP. One indicator of success is a stable or increasing breeding population of mallards (*Anas platyrhynchos*) in Minnesota (Minnesota Prairie Plan Working Group 2011). This species was selected as an indicator because it uses upland habitats for nesting and wetlands for rearing broods.

To attain this goal, it is important to understand such phenomena as demographic characteristics that contribute most to mallard population growth and habitats associated with mallard population metrics. For example, demographic processes that operate during pairing and brood-rearing life-cycle phases, especially nest success and hen and duckling survival, appear to greatly influence the growth rate of the midcontinent population of mallards (Hoekman et al. 2002). Further, the area of temporary and seasonal wetlands is positively related to the abundance of mallard pairs in North Dakota and westcentral Minnesota (Krapu et al. 1997), and the logarithm of the ponded area of temporary, seasonal, and semi-permanent wetlands is positively associated with wetland occupancy by mallard broods in the Dakotas (Walker et al. 2013).

Knowledge of habitat components important to mallards during breeding and brood-rearing phases has been used in the development of conservation plans. For example, the Minnesota Long Range Duck Recovery Plan (DRP, Anonymous 2006) identified landscape-level habitat components that purportedly are positively associated with waterfowl production. This plan operates under the assumptions that greatest waterfowl production occurs within prairie-wetland complexes that are 10.4–23.3 km^2 (4–9 mi^2) in area, and are comprised of ≥40% grassland and ≥20% wetlands. Also, >50% of wetlands should be temporary and seasonal and there should be 1 shallow lake >20.2 ha (50 ac) on each habitat complex. The intent of the MPCP was to establish Core Areas with a similar composition of wetland and grassland habitats (Minnesota Prairie Plan Working Group 2011), but such habitat characteristics varied substantially among these sites (Table 1).

Because of recent interest in the determining the success of the MPCP (G. Hoch, MN DNR, personal communication), I initiated a pilot study to determine whether it was feasible to collect sufficient count data to precisely estimate abundance, density, and probability of wetland

occupancy of pairs and broods of mallard and other waterfowl and waterbird species within MPCP Core Areas. Such data could be used to monitor population trends as a measure of success if this effort was conducted for a longer time period. Although MPCP Core Areas initially were selected because of existing native prairie, other grasslands, wetlands, and shallow lakes (Minnesota Prairie Plan Working Group 2011, p. 3). I expected a gradient of abundance metrics of pairs and broods of mallards, other waterfowl, and waterbirds that was attributable to site-specific differences in the area and composition of grasslands and wetlands (Table 1). More specifically, I expected that the ordinal ranking of Core Areas in terms of waterfowl abundance measurements to be positively associated with the corresponding habitat metrics, and that the differences in ranks of Core Areas could be useful for identifying site-specific habitat management needs.

The primary goals of this pilot project were to (1) measure the use of wetlands in MPCP Core Areas by mallards and other waterfowl and waterbirds, (2) critically examine which metrics that could be used to measure success in the MPCP and the limitations and challenges associated with interpreting those metrics over space and time, and (3) evaluate the feasibility of using ground-based wetland surveys to quantify the success of the MPCP in terms of populations of mallards and other waterfowl and waterbirds. In this study, feasibility included survey duration time, the selection and limitations of different study designs that could be used to survey wetlands in Core Areas; and whether sufficient data (i.e., sample sizes) could be collected to generate precise estimates of population parameters for comparisons over space and time.

Objectives

The specific objectives of this study were to:

(1) Rank the relative value of each MPCP Core Area to mallard pairs and broods based on knowledge of landscape-level habitat components that are important to these cohorts.

(2) Measure pair and brood use (presence-absence and abundance) of wetlands within Core Areas, and explain the variation in use based on several wetland- and landscape-level covariates.

(3) Estimate the *density* of pairs and broods of mallards and other waterfowl and waterbird species in Core Areas.

(4) Rank MPCP Core Areas based on empirical estimates of density and compare observed and predicted ranks to identify Core Areas that may not be functioning at expected levels with respect to a MPCP goal and guide future habitat-management projects (e.g., via predicted avian abundance-habitat relationships).

(5) Investigate the influence of survey-, habitat-, and weather-related covariates on detectability and use this information to improve survey protocols.

STUDY AREA

I conducted this study at a subset of MPCP Core Areas in the Prairie Parkland, Tallgrass Aspen Parkland, and Eastern Broadleaf Forest (Transition) ecological provinces (Figure 1).

METHODS

Site Selection

Originally, I planned to conduct surveys in the same Crew Areas during the pairing and brood-rearing phases of the study, and therefore predicted the value of Core Areas based on habitat characteristics important to both mallard pairs and broods, and acknowledged that the settling pattern of pairs likely would vary among years. Therefore, I stratified the 36 MPCP Core Areas by latitudinal zone (south, central, north), assigned 12 Core Areas to each zone, and numerically ranked these sites based on the composition of grassland and Types II-IV wetlands.

I then assigned the 12 Core Areas in each zone a class rank (high, medium-high, medium-low, and low) based on the corresponding numerical rank, and then randomly selected a Core Area from each latitudinal zone-class rank stratum (*n* = 12 Core Areas, Table 1, Figure 1).

Low counts of mallard pairs during the early phase of this pilot study forced a reconsideration of this approach to selecting sites at which to conduct brood surveys. Because of the need to observe a sufficient number of broods to both estimate wetland occupancy by this cohort and evaluate my methodology, I non-randomly selected 8 Core Areas (Table 2*b*, Figure 1) that likely had the greatest density of mallard broods.

Habitat Data

A GIS and pertinent data layers were used to quantify the areal extent of grassland and wetland habitats within each Core Area. Specifically, the MN DNR WMAs, CRP 2007, NLCD 2011, MN DNR Native Plant Communities, Big Stone NWR, BWSR, and NLCD 2011 layers (MN DNR, unpublished data) were used to calculate the areal extent of grasslands, and the Circular 39 Wetland data layer (MN DNR, unpublished data) was used to calculate this metric for wetlands. There are many grassland cover types in these GIS layers, but I used only those that were likely to attract nesting mallards in the GIS layer. My classification of wetlands followed Stewart and Kantrud (1971): temporary - ephemeral (Type I), temporary (Type II), seasonal (Type III), semi-permanent (Type IV), and permanent (Type V). All surveyed wetlands also were classified in the field according to Stewart and Kantrud (1971). I used the field classification if there was a discrepancy with the GIS classification.

Wetland Surveys

I divided the wetland surveys into 3 time periods based on the phenology of the mallard life cycle in Minnesota: migratory (29 April–28 May; migrants and breeding pairs were most likely to be counted), pair (4–25 June; focus was on counting pairs breeding locally, but broods that hatched early also were counted), and brood (25 June–23 July; focus was on counting broods, but adults were counted as well). During each period, field crews generally conducted surveys in the most southerly MPCP Core Areas first and then proceeded northward so that these efforts coincided with the appropriate mallard life-cycle phase of interest.

I chose to conduct surveys at Types I-V wetlands during the migratory and pair periods, but focused survey efforts on Types III-V wetlands during the brood period. I included Types IV and V wetlands during this study because (1) the alteration of many relatively small, ephemeral wetlands in Minnesota may have increased the use and value of relatively large, permanent wetlands to mallards (M. A. Hanson, Minnesota Department of Natural Resources [MNDNR], personal communication), and (2) these habitats are selected during the brood-rearing phase of the mallard life cycle (Raven et al. 2007, but see also Rotella and Ratti 1992). Personnel did not survey Type I wetlands and surveyed only a few Type II wetlands during the brood period because it was likely that these would be dewatered during summer and thus attract no ducklings. I used waterfowl surveys during the migration period to guide the survey methodology during the pair and brood periods. More specifically, I needed to develop an understanding of how many birds were likely to be detected per wetland and the time required to survey an adequate number of wetlands in a typical Core Area. Personnel attempted to survey as many wetlands in the selected Core Areas as possible during the pair and brood periods.

Personnel conducted wetland surveys using some of the methodology developed by Pagano and Arnold (2009*a*, *b*) and Walker et al. (2013). I attempted to maintain a relatively consistent field methodology throughout the study, but some differences occurred among the migration, pair, and brood periods as field techniques were being refined.

Personnel used a double-observer approach (Nichols et al. 2000) to conduct surveys. Birds were independently but simultaneously counted by 2 observers from both roadsides and by walking to wetlands (hereafter roadside and walk-in surveys, respectively) during all periods.

Roadside surveys were conducted at those wetlands that could be adequately observed from a public right-of-way, were not anomalous (e.g., quarry-pit wetland), or near a residence. Walk-in surveys were conducted at wetlands that could not be readily observed from public right-of-ways, were logistically feasible to access, were not anomalous, and to which field crews were permitted access (i.e., public and TNC lands). Both types of locations were surveyed to increase the number of wetlands examined and improve the representativeness of the wetland sample.

During surveys, personnel either stood at opposite ends of a vehicle (roadside) or stood 3–6 m away from each other (walk-in). Each observer used binoculars and a spotting scope to observe, identify, and count birds; and a Leupold ® RX-800/DNA rangefinders and a handheld compass to measure the distance and direction, respectively, to social groups and individual birds. The minimum duration of migrant and pair surveys was 2 min and brood survey was 10 min, but field personnel could conduct surveys for a longer time period if it was difficult to count all observable birds or if birds continued to emerge from vegetation during a survey. Personnel also conducted repeat-visit surveys (MacKenzie et al. 2006) during the brood period, with the 2 visits occurring within 4–36 hrs for a given wetland (Walker et al. 2013). A repeat-visit approach was used during the brood period because the detectability of this cohort is relatively low (e.g., Walker et al. 2013).

Personnel classified the social status of observed individual birds and aggregations of birds of 1 species as follows: group (>5 individuals, one or both sexes with no discernable pairs), flocked males (aggregations of ≤5), pairs, lone male, lone female, brood hen, and lone birds of unknown sex (see Dzubin 1969). Social groups that indicated paired breeding status were pairs, lone males, and flocked males (Dzubin 1969). Personnel classified the stage of development of ducklings based on feather development and body shape (Gollop and Marshall 1954). Bird counts, social classifications, development stages of ducklings, and distances and locations were recorded independently, but team members discussed their observations immediately after each survey to minimize species misidentifications and double-counts of birds, identify birds that were not detected by 1 observer, and reconcile discrepancies in assigned social classifications.

At the end of each survey, personnel also recorded the MPCP Complex name, date, survey location, observers, wetland type and identification number, ownership of land surrounding study wetlands (4 classes: public, private, The Nature Conservancy, mixed ownership), survey type (2 classes: roadside, walk-up), beginning and end times, weather variables (i.e., temperature, relative humidity, dew point, barometric pressure, wind speed and chill, heat stress index, precipitation, % cloud cover), and habitat characteristics (i.e., proportions of each study wetland that are covered along the edge by tall vegetation, proportion of the wetland observable from the survey point, proportion of the wetland that contained water [all 0–1.0 in 0.05 increments], whether or not the wetland was flooded beyond the normal edge). Locations of survey points were measured with Garmin ® Montana 650 GPS units. Most weather variables were measured with Kestrel ® 3000 Portable Weather Meters. Ocular estimates of *% cloud cover* and habitat characteristics were made jointly by crew members.

Data Analysis

First, the number of wetlands surveyed was summarized by type and period, and bird counts were summarized by MPCP Core Area, survey period, species, and social aggregation. This information was used to guide the selection of a response variable (*count* v. *occupancy*) for each species included in the modeling exercise, and to ascertain which waterfowl species and social aggregations (i.e., pairs, broods) had adequate information (i.e., number and distribution of detections) to include in this effort. Next, the distributional characteristics of predictive covariates were examined (see Giudice et al. 2012) to ascertain which should be transformed. These results along with findings of previous research, logic, and available sample sizes were used to ascertain

which covariates were most likely to predict the detectability (p) and wetland occupancy (ψ) of breeding pairs and broods of each species or grouping of species. Detectability is defined as the probability that a cohort of interest was present, available for detection, and detected during a survey of a wetland; and occupancy is defined the probability that a wetland is occupied by a cohort of interest (Mackenzie et al. 2006).

The influence of survey-, weather-, and habitat-related (landscape and wetland spatial scales) covariates on p and ψ were examined to address a secondary research goal. The survey-related covariates examined were individual observer (OBS); survey method (roadside v walk-in, METHOD, categorical predictor); Julian date (JULDATE), the start time (24-hr, START) and duration of surveys (min, SURVDUR), and in surveys conducted during the brood period, wetland visit (first v. second during brood surveys; VISIT). The weather-related covariates were the wind speed (km/hr, WIND) and temperature (^{o}C, TEMP) during each survey. Landscape-level habitat covariates were Core Area (COREA, categorical predictor), proportion of each Core Area comprised of medium-to-high quality grasslands (GRASS), and the density (per mi^2) of Wetland Types II-IV (DENSWET2-4) and V (DENSWET5) in each Core Area. The wetland-level covariates were Wetland Type (I–V, WETTYPE, categorical predictor); the proportions of wetland edges obscured by tall vegetation (TALLVEG); whether or not a wetland was flooded (FLOOD, categorical predictor); the ownership of land surrounding study wetlands (OWN, categorical predictor); the latitude of the survey point (LAT); and the proportion of the wetland area observable from the survey point (OAW, or observable area of wetland). The last covariate was developed as a surrogate measure of the surveyed area of a wetland basin, and is the product of the areal extent of a wetland (ac, measured in the GIS wetland layer) and a field estimate of the proportion of the wetland that is visible from the survey point. Because there was a strong positive skew in the OAW, it was necessary to log transform this covariate to improve its distribution. Log OAW was strongly correlated with the actual areal extent of wetlands, but the former covariate was a better metric for calculating the *densities* of birds and modeling the influence of the areal extent of survey area on the p and ψ.

Because of the low number of pair and brood detections in my data and the desire to examine the influence the many covariates on parameters of interest, a 2-stage approach was chosen to build models for both pairs and broods in separate analyses. In the first stage, a small set of *a priori* candidate models were developed to examine the influence of covariates on the observation process (detectability), and the state process (wetland occupancy) was held constant. All models were evaluated using information-theoretic methods, with candidate models having ΔAIC-values of ≤2 considered to have strong support relative to other models under consideration (Burnham and Anderson 2002). Further, I considered a covariate to be a significant predictor of p if it was included in the top-ranked model (Burnham and Anderson 2002) and the 95% asymptotic CIs associated with coefficient estimates of this covariate did not encompass 0.

The same structure of p from the best approximating model of the first stage continued to be used in the second stage, but the influence of several covariates on ψ was explored. As with the first stage, information-theoretic methods (Burnham and Anderson 2002) were used to identify the best approximating model, and 95% asymptotic CIs associated with each parameter in this model were examined to ascertain the precision of estimated covariate effects on ψ. The relatively strong support of a model does not indicate that it is necessarily reliable.

There were some differences in the analyses of data collected during the pair and brood periods. Both pair- and brood-count data were compiled as presence-absence data, but it was necessary to analyze these data somewhat differently. I treated the double-observation pair-count data generated by each member of a 2-person field crew at an individual wetland as that collected during independent visits. However, both double-observer and repeat-visit survey approaches were used simultaneously were used to count broods, so observations by each

individual on a 2-person field crew were treated as 2 independent sampling occasions, and each of the 2 visits was treated as a different sampling period.

Pair-count data were sparse, so a preliminary data analysis was performed to identify those species that likely had sufficient observations to develop models and generate estimates. Brood-count data were especially sparse in some MPCP Core Areas, so data from only those Areas in which ≥10 wetlands were surveyed ≥2 times were included in the analyses. Species-specific brood-count data were aggregated into 3 biologically plausible groups: all waterfowl species, dabbling ducks, and cavity-nesting ducks. A separate set of analyses was performed on each of these groups.

Although a similar model-building process was used for both pair- and brood-count data, a slightly different set of candidate models were examined during both the first and second analytical stages for each cohort. Specifically, the model set for first stage of the pair analysis consisted of a null model in which ψ and *p* were held constant and other models in which habitat- (TALLVEG, OAW), weather- (TEMP, WIND), and survey-related covariates (SURVDUR) were used to predict *p*. In contrast, models in first stage of the brood analysis examined the influence of wetland- (TALLVEG, log OAW), weather- (WIND, TEMP), and survey-level covariates (OBS, START, METHOD, VISIT, SURVDUR) on *p*.

In the second stage of the pair analysis, several *a priori* candidate models were developed to examine the influence of wetland- (log OAW, TALLVEG, WETTYPE) and Core Area or landscape-level covariates (COREA, GRASS, DENSWET2-4, DENSWET5) on ψ. In the brood analysis, the influence of similar wetland- (WETTYPE, TALLVEG, log OAW), landscape- (COREA, GRASS, DENSWET2-4, DENSWET5), and survey-level covariates (JULDATE) on ψ were examined with a similar set of *a priori* candidate models.

I assumed no local extinction (ϵ) or colonization (γ) of pairs occurred during any survey, and therefore did not estimate these parameters during the analysis of data from this cohort. For broods, ϵ and γ were considered nuisance parameters because most repeat-visits to individual wetlands occurred within 36 h, and therefore the probabilities of these parameters were always modeled as constants. I define extinction as the probability that an initially occupied site will become unoccupied during the sampling period (i.e., detected on the first visit to a wetland but not the second), and colonization as the probability that a previously unoccupied site becomes occupied during this period (i.e., not detected on the first visit but detected on the second).

The R programming language (version 3.0.3, R Development Core Team 2014) was used to conduct all analyses. The R function "scale" was used to center and scale all covariates except log OAW and categorical predictors prior fitting models to data. Two functions in the R package "unmarked" (Fiske and Chandler 2011) were used to fit hierarchical models of occurrence and abundance to pair- and brood-count data and generate parameter estimates. Specifically, the "occu" function (MacKenzie et al. 2002, 2006; Royle and Dorazio 2008) was used to fit models to pair-count data, and the "colext" function was used to fit dynamic-occupancy models (MacKenzie at al. 2003) to brood-count data.

Estimation of Pair and Brood Density

To meet the research goal of comparing the predicted and observed metrics of waterfowl abundance in MPCP Core Areas, it was necessary to estimate a parameter that could be extrapolated from the spatial scale of a surveyed patch (i.e., wetland) to that of the Core Area. Density fit that criterion, and was estimated in 2 ways for mallard pairs. First, the empirical estimate of density was computed as the sum of the count of indicated breeding pairs divided by the sum of the OAW in each Core Area. Second, a model-based density estimate was calculated by first modeling the wetland-level abundance of pairs as a function of individual Core Area, and incorporating the influence of survey duration and proportion of tall vegetation on detectability into the model. The wetland-level predictions from this model were summed across all of these habitat

units in a given Core Area, and then divided by the OAW following the same approach as the empirically based estimate of density. The model was fit in R using the "multinomPois" function (Royle 2004, Royle and Dorazio 2006) in the unmarked package. I only used a wetland-based empirical estimate of brood density, calculated as sum of the counts of broods divided by the sum of the OAW for a given Core Area (broods / survey ac). There was no attempt made to generate model-based estimates of density because brood-count data were sparse and there was great uncertainty associated with model-based estimates of occupancy.

Both pair and brood density were calculated at the spatial scale of the Core Area. Specifically, the number of pairs or broods per OAW in a given Core Area was multiplied by the total area of Type II-V wetlands in that site, and this value was divided by the total area (mi^2) of that site. This can be interpreted as a crude index of pair or brood density at the Core Area scale, and is referred to as the total area-based empirical approach.

Ranking MPCP Core Areas

I first developed predictive ranks of MPCP Core Areas for mallard pair and brood cohorts separately. The predictive ranking of Core Areas for pairs and broods were based on the proportions of these spatial units comprised of Type II–IV wetlands and grassland habitats of moderate-to-high quality to nesting waterfowl, respectively. I then produced empirical ranks of each Core Area based on the relative values of each approach used to estimate density of the cohorts of interest.

RESULTS

I did not analyze data from the migration period because field methodology still was being developed at that time. Survey data are available from 403 wetlands in 11 MPCP Core Areas examined during the pair period and 265 wetlands in 8 Core Areas during the brood period. I tabulated the number of wetlands surveyed by type, MPCP Core Area, and period (Table 2).

Roadside surveys comprised 83% and 87% of wetland surveys during the pair and brood periods, respectively, and walk-in surveys comprised the remainder. The wetlands surveyed during the pair period were mostly on private (68%) and public (24%) land, but some were located on mixed ownership (5%) and The Nature Conservancy (2%) properties, and 1% occurred on lands in which the ownership was not known. During the brood period, 71% of surveys were conducted on private land, 25% on public land, and 4% on wetlands surrounded by properties of mixed ownership. Water levels could not be adequately observed at some study wetlands, usually because of dense vegetation. Of the wetlands that could be observed adequately during the pair and brood surveys, field crews estimated that flooding was occurring at 14% and 7% of wetlands, respectively.

I included 352 of the 403 wetlands examined during the pair period in the analyses. Some wetlands were excluded because of a dewatered condition or missing covariate data. The MPCP Core Areas that met the criterion of ≥10 wetlands surveyed twice during the brood period were: Big Stone Moraine, Glacial Lakes, Lac qui Parle Prairie, and Lake Christina Hills. Data from 250 of the 265 wetlands surveyed during the brood period were included in the analyses.

After initially aggregating bird observations into 8 social classifications, I reclassified some of these groupings as indicated breeding pairs and broods for the analyses. I then tabulated counts of these 2 cohorts by species, MPCP Core Area, survey period, and proportion of surveyed wetlands with the cohorts of interest (Table 3). Personnel observed 10 waterfowl and 1 waterbird species in pairs during 4 – 25 June, and 12 waterfowl and 3 waterbird species with broods during 25 June – 23 July (Table 3).

Most wetlands had ≤1 indicated breeding pair of mallards (Figure 2) or blue-winged teal (*Anas discors*, Figure 3) or broods of all species (Figure 4). Further, the proportions of wetlands

with pairs of any species during 4–25 June or broods of any species during 25 June – 23 July were low (Table 3). The number of observations of the target cohorts of all species was too sparse to develop reliable models, especially for broods of any individual species. Nevertheless, the modeling exercise was conducted to gain an understanding of how models performed with the available data and how precisely parameters could be estimated. Relatively simple models were developed for the pairs of the 2 most abundant species, as well as aggregations of 3 species groups of broods: (1) all observed waterfowl (i.e., mallard, blue-winged teal, American green-winged teal [*Anas crecca*], gadwall [*Anas strepera*], northern shoveler [*Anas clypeata*], ring-necked duck [*Aythya collaris*], canvasback [*Aythya valisineria*], redhead [*Aythya americana*], lesser scaup [*Aythya affinis*], unidentified scaup [*Aythya sp.*], hooded merganser [*Lophodytes cucullatus*], wood duck [*Aix sponsa*], large Canada goose [*Branta Canadensis maxima*], trumpeter swan [*Cygnus buccinator*]), (2) dabbling ducks, species of the genus *Anas* (i.e., mallard, blue-winged teal, American green-winged teal, gadwall, northern shoveler), and (3) cavity nesters (i.e., hooded merganser, wood duck).

Density and Ranks of MPCP Core Areas

The calculated density of mallard pairs in different Core Areas ranged from 0 to 39.10 pairs/mi^2 using the data-based approach. In the model-based approach, density estimates of this cohort ranged from 0 to 107.31 pairs/mi^2. In the first approach to calculating the density of broods of all waterfowl species combined, estimates for different Core Area ranged from 0 to 0.27 broods/surveyed acre of wetland. The second approach produced density estimates of 0 to 35.87 broods/mi^2 in the Core Areas examined.

Rankings of these Core Areas based on the relative value of these density calculations are presented in Table 4, as are the ranks of these sites generated from predicted ranks (i.e., those based on the proportion of Core Areas comprised of wetlands and grasslands). The predicted ranks of Core Areas often were substantially different than empirically-based ranks for both cohorts. In contrast, rankings of Core Areas based on the 2 different methods of calculating density of each cohort often were qualitatively similar.

Pair Detection

Pairs of 2 waterfowl species, mallards and blue-winged teal, had sufficient count data with which to perform analysis. The best approximating model in the first analytical stage indicated that *detection* of mallard pairs was influenced by log survey duration, log wind speed, log observable area of wetlands, temperature, and the proportion of wetland edges obscured by tall vegetation (Appendix 1*a*). However, the associated coefficient estimates of only 2 covariates, log survey duration and the proportion of wetland edges obscured by tall vegetation, did not encompass 0. Log survey duration had a positive influence on detectability (Figure 5), but the proportion of wetland edges obscured by tall vegetation had a negative influence (Figure 6). Consequently, an *a posteriori* model in which *p* varied by log survey duration and the proportion of wetland edges obscured by tall vegetation and ψ was constant was developed and compared to other candidate models in an information-theoretic framework (Appendix 1*a*). This model was ranked higher than any *a priori* model.

In the first stage of the analysis of blue-winged teal pair data, only 1 approximating model that had relatively strong support (Appendix 2*a*). This model indicated that detectability was positively associated with log survey duration (Figure 7).

Overall estimates of the detectability of mallard and blue-winged teal pairs also were generated. These estimates are 0.912 (95% CI: 0.800–0.964) and 0.848 (95% CI: 0.669–0.940), respectively.

Brood Detection

Because few broods of any species were observed during 25 June – 23 July (Table 3) and these count data were not well-distributed across Core Areas or predictive covariates, it is unlikely that there was sufficient data to build reliable species-specific models. Therefore, species-specific count data were aggregated into 3 groups (i.e., all waterfowl species, dabbling ducks, and cavity nesting ducks) and separate analyses were performed on each.

In the first stage of analyses of brood data from all observed waterfowl species combined, the best approximating model (Appendix 3*a*) indicated that detectability was negatively associated with wind speed (Figure 8) and temperature (Figure 9), but positively associated with log OAW (Figure 10) and whether a brood was detected on a previous visit. Specifically, the detectability of broods was slightly greater on the second visit (1.00, 95% CI: 0.00–1.00) if it was detected on the first (0.926, 0.539–0.964). Detectability also varied by method (Figure 11), with greater estimates generated during roadside surveys than walk-in surveys.

There was no evidence that the covariates examined influenced the detectability of dabbling duck broods (Appendix 3*c*). The overall estimate of this parameter in the null model was 0.89 (95% CI: 0.76–0.95).

The analysis of cavity-nesting species suggested that detectability was negatively associated with temperature and wind speed, but positively associated with log OAW and whether a brood was observed at a wetland on the first of 2 visits (Appendix 3*e*). Detectability also varied by survey method, with a greater proportion of broods detected during roadside surveys than during walk-in surveys.

Detectability estimates of the broods of 6 species with adequate data also were generated using only double-observer data. These species-specific estimates are: mallard 0.769 (95% CI: 0.548–0.902), blue-winged teal 0.780 (95% CI: 0.611–0.889), hooded merganser 0.845 (95% CI: 0.844–0.954), large Canada goose 0.892 (95% CI: 0.784–0.950), wood duck 0.918 (95% CI: 0.812–0.967), and pied-billed grebe 0.930 (95% CI: 0.798–0.978). The substantial overlap of the 95% CIs associated with these point estimates suggests that there was no evidence of species-specific differences in detectability.

Pair Occupancy

Based on results of the first stage of analyses, I considered the covariates log survey duration and proportion of wetland edges obscured by tall vegetation to be important predictors of the detectability of mallard pairs (Figures 5 and 6, Appendix 1*a*). Consequently, these covariates were used as predictors of detection in efforts to model wetland occupancy. Two candidate models had relatively strong support, with ΔAIC-values of ≤2 (Appendix 1*b*). The top-ranked model indicated that occupancy varied among MPCP Core Areas, but the 95% CIs associated with these point estimates overlapped substantially (Figure 12). The second-ranked model indicated that occupancy was positively associated with the proportion of Core Area comprised of grassland but negatively associated with the density of Type V wetlands. The density of Type II–IV wetlands also influenced occupancy under this model, but the 95% CIs of the coefficient estimate of this covariate encompassed 0, so I could not make an inference about the relationship between this habitat attribute and the occupancy of mallard pairs.

In the second analytical stage of blue-winged teal pair data, log survey duration was used as a predictor of detection in the modeling of wetland occupancy (Figure 7, Appendix 2*a*). The only model with relatively strong support indicated that the probability of occupancy varied among MPCP Core Areas (Appendix 2*b*). However, this parameter was inestimable for some Core Areas, probably because observational data for this species were sparse. Thus, I will not discuss the relative rankings of Core Areas from the perspective of blue-winged teal pairs.

Overall estimates of occupancy for both mallards and blue-winged teal also were generated. These estimates are 0.089 (95% CI: 0.063–0.123) and 0.055 (95% CI: 0.035–0.085) for mallard and blue-winged teal pairs, respectively.

Brood Occupancy

In the second analytical stage of brood data from all observed waterfowl species aggregated, 4 candidate models had relatively strong support (Appendix 3*b*). Two models indicated that occupancy indicated that wetland type was an important influence on this parameter. I consider the second-ranked model, which indicated that wetland occupancy was constant, to be of most interest because it had 2 fewer parameters than the top-ranked model and a ΔAIC-value of only 1.2. The overall probabilities of occupancy, extinction, and colonization were 0.29 (95% CI: 0.23–0.36), 0.38 (95% CI: 0.27–0.50), and 0.10 (95% CI: 0.05–0.16), respectively. The estimate of wetland occupancy used data from the 4 Core Areas with a sufficient number of wetlands surveyed (Figure 13).

Two models in the analyses in which all observed dabbling duck species were aggregated had ΔAIC-values of <2, and thus had relatively strong support (Appendix 3*d*). The top-ranked model indicated that wetland occupancy was negatively associated with the proportion of grassland in Core Areas**,** but positively associated with the density of Type II–V wetlands. The second-ranked model had a similar ΔAIC-value and the same number of parameters as the top-ranked model, but better addressed the research objective of discerning differences in wetland occupancy among Core Areas. Wetland occupancy estimates in Core Areas ranged from 0 (95% CI: 0.00–0.01) to 0.21 (95% CI: 0.11–0.37), and probabilities of extinction and colonization were 0.49 (95% CI: 0.26–0.72) and 0.03 (95% CI: 0.01–0.06), respectively.

The top-ranked model in the analyses of cavity-nester broods indicated that wetland occupancy was positively associated with log OAW and the proportion of the wetland edge obscured by tall vegetation, but negatively associated with the proportion of grassland in the Core Area and the density of Types II-IV wetlands (Appendix 3*f*). The second- and third-ranked models were competitive because both had ΔAIC-values of only 0.53 and had 2 fewer parameters than the top-ranked model. However, I preferred the third-ranked model because it used the covariate Core Area to explain variation in occupancy, which allowed the discernment of differences among sites. Further, the negative relationship between occupancy and wetland density in the top-ranked model appeared spurious. Occupancy estimates in the preferred model ranged from 0.05 (95% CI: 0.01 0.19) to 0.23 (95% CI: 0.16–0.32) among Core Areas, and the estimates of extinction and colonization were 0.33 (95% CI: 0.20–0.50) and 0.06 (95% CI: 0.03–0.11), respectively.

Weather

Statewide ice-out dates were 9 d later than the long-term median date (MNDNR 2015*b*). Monthly weather characteristics during the study indicate that many MPCP Core Areas were located in divisions that were slightly cooler and had greater precipitation than normal during much of the field season (Table 5, MNDNR 2015*a*). Rainfall amounts during June 2014 were approximately 3 times greater than normal (MNDNR 2014*b*). A heavy rainfall event occurred in substantial portions of Minnesota during 11–12 May 2014 (MNDNR 2014*a*).

DISCUSSION

Challenges of Assessing MPCP Success

A primary goal of MPCP is to protect, restore, and enhance grasslands, wetlands, and other habitats in the prairie and transition zones (Minnesota Prairie Plan Working Group 2011). Unfortunately, current and accurate land-cover data were not available during this study.

Similarly, the dates, locations, and areal extents of all prior and planned habitat restoration and enhancement efforts in each of the 36 Core Area and associated corridors had not been incorporated into a central database. Such data is needed as a baseline for planning surveys and evaluating the current indicators of success of the MPCP, in terms of both of (1) habitat preservation and improvement and (2) ecosystem function.

The lack of accurate spatial data also hindered my efforts to select a representative sample of wetlands for surveys, decreased the efficiency of surveys (e.g., some wetlands displayed in the Circular 39 GIS layer were dry or had been altered to the extent that a new classification was warranted), and did not facilitate the accurate calculations of the areal extent of important habitats, association of habitat attributes to avian-count metrics, or extrapolation of count data from wetlands to Core Areas. I did not classify terrestrial habitats as part of the survey methodology, and therefore did not detect problems with the available grassland habitat data. However, it is likely that the areal extent of grassland habitats in MPCP Core Areas was less than that indicated by the GIS layer, given the large-scale conversion of grasslands to croplands (Lark et al. 2015) and recent decrease in the area enrolled in the Conservation Reserve Program (MacDonald 2013).

It would be beneficial to periodically evaluate the success of the MPCP to provide useful feedback on the responses of populations of indicator species to management actions, but the selected measures of success must be realistic, measurable, and biologically meaningful. However, a current measure of related to ecosystem function (i.e., stable-to-increasing populations of indicator species at the statewide level [Minnesota Prairie Plan Working Group 2011, pp. 47–48]) appears unrealistic because it is unlikely that management actions on Core Areas (about 3% of the land base of Minnesota) could substantially influence population trends at a statewide scale. Further, any assessment of the population responses of migratory species to management actions could be confounded by functional processes that occur at multiple spatial and temporal scales. Preferably, baseline population estimates of indicator species would have been estimated and the areal extents of important habitats, prior habitat management activities, and administrative actions (e.g., protection, restoration, creation, enhancement) would have been measured accurately at the time Core Areas and Corridors were established, and then periodically assessed as restorative efforts occurred. These measurements were not taken, so alternative approaches must be used to evaluate the success of the MPCP. This could be accomplished by comparing population parameter estimates of indicator species within managed sampling units (i.e., treatments) located inside Core Area to those outside the boundaries.

I initiated a pilot study to measure the use of wetlands in MPCP Core Areas by waterfowl, and examine the feasibility of using the mallard populations at these sites as an indicator of success under the MPCP. Field crews surveyed 403 wetlands during the pair period and 265 wetlands during the brood period across a broad geographic area within Minnesota to try to meet these goals, but counts of mallards and other waterfowl and waterbirds (Table 3) were substantially lower than I anticipated. Sparse count data of some indicator species could arise in a broad-scale wildlife-monitoring program designed to assess the performance of conservation efforts. That I was able to document pairs of 18 species and broods of 15 species of waterfowl and waterbirds using Core Areas (Table 3) supports the idea of conserving and improving habitats at these sites. However, avian habitat use during 1 field season does not provide information regarding the specific demographic vital- rates influencing site-specific population dynamics. The most important findings of this pilot project may be (1) it is challenging to collect sufficient mallard pair and brood count data over a set of Core Areas that vary in size, shape, habitat composition, and accessibility, and (2) there are challenges and limitations to using migratory species count-data as a measure of program success.

A major challenge of the MPCP that will need to be addressed is the development of a more realistic set of program goals. This need is indicated by the following observations. First, it

is unlikely that habitat restoration and management activities that occur on 3% of the Minnesota land base would drive the statewide population growth of mallards. Second, any assessment of program success based on population levels or trends of indicator species that are migratory likely will be confounded by the influence of variables that operate during the non-breeding season and outside of the Core Areas. Third, pair- and brood-count data collected during 1 field season from a subset of available wetlands may be difficult to interpret and therefore not provide the information needed to adequately evaluate success, given the lack of baseline population and reliable spatial habitat data.

To develop a set of more realistic set of population goals for indicator species, researchers will need to identify a parameter that can be readily estimated at a reasonable cost. The use of the parameter density (number of cohorts / unit area) to compare Core Areas is appealing because it can be extrapolated from the spatial scale of wetlands to that of Core Areas, which permits the comparison of larger spatial units that vary greatly in areal extent and shape. However, this parameter is difficult to reliably estimate because precipitation and evapotranspiration substantially will alter the areal extent of wetlands during the survey period, and the obscuring effects of dense vegetation and topography will not permit observers to estimate the areal extent of wetlands at the time of surveys. Because I typically generated counts of 0 or 1 pair or broods at most wetlands, it was preferable to measure a binary abundance metric (i.e., presence-absence) for these cohorts and estimate wetland occupancy adjusted for imperfect detection. Occupancy modeling based on repeated surveys has become a popular monitoring tool, especially for relatively rare species (see MacKenzie et al. 2006), but the use of this parameter to measure success of the MPCP is not necessarily straightforward. For example, 2 Core Areas with an equal areal extent of wetlands and waterfowl detectability, immigration, and emigration may have an equal number of pairs and broods during year 1. If 1 Core Area has average hen and duckling mortality but the other has unusually high mortality of these cohorts, it is intuitive that the second Core Area likely would have lower abundance in year 2 (assuming equal immigration and emigration between sites and years) and would not contribute to the MPCP goal of a stable-to-increasing statewide mallard population. Using occupancy or density to monitor success of the MPCP is further complicated by differences in habitat composition and accessibility among Core Areas, the annual fluctuation in areal extent in Type I-III wetlands attributable to climactic variation, and other factors operating at multiple spatial scales (e.g., wetland, landscape, regional) that affect the spatial distributions of waterfowl population.

Using statewide population trends of an indicator species may not be a realistic means of ascertain the success of the MPCP. It may be more tractable to examine differences in population parameter estimates among smaller sample units (e.g., Core Areas, control and habitat treatment sites within Core Areas) as a way of ascertaining success. However, it will be necessary to measure parameters of interest over a realistic timeframe because a staggered response-time of populations to management actions is likely, local waterfowl populations often fluctuate, and the many variables that influence the local population dynamics of migratory species operate at multiple spatial and temporal scales.

Researchers should consider the trade-offs associated with various sampling designs, survey methodologies, sample size needs, and project costs when attempting to ascertain the success of large-scale conservation projects, such as the MPCP. For example, there was a trade-off between the number of wetlands sampled, the number of visits to study wetlands, and costs in this pilot study. If estimating temporal trends in occupancy was an objective in a longer-term study, there also would be a tradeoff between the study length (i.e., field seasons) and the number of wetlands sampled. In a situation such as that I encountered (i.e., reasonably high detection probabilities but only low-to-moderate occupancy probabilities), researchers should survey a greater number of wetlands per Core Area, as opposed to more intensively sampling fewer wetlands. Unfortunately, it would be difficult to survey enough wetlands in smaller or drier Core Areas to generate precise estimates of wetland occupancy.

Despite the effort and resources expended on this project (i.e., 3 2-person crews each using a vehicle for 3 months) and surveys conducted at many of the accessible roadside wetlands in selected Core Areas, precise estimates of occupancy could not be generated. MN DNR personnel surveyed 403 wetlands during the pair period (4 – 25 June) and 265 wetlands during the brood period (25 June – 23 July), but researchers in the Dakotas were able to visit more wetlands and count more waterfowl, probably because the densities of both were substantially greater in the Dakotas than in MPCP Core Areas. Specifically, a 2-person team surveyed 1182 wetlands for pairs during 3–18 May (Pagano and Arnold 2009*a*), and later surveyed 787 wetlands for broods in North Dakota during 2–25 June and 2–25 July of 1 field season (Pagano and Arnold 2009*b*) and an unspecified number of field crews surveyed 3,226 wetlands in the Dakotas for broods during 3 field seasons (Walker et al. 2013). MN DNR crews observed only 54 mallard and 30 blue-winged teal pairs during the pair period, and 12 mallard and 21 blue-winged teal broods during the brood period, but Pagano and Arnold (2009*a*) detected 656 mallard and 1066 blue-winged teal pairs during 3–18 May, and Pagano and Arnold (2009*b*) observed 306 mallard and 503 blue-winged teal broods during 2–26 June and 2–26 July. Sufficiently precise wetland occupancy estimates for larger Core Areas could have been generated by surveying more wetlands, but costs would have increased substantially.

MPCP Core Area Rankings Based on Density Estimates of Mallard Pairs and Waterfowl Broods

I report both the predicted and empirical ranks of Core Areas, but these rankings should be viewed with skepticism. The predicted ranks were based on scientific knowledge of mallard habitat associations (see Krapu et al. 1997, Walker et al. 2013), but some empirical ranks were based on density estimates calculated from sparse count data, Core Areas with relatively few wetlands, and subjective estimates of the observable area of study wetlands. Further, both predicted and empirical rankings were based on measurements of the areal extent of important habitats (i.e., wetlands, grasslands) that are of questionable accuracy. Consequently, my initial rankings of Core Areas should not be used to guide habitat management projects.

Density estimates of mallard pairs calculated from the data-based approach (range 0 to 39.10 pairs/mi^2) appeared more plausible than the model-based approach (0 to 107.31 pairs/mi^2). Although the spatial areas of inference are not the same and survey methodologies are dissimilar, the density of mallard pairs in the Four Square Mile Surveys (range: 1.5 to 5.8 mallard pairs/mi^2, Anonymous 2014) was similar to some but not all of my Core Area density estimates. The model-based pair-density estimate at one small Core Area (Rush Lake) appeared to be spurious. This result may have generated from a relatively high count of pairs on the few wetlands accessible to field crews. Unfortunately, it is not possible to make a meaningful comparison between my estimates of young-of-the-year density because the density metrics used by the 2 surveys were different (broods/mi^2 v recruits/ mi^2).

It is interesting that the different methods used to empirically estimate density generally produced similar ranks of Core Areas (Table 4), but that the predicted ranks in some cases were substantially different than empirically-based ranks. It may be that differences between predicted and empirical ranks occurred because (1) the habitat variables important to mallard pairs and broods in other locations were not useful for predicting the abundance or wetland occupancy of these cohorts in western Minnesota, or my ranking system was not refined enough to capture factors influencing mallard settling or reproductive success, (2) the habitat information garnered from available wetland and grassland GIS layers was inaccurate and therefore not useful for making predictions, or (3) unusual weather patterns immediately prior to and during the field season (MNDNR 2014*a*, *b*; 2015*a*, *b*) may have contributed to unexpected spatial distributions of settling pairs of mallards and the low production of waterfowl broods.

Modeling and Estimation of Occupancy

The results of modeling and estimation efforts should be viewed with caution for several reasons. Field crews were able to count pairs and broods at fewer wetlands within Core Areas than some recent studies (e.g., Pagano and Arnold 2009*a*, *b*; Walker et al. 2013), and this sparse data did not facilitate the reliable estimation of occupancy. Also, multiple candidate models in most analyses that had ΔAIC-values ≤2 (Appendices 2–4), which suggests the existence of substantial modeling uncertainty. Last, data were collected during a field season with an anomalous weather pattern (MNDNR 2014*a*, *b;* 2015*a*, *b*), which likely affected the spatial distribution patterns of the settling pairs and the survival and thus abundance of clutches and young. Thus, it may be necessary to conduct waterfowl surveys in Minnesota for multiple field seasons to obtain sufficient data to accurately estimate occupancy.

Pair Occupancy

The top-ranked mallard-pair model suggests that wetland occupancy varied among MPCP Core Areas, but there was no significant difference among most of the site-specific estimates of this parameter. The top-ranked blue-winged teal-pair model suggests that wetland occupancy is associated with both landscape- (proportion of grassland, densities of Types II-IV and Type V wetlands in each Core Area) and wetland-level covariates (the proportion of the wetland area observable from the survey point, the proportion of wetland edge obscured by tall vegetation, wetland type). The inability to detect significant differences in wetland occupancy rates of pairs among Core Areas may be attributed to the relatively small pair counts (n = 54 mallards and 30 blue-winged teal [Table 3], with overall wetland occupancy estimates of 8.9% and 5.5%, respectively). The low pair-count data also may be attributed to the somewhat late timing of my surveys; *i.e.*, some pair bonds may have ended and males may have moved to molting areas and thus were not available to be counted (Dzubin 1969) when surveys were conducted. Also, pair counts in the Core Areas may have been lower than anticipated because duck density in Minnesota probably is less than in the Dakotas (see Cordts 2015, Zimpfer et al. 2015). Last, low pair counts may have occurred in-part because none of the Core Areas surveyed met both the 20% wetland and 40% grassland criteria of the Duck Recovery Plan (Anonymous 2006, Table 1).

Brood Occupancy

There was substantial uncertainty with regard to the influences of different covariates on occupancy in any of my 3 aggregations of waterfowl species. This uncertainty may be attributed to sparse brood-count data and species-specific differences in niche occupation. Although some well-supported brood models suggest that the occupancy rates of the dabbling duck and cavity-nester aggregations differed among MPCP Core Areas, the substantial overlap of most 95% CIs associated with 4 of these site-specific parameter estimates suggest that differences were not discernable.

Detectability

Although modeling and estimating detectability usually is not a primary objective of waterfowl surveys, a precise and reliable estimate of this parameter is required to generate reliable estimates of wetland occupancy. Further, survey methodology could be improved and standardized with knowledge of the time during a survey at which detectability approaches 1.0, the approaches that generate sufficiently high estimates of this parameter, and knowledge of the environmental predictors that influence this parameter. For example, surveys targeting mallard and blue-winged teal pairs probably would generate detection probabilities of 1.0 at 12 and 17 min, respectively. Using the double-observer approach to count pairs also should produce high detectability estimates, given the estimates generated in this (91% and 85% for mallard and blue-winged teal pairs, respectively) and another study (91% for 8 waterfowl species combined [Pagano and Arnold 2009*a*]). Similarly, the use of both double-observer and repeat-visit surveys for broods allowed the indirect examination of the availability of broods to be counted, which is an important part of the observation process. This approach also permitted high estimates of brood detectability to be generated for both the first and second visits (93% and 100%, respectively)

when this cohort was available to be observed on both visits. The type of survey conducted should be incorporated into models, given that greater brood detectability estimates are generated from roadside surveys than walk-in surveys (Pagano and Arnold 2009*b*, this study)

Habitat characteristics and weather attributes also may influence the detectability of waterfowl, and therefore should be measured and included as explanatory covariates in models. For example, my results and those of Pagano and Arnold (2009*a*) suggest that the proportion of wetland edges obscured by tall vegetation had a negative influence on the detectability of pairs (Figure 6), and a similar relationship was observed for waterfowl broods (Giudice 2001, Walker et al. 2013). This vegetative structure likely provides visual concealment and is commonly used as hiding cover by waterfowl. Researchers should consider excluding wetlands with especially dense emergent vegetation from their surveys (Nichols et al. 2000) because it is not feasible to count waterfowl using such habitats (see Giudice 2001).

The areal extent of surveyed wetlands also may have an effect on waterfowl detectability. Assuming a positive relationship between the observable area of wetland and the total area of the wetland, it appears that broods were more likely to be detected on relatively large wetlands (Figure 10). It may be that broods using larger wetlands were more tolerant of disturbance by field personnel than those using smaller wetlands; e.g., broods were less likely to move into hiding cover when personnel were present at larger wetlands. In contrast, 2 other studies observed a negative relationship between the detectability of broods and wetland size, but there likely were differences in the areal extents of wetlands between my study sites and those of Pagano and Arnold (2009*b*) and Walker et al. (2013). Further, I did not limit the size of wetlands examined, but Pagano and Arnold (2009*b*) only examined wetlands of ≤5 ha.

The weather covariates that influenced brood detectability varied among different waterfowl guilds (Appendix 3*a*, *c*, and *e*), but in the analysis of broods of all species combined, this parameter appeared to be affected by wind speed (Figure 8) and temperature (Figure 9). More specifically, wind speed likely had a negative influence on detectability because broods were obscured by the associated disturbance of the water surface and behavioral responses to this covariate (e.g., hiding from this disturbance in vegetative cover). Temperature may have negatively influenced detectability in several ways. Detectability could have been reduced when ambient temperatures were high if broods were less active or used shaded and concealed locations within wetlands, or if field personnel were fatigued or distracted by this condition. Walker et al. (2013) also observed a negative relationship between wind speed and the detection probability of gadwall and blue-winged teal. In contrast, Pagano and Arnold (2009*b*) found that wind speed and temperature were positively associated with detectability, but noted that this was dissimilar to results of other studies (e.g., Giudice 2001).

Researchers should conduct waterfowl surveys at the most appropriate time within their life cycle (e.g., when most observable) to increase the likelihood of generating representative count data for the cohorts of interest. Typical dates on which life-cycle phases occur at selected study sites usually are used to determine the timing of investigations, but there can be temporal variation in the phenology of some phases (e.g., Oja and Poysa 2007). Thus, it may be somewhat challenging to establish a sampling period *a priori* because of the difficulty in predicting annual variation in environmental conditions (e.g., weather patterns, phenology of ice-out) and its influences on the phenology of waterfowl life-cycles. It may be necessary to alter study plans when unusual environmental conditions occur to ensure that a sufficient number of waterfowl will be observed.

Personnel conducted pair surveys relatively late (4–25 June), in-part because of the late spring and ice-out dates of wetlands in Minnesota during 2014 (MNDNR 2015*a*, *b*) and the time required to complete surveys during the migration period. It may be that more waterfowl and waterbirds would have been observed during pair surveys conducted earlier (e.g., 3–18 May [Pagano and Arnold 2009*a*], 27 April–15 May and 20 May–6 June [Anonymous 2014]) because

detectability likely decreased as emergent vegetation grew more densely during as the field season progressed. However, personnel also observed relatively few broods of any species, despite the substantial overlap between the dates of my brood surveys (25 June–23 July) and those of Pagano and Arnold (2009*b;* 2–26 June and 2–26 July) and Walker et al. (2013; 20 July–5 August). Thus, variables other than survey period (e.g., geographic pattern of settling pairs, seasonal weather) may have had some negative influence on the number of observed waterfowl at my study sites.

Researchers should conduct surveys when cohorts of interest are most detectable, which I expect is when waterfowl generally are most active (i.e., early morning, late evening). Reasonably high detectability estimates were generated in this pilot study, but few pairs and broods were observed during the typical survey hours (i.e., approximately 0700–1900). More birds may have observed if a greater proportion of the surveys were conducted only during the early morning and late evening. However, the obscuring effect of shadows and increased cost associated with mileage and travel time when driving to and from study sites twice per day must be considered if surveys are limited to these 2 periods. Further, the within-day temporal patterns of brood detectability may vary among species (Pagano and Arnold 2009*b*, Walker et al. 2013).

MANAGEMENT IMPLICATIONS

The establishment of MPCP Core Areas and Corridors could be an effective means of conserving and maintaining native prairie biotic communities and ecosystem processes, but this plan should be assessed (sensu Reynolds et al. 2001) periodically to ensure that goals and objectives are being met. However, results of this study indicate that an assessment of broad-scale conservation plans such as the MPCP will be complex. Such assessments could be simplified somewhat if the original objectives of the MPCP were replaced with metrics of success that were more realistic and easily measured. The development of new objectives and criteria to measure success will require critical thought by scientists and managers, and some knowledge of habitat conditions and the historical and current populations of indicator species in Core Areas. Further, there will be a need for monitoring programs to track the response of populations of indicator species to habitat management projects at the appropriate temporal and spatial scales. Identifying this temporal scale will require an understanding of the timeframe during which the population of interest is likely to respond to management actions. Selecting the best spatial scale at which to measure success may be especially challenging, in-part because of the need to develop and implement a waterfowl monitoring method that permits a meaningful extrapolation of population parameter estimates from the spatial scale of wetlands to that of Core Areas, which differ in total areal extent and in the composition, spatial configuration, and area of important habitats. It also will be a challenge to understand responses of low-density populations to habitat management, given that both pre- and post-treatment parameter estimates likely will have relatively wide 95% CIs.

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Table 1. The landscape-level habitat characteristics of Minnesota Prairie Conservation Plan (MPCP) Core Areas.

MPCP Core Area	Latitudinal Zone	Predicitive Class Rank [a]	Total area (mi^2)	Proportion Type II-IV wetlands [b]	Proportion grassland [c]
Antelope Hills	S	H	38.97	0.088	0.197
Lac Qui Parle Prairie [e]	S	H	166.72	0.107	0.219
Upper Minnesota River Valley	S	H	235.02	0.041	0.109
Chanarambie Creek [d]	S	MH	27.67	0.019	0.212
Prairie Coteau / Rock River	S	MH	38.61	0.037	0.116
Yellow Medicine Coteau	S	MH	24.99	0.035	0.15
Cottonwood River Prairies [d]	S	ML	7.63	0.055	0.12
Hole-In-The-Mountain	S	ML	70.83	0.023	0.116
Shaokatan Prairies	S	ML	16.83	0.021	0.144
Des Moines River Valley	S	L	135.81	0.016	0.081
Plum Creek	S	L	9.87	0.003	0.098
Red Rock Ridge [d]	S	L	19.08	0.005	0.09
Blue Stem Prairie	C	H	36.96	0.123	0.168
Felton Prairie [d]	C	H	42.86	0.149	0.235
Rothsay Prairie	C	H	35.35	0.202	0.34
Big Stone Moraine [d]	C	MH	35.76	0.108	0.157
Glacial Lakes [d, e]	C	MH	264.16	0.15	0.104
Waubun Prairie	C	MH	32.26	0.217	0.154
Lake Christina Hills [e]	C	ML	43.67	0.087	0.036
Big Stone Lake Prairie – North [e]	C	ML	14.81	0.045	0.174
Blanket Flower Prairie [c]	C	ML	11.77	0.02	0.189
Big Stone Lake Prairie – South [e]	C	L	17.45	0.014	0.183
Lake Traverse Prairie [e]	C	L	26.7	0.019	0.158
Reisdorah Prairie [d, e]	C	L	10.24	0.02	0.122
Aspen Parkland [d]	N	H	465.5	0.244	0.165
Glacial Ridge	N	H	185.36	0.173	0.156
Syre Prairie	N	H	31.5	0.152	0.248
Rush Lake [d]	N	MH	14.34	0.209	0.228
East Park - Thief Lake [d]	N	MH	139.72	0.131	0.079
Espelie	N	MH	10.026	0.374	0.12
Florian	N	ML	16.32	0.128	0.145
Pembina Prairie	N	ML	64.78	0.105	0.137
Wambach Santee Prairie	N	ML	71.35	0.08	0.135
Agassiz Dunes	N	L	41.54	0.053	0.097
New Solum Prairie [d]	N	L	39.87	0.147	0.073
Chester Hills Prairie	N	L	27.55	0.137	0.066

[a] H = high, MH = Medium-high, ML = Medium-low, L= Low

[b] The proportion of the total area of MPCP Core Areas comprised of Type II-IV wetlands (Stewart and Kantrud 1971) was estimated from the Circular 39 Wetland GIS layer.

[c] The proportion of MPCP Core Areas comprised of medium-to-high quality grasslands was estimated from the 2011 Cropland GIS layer.

[d] MPCP Core Area was examined during the pair period.

[e] MPCP Core Area was examined during the brood period.

Table 2. The number of wetlands of 5 types surveyed in Minnesota Prairie Conservation Plan Core Areas during 2 periods during the *a*) pair (4 – 25 Jun) and *b*) brood periods (and 25 Jun – 23 Jul) of 2014.

a) Period		Number of wetlands surveyed of 5 types [a]				
Pair (4 – 25 Jun)	Core Area	I	II	III	IV	V
	Aspen Parkland	26	30	20	2	39
	Blanket Flower Prairie			6		3
	Chanarambie Creek	1		8	1	4
	Cottonwood River	3	1	15	4	1
	East Park – Thief Lake	13	7	6	1	6
	Felton Prairie	1	5	12	1	3
	Glacial Lakes	7	9	31	15	12
	New Solum Prairie	4	14	11	3	2
	Red Rock Ridge			5	1	
	Reisdorah Prairie		1	9	2	1
	Rush Lake	1		16	3	4
Total		56	67	139	33	75
b) Brood (25 Jun – 23 Jul)	Big Stone Lake Prairie - North			1	2	1
	Big Stone Lake Prairie - South			5		
	Big Stone Moraine		1	22	9	7
	Glacial Lakes			62	42	24
	Lake Christina Hills			21	17	22
	Lake Traverse Prairie				1	2
	Lac Qui Parle Prairie		1	3	16	3
	Reisdorah Prairie			1	1	1
Total		0	2	115	88	60

[a] Wetland classifications followed Stewart and Kantrud (1971).

Table 3. Counts of the pair and broods cohorts of waterfowl and waterbird species observed during surveys conducted at Minnesota Prairie Conservation Plan Core Areas during the pair (4 – 25 Jun) and brood periods (25 Jun – 23 Jul) of 2014.

	Pair period (4 – 25 Jun)			Brood period (25 Jun – 23 Jul)		
Species [a]	# Pairs	# Broods	Proportion of wetlands with pairs	# Pairs	# Broods	Proportion of wetlands with broods
AGWT	4	0	0.005	2	2	0.008
BUFF	1	0	0.002	0	0	0
BWTE	30	1	0.057	40	21	0.057
CANV	0	0	0	2	4	0.015
COLO	4	0	0.010	4	2	0.008
GADW	0	0	0	15	1	0.004
HOME	2	3	0.005	11	48	0.147
LCGO	8	5	0.015	20	31	0.083
LESC	0	0	0	4	2	0.004
MALL	54	1	0.079	29	12	0.030
NSHO	8	0	0.012	3	0	0
PBGR	0	0	0	2	18	0.053
REDH	0	0	0	21	3	0.011
RNDU	17	0	0.020	15	2	0.008
RNGR	0	0	0	1	1	0.004
RUDU	0	0	0	10	0	0
TRUS	2	0	0.005	2	2	0.008
WODU	15	1	0.020	59	26	0.075

[a] Species-specific acronyms are: AGWT = American green-winged teal, BUFF = bufflehead, BWTE = blue-winged teal, CANV = canvasback, COLO = common loon, GADW = gadwall, HOME = hooded merganser, LCGO = large Canada goose, LESC = lesser scaup, MALL = mallard, NSHO = northern shoveler, PBGR = pied-billed grebe, REDH = redhead, RNDU = ring-necked duck, RNGR = red-necked grebe, RUDU = ruddy duck, TRUS = trumpeter swan, WODU = wood duck.

Table 4. The predicted, empirical, and model-based (pairs only) rankings of the value of a subset of Minnesota Prairie Conservation Plan Core Areas to mallard pairs and broods of all waterfowl species during the *a*) pair (4–25 Jun) and *b*) brood periods (25 Jun–23 Jul) of 2014.

Core Area	Region	Predicted rank	Empirical rank	Model-based rank
a. Pair period (4–25 Jun)				
Aspen Parkland	North	1	11	11
Rush Lake	North	2	3	1
Glacial Lakes	Central	3	8	8
Felton Prairie	Central	4	4	3
New Solum Prairie	North	5	7	6
East Park – Thief Lake	North	6	10	9
Cottonwood River	South	7	6	7
Reisdorah Prairie	Central	8	1	2
Blanket Flower Prairie	Central	9	5	5
Chanarambie Creek	South	10	9	10
Red Rock Ridge	South	11	2	4
b) Brood period (25 Jun–23 Jul)				
Lac Qui Parle Prairie	South	1	1	
Big Stone Lake Prairie – South	Central	2	7.5	
Big Stone Lake Prairie - North	Central	3	5	
Lake Traverse Prairie	Central	4	6	
Big Stone Moraine	Central	5	3	
Reisdorah Prairie	Central	6	7.5	
Glacial Lakes	Central	7	4	
Lake Christina Hills	Central	8	2	

Table 5. The monthly departure from normal measurements of temperature (°C) and precipitation (cm) of divisions[a] that encompass the Minnesota Prairie Conservation Plan Core Areas sampled during the 2014 field season.

Division [a]	MPCP Core Areas	Weather variable	Apr	May	Jun	Jul
Northwest	Aspen Parkland, Blanket Flower Prairie, East Park – Thief Lake, Felton Prairie, New Solum Prairie, Rush Lake	*Temperature*	-2.77	-0.50	+0.33	-1.17
		Precipitation	+4.60	-0.53	+6.53	-1.73
West-central	Big Stone Lake Prairie – North, Big Stone Lake Prairie – South, Big Stone Moraine, Glacial Lakes, Lac Qui Parle Prairie, Lake Christina Hills, Lake Traverse Prairie, Reisdorah Prairie	*Temperature*	-2.45	-0.67	+0.05	-1.61
		Precipitation	+3.43	+0.86	+9.73	-4.32
Central	Glacial Lakes	*Temperature*	-2.84	-0.83	+0.22	-1.44
		Precipitation	+7.52	+3.86	+11.20	-2.51
Southwest	Chanarambie Creek, Red Rock Ridge	*Temperature*	-1.73	-0.56	-0.11	-2.11
		Precipitation	-1.09	-3.53	+15.37	-4.37
South-central	Cottonwood River	*Temperature*	-1.55	-0.56	+0.27	-3.50
		Precipitation	+4.14	-3.73	+14.02	-5.44

[a] Division boundaries are available at: www.cpc.ncep.noaa.gov/products/analysis_monitoring/regional_monitoring/CLIM_DIVS/minnesota.gif

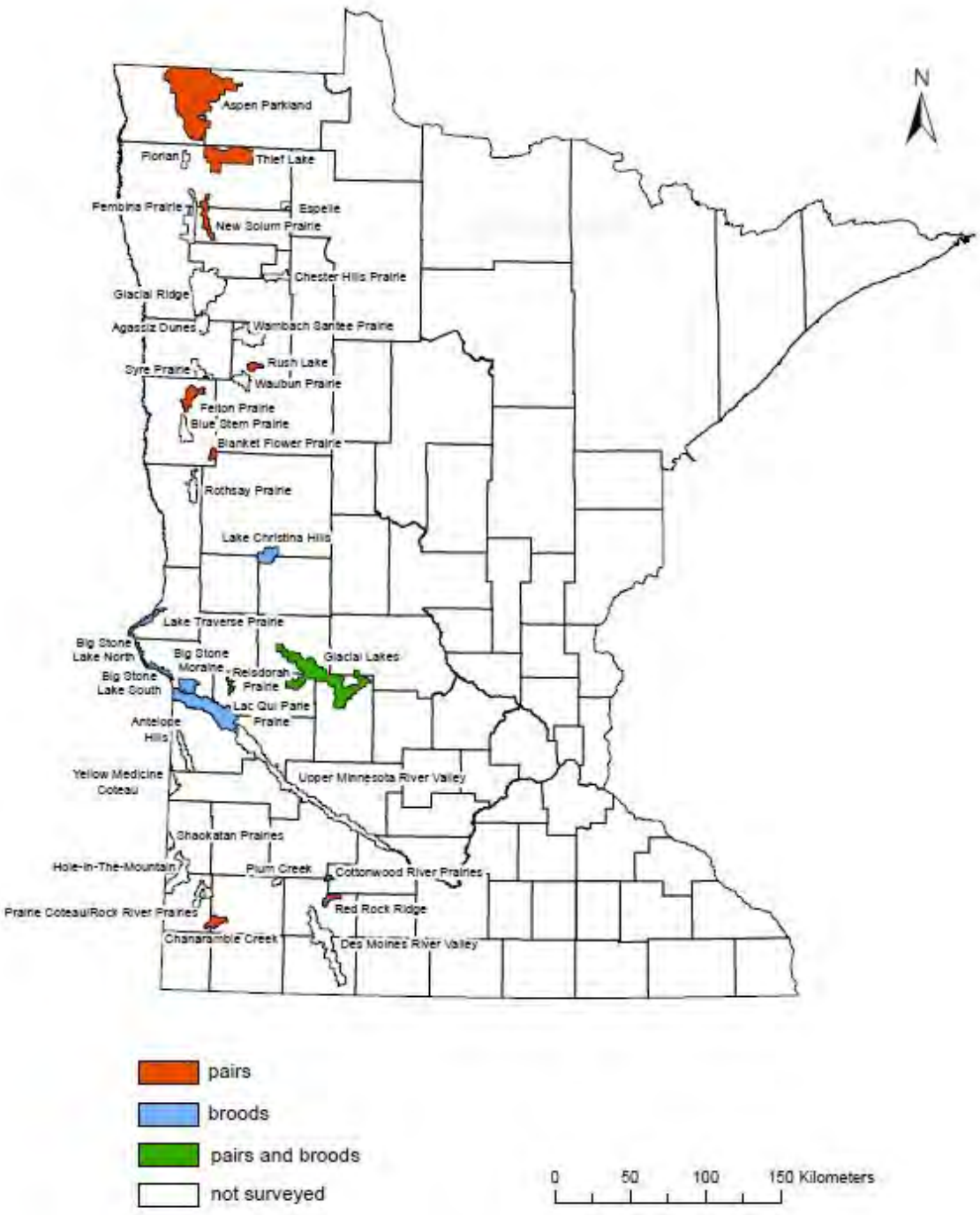

Figure 1. Distribution of core areas (n = 36) designated in the Minnesota Prairie Conservation Plan. Observers surveyed nine core areas for pairs, six for broods, and two for both during the 2014 breeding season. Seventeen core areas were not surveyed.

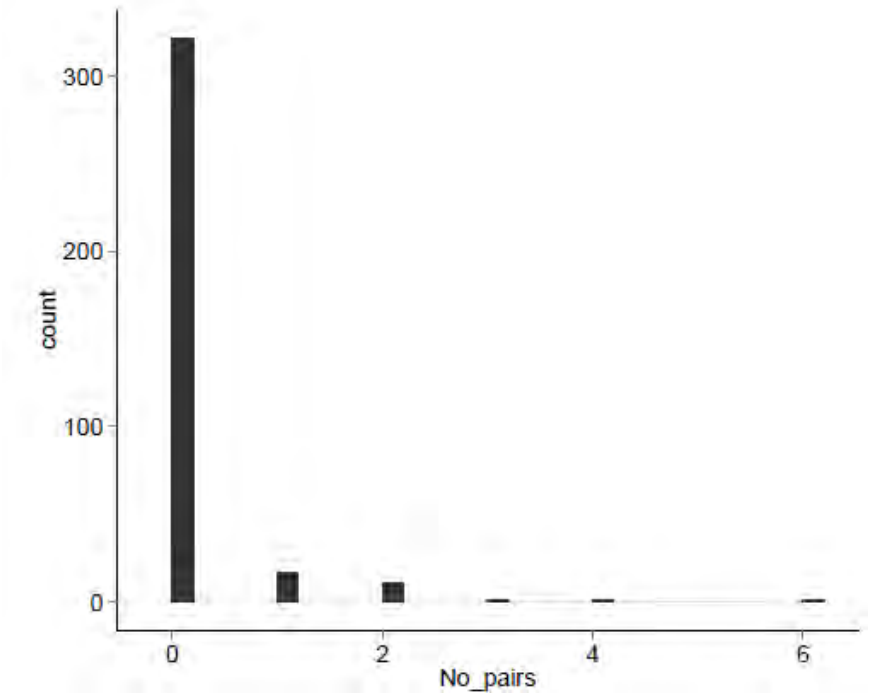

Figure 2. Frequency distribution of the number of indicated breeding pairs (No_pairs) of mallards observed at 352 wetlands within 11 Minnesota Prairie Conservation Plan Core Areas, 2014.

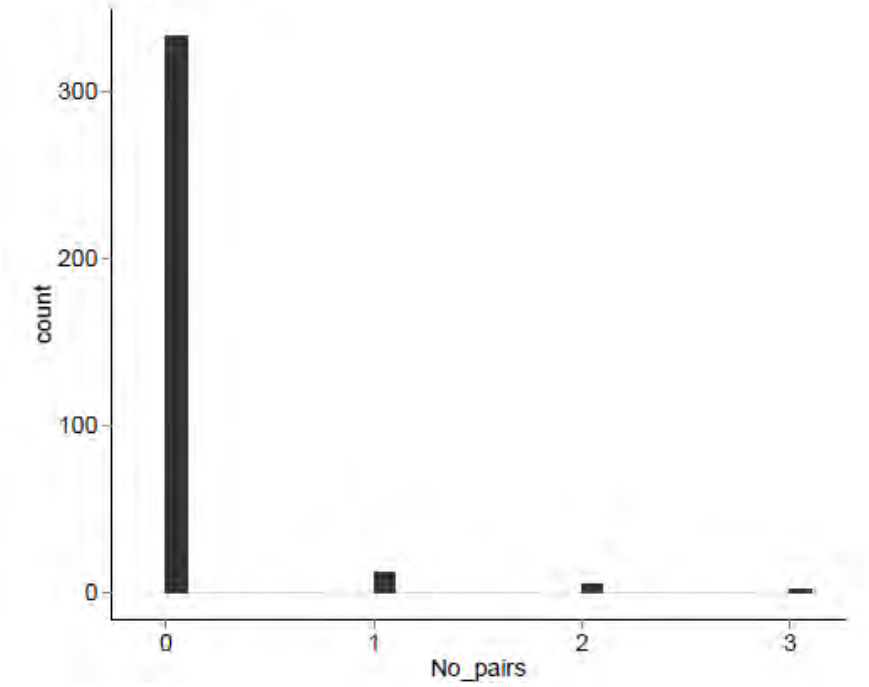

Figure 3. Frequency distribution of the number of indicated breeding pairs (No_pairs) of blue-winged teal observed at 352 wetlands within 11 Minnesota Prairie Conservation Plan Core Areas, 2014.

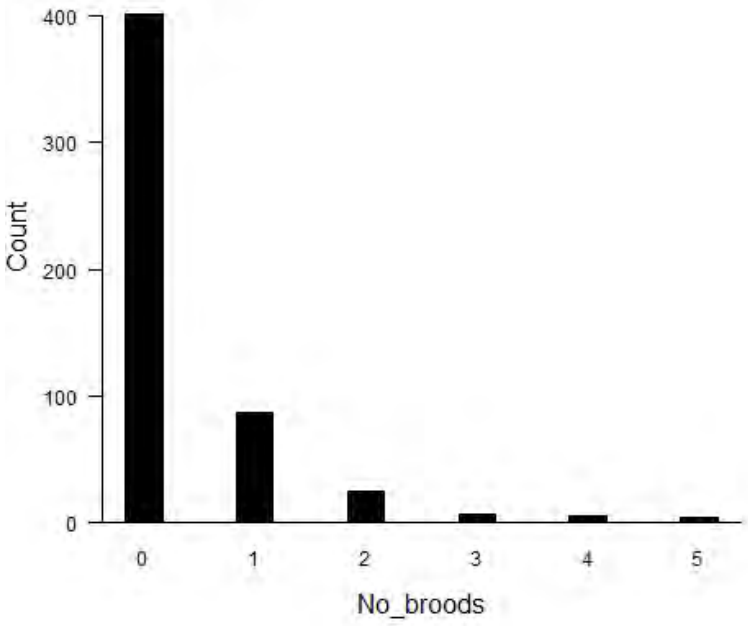

Figure 4. Frequency distribution of broods of all waterfowl species observed during ≥2 visits to 265 wetlands within 4 Minnesota Prairie Conservation Plan Core Areas, 2014.

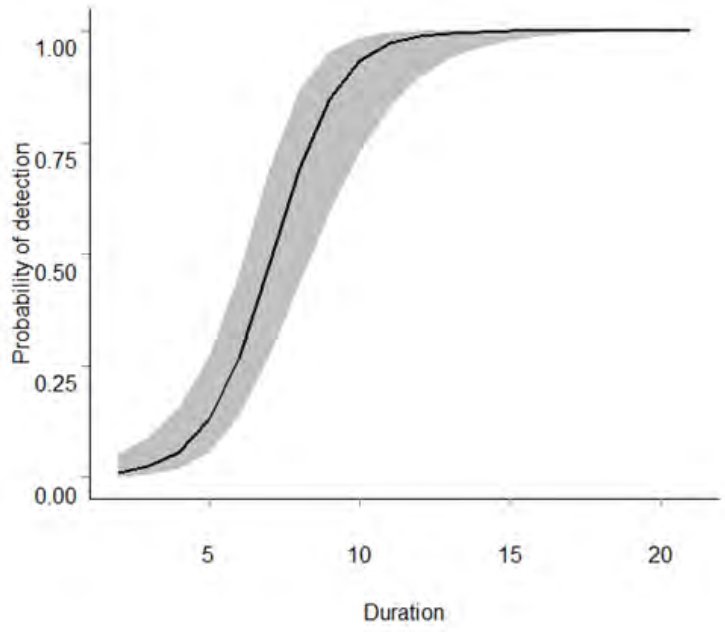

Figure 5. Modeled relationship between the probability of detection of indicated breeding pairs of mallards and survey duration (Duration [min]). Data used in this model were collected at 11 Minnesota Prairie Conservation Plan Core Areas during 2014.

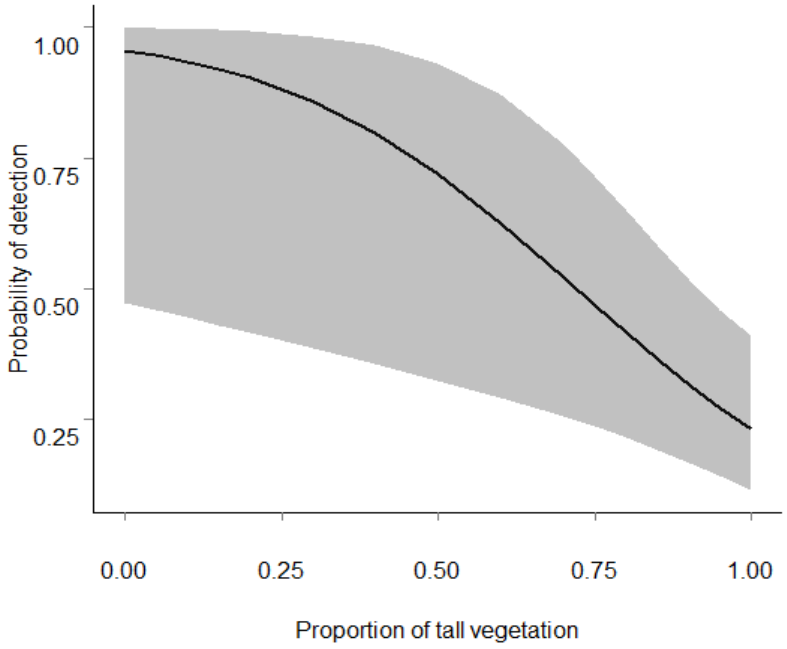

Figure 6. Modeled relationship between the probability of detection of indicated breeding pairs of mallards and the proportions of wetland edges obscured by tall vegetation. Data used in this model were collected at 11 Minnesota Prairie Conservation Plan Core Areas during 2014.

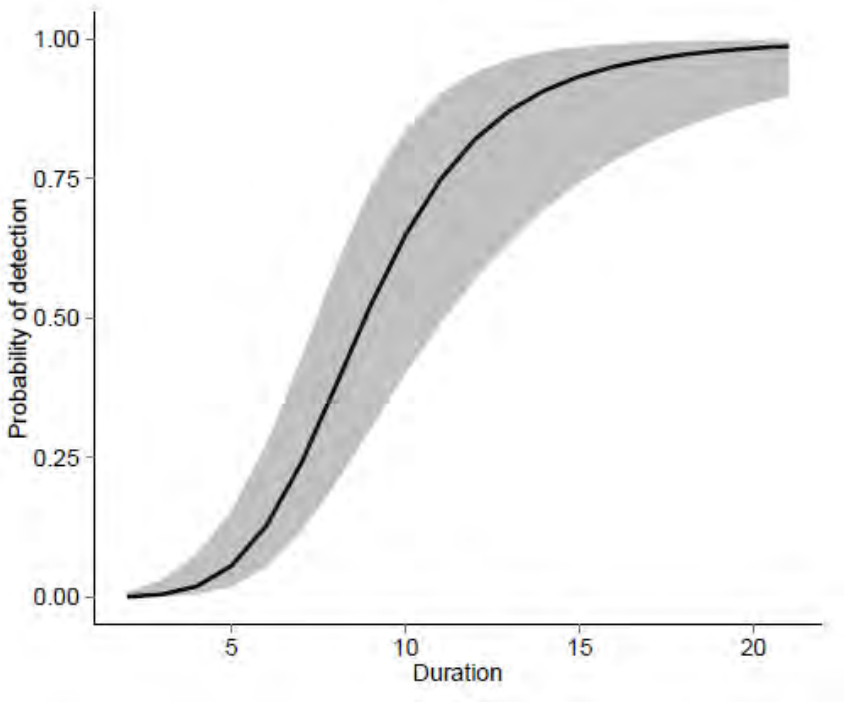

Figure 7. Modeled relationship between the probability of detection of indicated breeding pairs of blue-winged teal and survey duration (Duration [min]). Data used in this model were collected at 11 Minnesota Prairie Conservation Plan Core Areas during 2014.

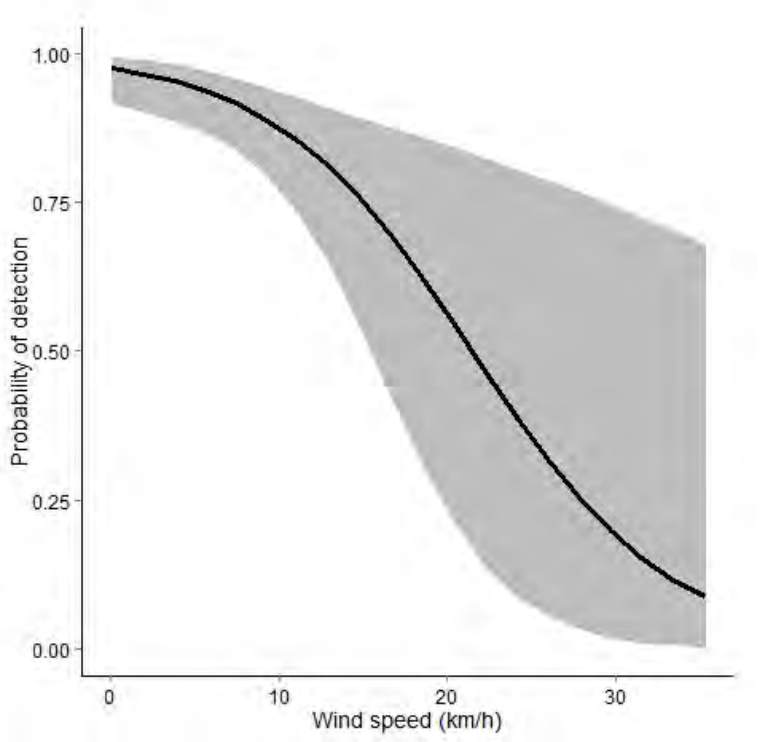

Figure 8. Modeled relationship between the probability of detection of all species of waterfowl broods and *wind speed* (km/h). Data used in this model were collected at 4 Minnesota Prairie Conservation Plan Core Areas during 2014.

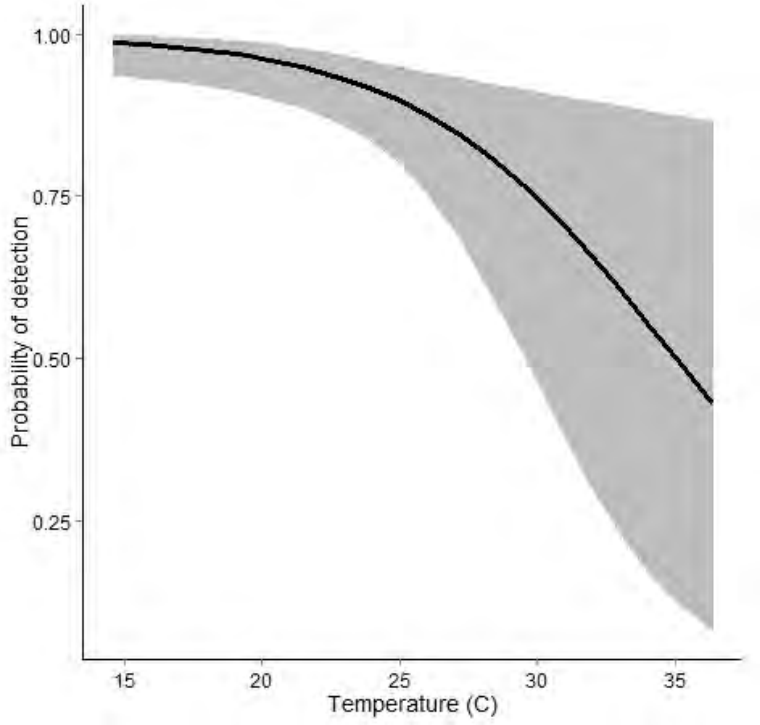

Figure 9. Modeled relationship between the detection probability of all species of waterfowl broods and *ambient temperature* (°C). Data used in this model were collected at 4 Minnesota Prairie Conservation Plan Core Areas during 2014.

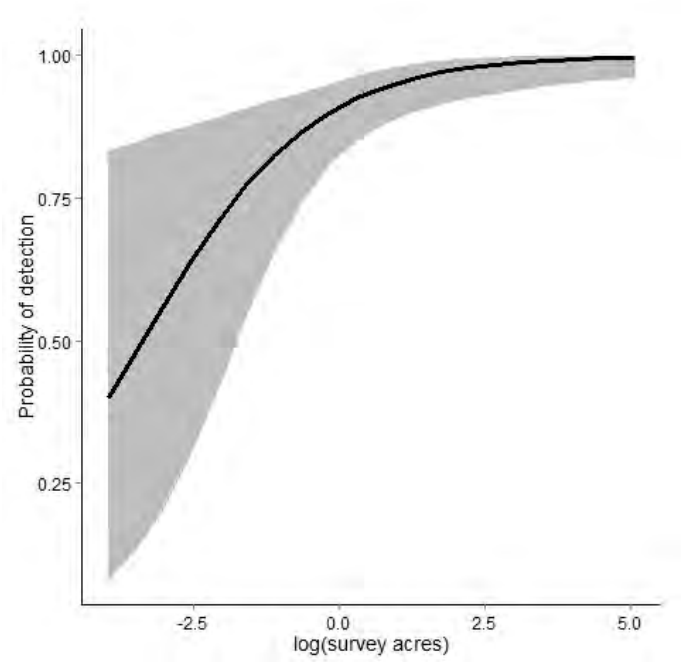

Figure 10. Modeled relationship between the probability of detection for all species of waterfowl broods and log observable area of wetlands (survey acres). Data used in this model were collected at 4 Minnesota Prairie Conservation Plan Core Areas during 2014.

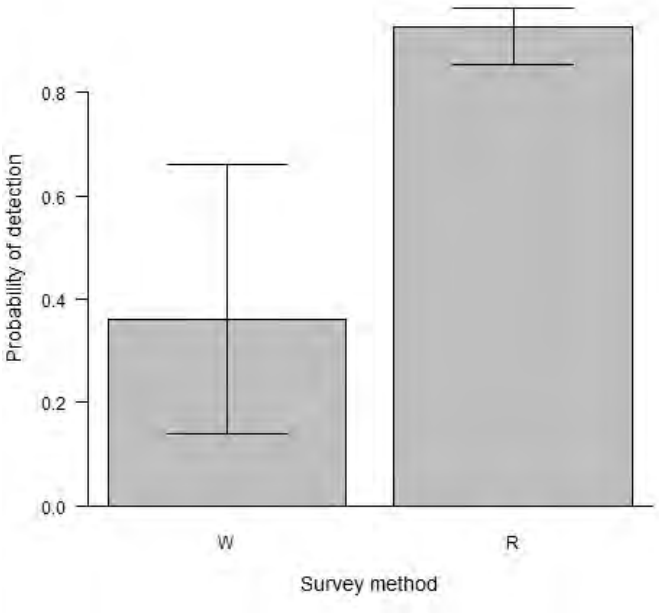

Figure 11. Modeled relationship between the probability of detection for all species of waterfowl broods and 2 survey methods: walk-in (W) and roadside (R). Data used in this model were collected at 4 Minnesota Prairie Conservation Plan Core Areas during 2014. Bars indicate 95% confidence intervals around the coefficient estimates.

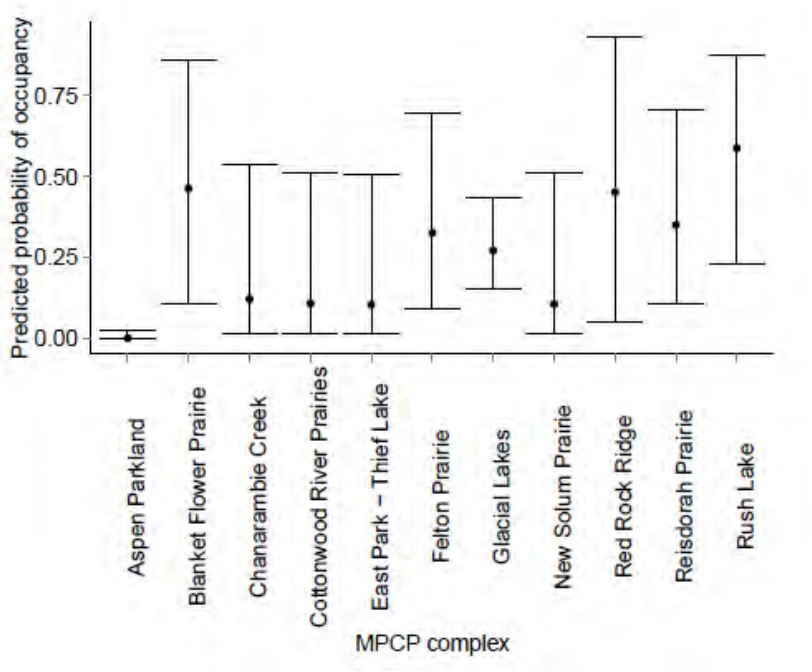

Figure 12. The estimated wetland occupancy of 11 Minnesota Prairie Conservation Plan (MPCP) Core Areas by indicated breeding pairs of mallards, 2014. Bars indicate 95% confidence intervals around the coefficient estimates.

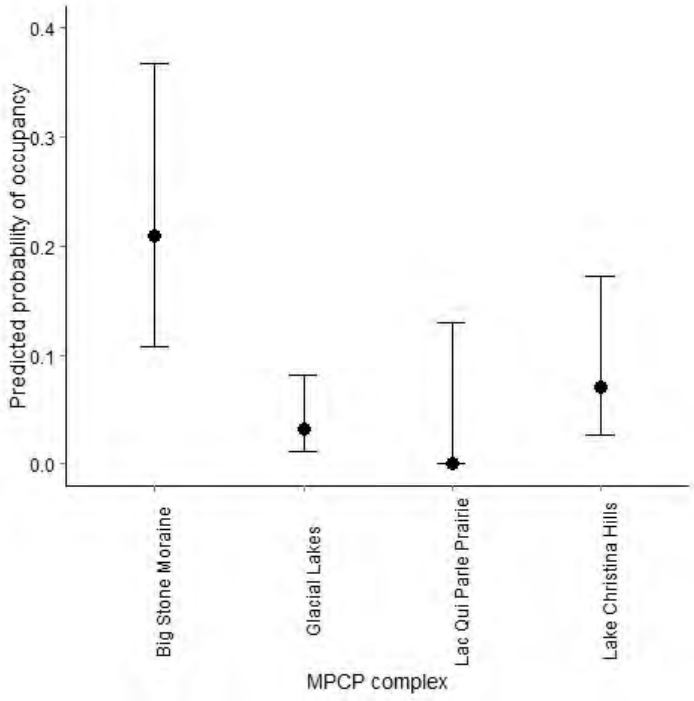

Figure 13. The estimated wetland occupancy within 4 Minnesota Prairie Conservation Plan (MPCP) Core Areas by waterfowl broods, 2014. Bars indicate 95% confidence intervals around the coefficient estimates.

Appendix 1. Results of a 2-stage analysis in which the detection (*p*) and wetland *occupancy* (Ψ) of breeding pairs of mallards observed at 11 Minnesota Prairie Conservation Plan Core Areas during 2014 were modeled. Approximating models that explain variation in the probabilities of the *p* of this cohort were developed in the first stage *a*). Models that explain variation in the Ψ of wetlands by this cohort were developed for the second stage *b*), and used the parameterization of *p* from the top-ranked model of the first stage.

Model [a]	K [b]	AIC	ΔAIC	ω [c]
a) Detection models (occupancy constant)				
$p_{\text{Log SURVDUR + TALLVEG}}$	4	215.8	0	0.58
$p_{\text{Log SURVDUR + Log OAW + TALLVEG + TEMP + Log WIND}}$	7	216.5	0.7	0.42
$p_{\text{Log SURVDUR}}$	3	227.2	11.4	0.01
$p_{\text{Log OAW + TALLVEG}}$	4	242.0	26.2	0
$p_{.}$	2	248.2	32.4	0
$p_{\text{TEMP+ Log WIND}}$	4	249.0	33.2	0
b) Occupancy models (detection varies by Log SURVDUR + TALLVEG)				
Ψ_{CoreA}	14	205.7	0	0.61
$\Psi_{\text{GRASS + DENSWET2-4 + DENSWET5}}$	7	207.4	1.7	0.26
$\Psi_{\text{GRASS + DENSWET2-4 + DENSWET5 + Log OAW + TALLVEG + WETTYPE}}$	14	210.9	5.2	0.05
$\Psi_{\text{Log OAW + TALLVEG + WETTYPE}}$	11	211.0	5.3	0.04
$\Psi_{\text{CoreA + Log OAW + TALLVEG + WETTYPE}}$	17	211.4	5.7	0.04
$\Psi_{.}$	4	215.8	10.1	0

[a] Acronyms of covariates are: SURVDUR = duration of survey, OAW = observable area of wetland, TALLVEG = proportion of wetland edges obscured by tall vegetation, TEMP = temperature, WIND = wind speed, OBS = observer, CoreA = MPCP Core Area, GRASS = proportion of Core Area comprised of grassland, DENSWET2-4 = density of Types II-IV wetlands, DENSWET5 = density of Type V wetlands, WETTYPE = wetland type.

[b] Number of parameters in the model.

[c] Model weights.

Appendix 2. Results of a 2-stage analysis in which the *detection* (*p*) and wetland *occupancy* (Ψ) of breeding pairs of blue-winged teal observed at 11 Minnesota Prairie Conservation Plan Core Areas during 2014 were modeled. Approximating models that explain variation in the probabilities of the *p* of this cohort were developed in the first stage *a*). Models that explain variation in the Ψ of wetlands by this cohort were developed for the second stage *b*), and used the parameterization of *p* from the top-ranked model of the first stage.

Model [a]	K [b]	AIC	ΔAIC	ω [c]
a). Detection models (occupancy constant)				
$p_{\text{Log SURVDUR}}$	3	160.3	0	0.98
$p_{\text{Log SURVDUR + Log OAW + TEMP + Log WIND}}$	7	168.0	7.7	0.02
$p_{.}$	2	180.7	20.4	0
$p_{\text{Log OAW + TALLVEG}}$	4	181.6	21.3	0
$p_{\text{TEMP + Log WIND}}$	4	183.0	22.7	0
b). *Occupancy* models (detection varies by log SURVDUR)				
$\Psi_{\text{GRASS + DENSWET2-4 + DENSWET5 + Log OAW + TALLVEG + WETTYPE}}$	13	150.2	0	0.61
$\Psi_{\text{Log OAW + TALLVEG + WETTYPE}}$	10	151.4	1.2	0.33
$\Psi_{\text{GRASS + DENSWET2-4 + DENSWET5}}$	6	155.0	4.8	0.05
$\Psi_{.}$	3	160.3	10.1	0.00

[a] Acronyms of covariates are: SURVDUR = duration of survey, OAW = observable area of wetland, TALLVEG = proportion of wetland edges obscured by tall vegetation, TEMP = temperature, WIND = wind speed, OBS = observer, CoreA = MPCP Core Area, GRASS = proportion of Core Area comprised of grassland, DENSWET2-4 = density of Types II-IV wetlands, DENSWET5 = density of Type V wetlands, WETTYPE = wetland type.

[b] Number of parameters in the model.

[c] Model weights.

Appendix 3. Results of a 2-stage analysis in which the *detection* (*p*) and wetland *occupancy* (Ψ) of 3 different species aggregations of waterfowl broods observed at 4 Minnesota Prairie Conservation Plan Core Areas during 2014 were modeled. Approximating models that explain variation in the probabilities of the *p* of broods of all observed waterfowl species were developed in the first stage *a*). Models that explain variation in the Ψ of wetlands by of broods of all observed waterfowl species were developed for the second stage *b*), and used the parameterization of *p* from the top-ranked model of the first stage. Models that explain variation in the probabilities of the *p* of broods of dabbling ducks were developed in the first stage *c*). Models that explain variation in the Ψ of wetlands by of broods of dabbling ducks were developed for the second stage *d*), and used the parameterization of *p* from the top-ranked model of the first stage. Models that explain variation in the probabilities of the *p* of broods of cavity-nesting ducks were developed in the first stage *e*). Models that explain variation in the Ψ of wetlands by of broods of cavity-nesting ducks were developed for the second stage *f*), and used the parameterization of *p* from the top-ranked model of the first stage. Overall *extinction* (ϵ) and *colonization* rates (γ) were estimated in all models.

Species aggregation [a]	Model [b]	K[c]	AIC	ΔAIC	ω [d]
a) All waterfowl	Detection models (occupancy, extinction, and colonization are constant)				
	p Log OAW + TEMP + WIND + METHOD + VISIT	9	579.5	0	0.95
	p SURVDUR + Log OAW + TALLVEG + TEMP + WIND + METHOD + START + VISIT	13	586.5	7.0	0.03
	p VISIT	5	588.6	9.1	0.01
	p Log OAW + TEMP + WIND + METHOD	8	590.1	10.6	0
	p OBS	10	590.2	10.7	0
	p Log OAW + TALLVEG	6	595.4	15.9	0
	p .	4	597.5	18.0	0
	p TEMP + WIND	6	600.2	20.7	0
	p SURVDUR + START	7	600.3	20.7	0
b)	Occupancy models (extinction and colonization are constant)				
	Ψ WETTYPE p Log OAW + TEMP + WIND + METHOD + VISIT	11	578.3	0	0.49
	Ψ. p Log OAW + TEMP + WIND + METHOD + VISIT	9	579.5	1.2	0.27
	Ψ Log OAW + TALLVEG + WETTYPE p Log OAW + TEMP + WIND + METHOD + VISIT	13	580.3	2.0	0.18
	Ψ GRASS + DENSWET2-4 + DENSWET5 p Log OAW + TEMP + WIND + METHOD + VISIT	12	585.3	7.0	0.02
	Ψ COREA p Log OAW + TEMP + WIND + METHOD + VISIT	12	585.3	7.0	0.02
	Ψ COREA + Log OAW + TALLVEG + WETTYPE p Log OAW + TEMP + WIND + METHOD + VISIT	16	585.6	7.3	0.01
	Ψ Log OAW + TALLVEG + WETTYPE + GRASS + DENSWET2-4 + DENSWET5 p Log OAW + TEMP + WIND + METHOD + VISIT	16	585.6	7.3	0.01
	Ψ. p.	4	597.5	19.2	0
c) Dabbling ducks	Detection models (extinction and colonization are constant)				
	Ψ. p.	4	242.5	0	0.38
	Ψ. p TEMP + WIND	6	243.7	1.2	0.20
	Ψ. p VISIT	5	244.1	1.6	0.17
	Ψ. p Log OAW + TALLVEG	6	245.0	2.5	0.11
	Ψ. p Log OAW + TEMP + WIND + METHOD + VISIT	9	246.8	4.3	0.04
	Ψ. p SURVDUR + START	7	248.2	5.6	0.02
	Ψ WETTYPE p Log OAW + TEMP + WIND + METHOD + VISIT	11	249.5	6.9	0.01
	Ψ. p SURVDUR + Log OAW + TALLVEG + TEMP + METHOD + START	13	250.3	7.8	0.01
	Ψ Log OAW + TALLVEG. p Log OAW + TEMP + WIND + METHOD + VISIT	11	250.5	8.0	0.01
	Ψ TALLVEG + WETTYPE p Log OAW + TEMP + WIND + METHOD + VISIT	12	251.4	8.9	0
	Ψ. p OBS	10	252.0	9.5	0

Appendix 3. continued

d)	Occupancy models (extinction and colonization are constant)				
	$\Psi_{\text{GRASS + DENSWET2-4 + DENSWET5}}\ p_{.}$	7	234.2	0	0.46
	$\Psi_{\text{COREA}}\ p_{.}$	7	234.2	0	0.46
	$\Psi_{\text{Log OAW + TALLVEG + WETTYPE + GRASS + DENSWET2-4 + DENSWET5}}\ p_{.}$	11	239.4	5.2	0.03
	$\Psi_{\text{COREA + Log OAW + TALLVEG + WETTYPE}}\ p_{.}$	11	239.4	5.2	0.03
	$\Psi_{.}\ p_{.}$	4	242.5	8.3	0.01
	$\Psi_{\text{JULDATE + WETTYPE}}\ p_{.}$	7	247.1	12.9	0
	$\Psi_{\text{Log OAW + TALLVEG + WETTYPE}}\ p_{.}$	8	249.1	14.9	0
e) Cavity-nesting ducks	Detection models (extinction and colonization are constant)				
	$\Psi_{.}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	9	447.2	0	0.41
	$\Psi_{.}\ p_{\text{TEMP + WIND}}$	6	448.3	1.1	0.24
	$\Psi_{.}\ p_{\text{SURVDUR + Log OAW + TALLVEG + TEMP + WIND + METHOD + START + VISIT}}$	13	449.7	2.5	0.12
	$\Psi_{.}\ p_{.}$	4	450.1	2.9	0.10
	$\Psi_{.}\ p_{\text{Log OAW + TALLVEG}}$	6	450.6	3.4	0.07
	$\Psi_{.}\ p_{\text{VISIT}}$	5	451.3	4.1	0.05
	$\Psi_{.}\ p_{\text{SURVDUR + START}}$	7	455.1	7.8	0.01
	$\Psi_{.}\ p_{\text{OBS}}$	10	455.5	8.2	0.01
f)	Occupancy models (extinction and colonization are constant)				
	$\Psi_{\text{GRASS + DENSWET2-4 + DENSWET5 + Log OAW + TALLVEG}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	14	445.5	0	0.21
	$\Psi_{\text{GRASS + DENSWET2-4 + DENSWET5}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	12	446.1	0.5	0.16
	$\Psi_{\text{COREA}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	12	446.1	0.5	0.16
	$\Psi_{\text{Log OAW + TALLVEG}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	11	446.1	0.5	0.16
	$\Psi_{\text{Log OAW + TALLVEG + WETTYPE + GRASS + DENSWET2-4 + DENSWET5}} p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	16	448.2	2.6	0.06
	$\Psi_{\text{COREA + Log OAW + TALLVEG + WETTYPE}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	16	448.2	2.6	0.06
	$\Psi_{\text{TALLVEG + WETTYPE}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	12	448.3	2.8	0.05
	$\Psi_{\text{WETTYPE}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	11	448.4	2.8	0.05
	$\Psi_{\text{Log OAW + TALLVEG + WETTYPE}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	13	448.6	3.1	0.05
	$\Psi_{.}\ p_{.}$	4	450.1	4.6	0.02
	$\Psi_{\text{JULDATE + WETTYPE}}\ p_{\text{Log OAW + TEMP + WIND + METHOD + VISIT}}$	12	450.2	4.6	0.02

[a] Species aggregations are as follows: all waterfowl = mallard, blue-winged teal, American green-winged teal, northern shoveler, gadwall, wood duck, hooded merganser, ring-necked duck, redhead, canvasback, lesser scaup, unidentified scaup, large Canada goose, and trumpeter swan; dabbling ducks = mallard, blue-winged teal, American green-winged teal, northern shoveler, and gadwall; cavity nesters = wood duck and hooded merganser.

[b] Acronyms of covariates are: SURVDUR = duration of survey, OAW = observable area of wetland, TALLVEG = proportion of wetland edges obscured by tall vegetation, TEMP = temperature, WIND = wind speed, OBS = observer, CoreA = MPCP Core Area, JULDATE = Julian date of survey, GRASS = proportion of Core Area comprised of grassland, DENSWET2-4 = density of Types II-IV wetlands, DENSWET5 = density of Type V wetlands, WETTYPE = wetland type.

[c] Number of parameters in the candidate model.

[d] Model weights.

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