

Summaries of Wildlife Research Findings **2017**



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

SUMMARIES OF WILDLIFE RESEARCH FINDINGS 2017

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CHRONIC WASTING DISEASE SURVEILLANCE IN MINNESOTA'S WILD DEER HERD

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SUMMARY OF FINDINGS

Fall 2017 marked the first time mandatory testing for chronic wasting disease (CWD) was utilized as a precautionary sampling method for hunter-harvested white-tailed deer (*Odocoileus virginianus*) in high risk areas of Minnesota. This testing occurred during opening weekend of the firearms deer season, November 4-5, 2017, in 21 deer permit areas (DPAs). Testing in north-central Minnesota was centered on a recently discovered CWD-positive deer farm in Crow Wing County. A total of 7,945 deer were tested and no CWD was detected. Similarly, testing in central Minnesota was centered on a second CWD-positive deer farm in Meeker County. A total of 2,623 deer were tested and no CWD was detected. In southeast Minnesota, 1,341 deer were tested in DPAs surrounding the CWD Management Zone (DPA 603), and 1,185 deer from within DPA 603. Six new cases of CWD were confirmed within DPA 603, bringing the total number of infected deer to 17 from fall 2016 to present. Bans on recreational feeding and attractants were enacted in 16 counties to curtail transmission and disease spread. In late 2017, a third positive deer farm was detected in Winona County; upon depopulation of this herd, there was a 100% infection rate. Surveillance conducted by Minnesota Department of Natural Resources (MNDNR) from hunter-harvested deer will continue in these areas, and more, in fall 2018.

INTRODUCTION

Chronic wasting disease is a transmissible spongiform encephalopathy (TSE) that affects elk (*Cervus canadensis*), mule deer (*Odocoileus hemionus*), white-tailed deer, caribou/reindeer (*Rangifer tarandus*), and moose (*Alces alces*). TSEs are infectious diseases caused by malformed prion proteins, which accumulate in the central nervous system, resulting in cell death and a microscopic "sponge-like" appearance of affected tissues. Incubation time of the disease can range from 1.5 to nearly 3 years, although infected animals have been found to shed prions in their feces up to a year before showing signs of illness (Tamguney et al. 2009, Haley et al. 2011). While infectious prions are primarily found in nervous system and lymphatic tissues, they have also been detected in blood, urine, muscle, and antler velvet. Clinical signs are non-specific and may include a loss of body condition and weight, excessive salivation, ataxia, and behavioral changes. There is currently no known treatment or vaccine for the disease, despite considerable efforts in research and development, and it is always fatal. When clinical signs of infection begin, death will occur within months. Experimental and circumstantial evidence suggest that transmission of the disease is primarily through direct contact with infected animals or their contagious 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). Evidence from studies of scrapie in sheep and other experimental systems suggest that infectious prions in the environment can remain viable as infectious agents for years (Georgsson et al. 2006, Saunders et al. 2008). Practical decontamination of suspected

environmental reservoirs of infectious prions is currently not possible, as the agent is extremely durable to degradation.

The Centers 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, new research conducted by the Canadian Food Inspection Agency has demonstrated that by orally administering muscle under experimental conditions from cervids (deer and elk) naturally infected with CWD, the disease can be transmitted to macaques. This finding has sparked renewed concerns about potential human health risks to eating CWD-contaminated venison (Czub, S. 2017, May). In separate work, also focused on susceptibility of CWD transmitted to macaques, the research group found no evidence of successful transmission (Race et al. 2018). These results effectively muddy the waters, and make it unclear exactly what the CWD transmission risk is to primates. Despite this uncertainty, researchers still advise caution and suggest people do not consume game animals confirmed with CWD.

Recently, in late November 2017, the Minnesota Board of Animal Health (BAH) announced CWD was found on a deer farm in Winona County in southeast MN. One adult male white-tailed deer was found dead on the farm and through routine surveillance was confirmed to have CWD. An additional male deer harvested on the farm also tested positive for the disease on December 8, 2017. The remaining 7 animals on this farm were euthanized, all of them tested positive for CWD (100% infection rate). One additional MN deer farm was identified to have received 3 animals from this Winona County positive farm within the past 5 years and was considered a trace-out. The owner of the trace-out farm agreed to depopulate these 3 animals; all were not detected for the disease.

Currently, Minnesota has approximately 398 cervid farms (Minnesota Board of Animal Health, 2018). The current statewide population estimate of wild deer is approaching one million. There is an element of inherent disease transmission risk between farmed and wild cervids. Overall, risk is difficult to quantify because wild deer populations are unevenly distributed across the landscape and range in densities from < 1 to 15 deer/km^2 (i.e., 1 to 40 deer/mi^2), fences on these facilities vary in construction quality, and the direct/indirect contact rates between farmed and wild cervids are unknown. In addition, deer farms are sporadically distributed on the landscape and are independent of wild deer densities. In Minnesota, farmed cervids are classified as livestock and managed by the Minnesota Board of Animal Health. MNDNR has no authority to oversee or regulate the farms or the animals. An overview of farmed cervid management in Minnesota can be found at <https://www.auditor.leg.state.mn.us/ped/2018/deerfarmssum.htm>

Since 2002, MNDNR has conducted CWD surveillance and sampled $>64,000$ wild deer across the state. The first occurrence of this disease in a wild deer was in 2010, when one adult female was found with CWD near an infected farmed elk facility in Pine Island. Aggressive surveillance efforts from 2011-2013 tested an additional $>4,000$ deer in the surrounding area, and failed to detect another case of CWD in the wild. More recently, in fall 2016, CWD was first detected in Fillmore county wild deer, which initiated the CWD response plan. MNDNR has been closely monitoring disease spread in the neighboring states and conducting additional surveillance efforts near those borders to ensure early detection of the disease. In April 2018, a sick deer was found positive for CWD in Eau Claire County Wisconsin, which will lead to an increase in surveillance efforts on Minnesota's side of the border in fall 2018.

METHODS

To prevent further disease transmission, MNDNR banned recreational deer feeding and use of attractants for deer in a 5-county area in southeastern Minnesota and deer feeding in 11 other counties in central and north-central Minnesota. (Figure 1). MNDNR also implemented carcass

import restrictions in 2016 that prohibit whole carcasses of deer, elk, moose, or caribou from entering the state.

Mandatory hunter-harvested surveillance was conducted at deer sampling stations during the first two days of the 2017 regular firearms deer season, in 21 DPAs in Minnesota (Figure 2). A total of 46 sampling stations were staffed with MNDNR personnel and veterinary or natural resources students to collect hunter information and remove medial retropharyngeal lymph nodes from harvested deer. These stations were distributed across 3 surveillance areas: north-central (DPAs 155, 171, 172, 242, 246, 247, 248, and 249), central (DPAs 218, 219, 229, 277, 283, and 285), and the southeast (343, 345, 346, 347, 348, and 349), as well as the CWD management zone, DPA 603. Stations were selected based on deer volume and distribution throughout each surveillance area to meet sampling goals: 3,600 samples for the north-central, 1,800 samples for the central, and 1,800 samples for the southeast. Hunters were required to present all adult deer (>1 year old) to be tested for CWD, if it was harvested on November 4-5, 2017 from within our surveillance DPAs.

All deer harvested within DPA 603, including archery, firearm and muzzleloader hunting seasons, were subject to additional restrictions: all adult deer (>1 year old) were required to be submitted for testing and special tagging, collection of muscle tissue for genetic analysis, and provide a tooth to determine exact age of adults (> 2 years old). Whole carcasses were prohibited from leaving DPA 603 until a test result of “not detected” was confirmed; test turnaround times were 3-4 business days. However, the following parts were permitted to leave the area without restriction: entire quarters, deboned meat, antlers or skull plate with antlers attached that has all brain material removed, as well as hides and teeth. The head, spinal column, and remaining carcass pieces were to be kept within DPA 603 until test results were known in order to reduce further risk of transmission across the state. All fawns harvested during the firearms season were verified of age class at a sampling station by MNDNR staff and properly tagged; they were not tested and carcasses could leave DPA 603 immediately. A MNDNR-leased refrigerated semi-trailer, kept at 33-38°F, was provided for hunters to use, along with a dumpster to dispose of butchering remains; both items provided hunters viable options after harvesting deer to comply with carcass export restrictions. Hunters checked their results on the MNDNR website using either their MNDNR number from their hunting license or the special carcass tag number assigned to their deer. During non-firearms seasons, when MNDNR staff were not present at sampling stations, head collection boxes were provided at 5 locations throughout DPA 603 with supplies for the hunter to leave information about where the deer was harvested, as well as the head for testing.

All samples were inventoried, entered into a digital database, and sent to Colorado State University (CSU) for enzyme-linked immunosorbent assay (ELISA) testing. Any presumptive suspect-positive deer from ELISA testing was confirmed using immunohistochemistry (IHC) testing at CSU. In addition to taking biological samples, hunter information was recorded at the time of sampling and included: hunter's name, telephone number, MNDNR number, and location of harvest down to Township, Range, and Section. Maps were provided to assist the hunters in identifying the location of the harvest site. Hunters were given an embroidered cooperator's patch, and notified that they would not be contacted with test results unless their deer tested positive.

In addition, 55 Minnesota taxidermists were trained and supplied with collection materials to target trophy bucks harvested from within our surveillance areas throughout all deer hunting seasons; however only 42 in-network, trained taxidermists collected any samples. We offered compensation to these taxidermists of \$5/head or \$10/lymph nodes for deer harvested within our DPAs of interest. This compensation offer was extended for additional samples from adult deer harvested in select DPAs beyond the mandatory opening weekend collection, if the hunter gave explicit permission. Any hunter that showed up to a sampling station with a trophy deer,

and didn't want the cape ruined from the sampling process, had options to fill out additional paperwork to follow thru with the mandatory sampling procedure. If the hunter chose to use an in-network taxidermist, then samples were collected by that taxidermist. However, if the hunter planned to use a different taxidermist, then the hunter was responsible for bringing the caped head to the nearest area wildlife office to fulfill the sampling requirements.

Due to the discovery of additional positive deer within DPA 603 during the fall, additional efforts were made to collect supplemental samples from DPA 603 and the surrounding area during winter 2018 (Jan-March). First, a special late season hunt was established, January 6, 2018 – January 14, 2018 that included DPA 603 and an expanded hunt boundary (Figure 3). This hunt allowed for the use of any unfilled 2017 deer license or the purchase of a special disease management license (available for a reduce cost of \$2.50) and an unlimited bag of either-sex deer. The same carcass movement restrictions and testing requirement from the fall hunt were in place during this special late hunt.

Following the late hunt, shooting permits were offered to landowners within 2 miles of a known positive deer during February 10, 2018 to March 10, 2018. Landowners inside this area were not required to own a minimum amount of acreage to qualify for a shooting permit, but did have to abide by city and state ordinances for discharging firearms. There were no limits to the number or sex of deer that could be harvested from the owner's property and they could designate as many shooters under their permit as desired. The use of high powered center fire rifles under this permit was allowed. The shooter was required to contact MNDNR staff within 24 hours of harvesting by calling a MNDNR CWD hotline and then provide the head of the deer at a collection box located at the Preston DNR Forestry Office within 48 hours. Each carcass was assigned a unique identification tag, and landowners were directed not to transport carcasses outside the surveillance area until a test result of "not detected" was confirmed. The same carcass export restrictions applied to these deer.

Opportunistic deer that were reported by the public, such as vehicle-kills, sick, and found dead deer within DPA 603 were also collected and sampled for CWD

On a statewide basis, MNDNR routinely samples any cervid exhibiting clinical symptoms of CWD infection. We have disseminated information to wildlife staff regarding clinical signs of infection for symptomatic deer. These staff are also provided with the necessary equipment and training for lymph node removal and data recording. The number of samples expected through these efforts is estimated to be less than 100 animals annually, since few reports of deer with clinical signs are received.

RESULTS AND DISCUSSION

During fall 2017, mandatory statewide surveillance efforts sampled a total of 13,094 wild deer, including 481 collected through participating taxidermists. In the north-central zone, 7,945 (2.2x goal) samples were collected with a 92% compliance rate based on registration, and had no detection of CWD. In the central zone, 2,623 (1.5x goal) samples were collected with a 90% compliance rate and no detection of CWD (Figure 4). In the southeast, 1,341 samples were collected, which was 40% short of surveillance goal and had a 68% compliance rate (Figure 5). A total of 1,185 adult deer were sampled from within DPA 603, with a 104% compliance rate, which included the discovery of 6 additional positive deer. Of the 6 deer that tested positive for CWD, all were adult males and 4 were located in the initial core infection area near Preston, MN and 2 were located about 6 miles west, within Forestville/Mystery Cave State Park. Detecting the disease this far to the west in DPA 603 could mean detecting the disease was missed during surveillance efforts of fall 2016/winter 2017, or due to deer dispersal from the core area.

During the special late hunt, a total of 275 deer were sampled for CWD; all were not detected for the disease. A total of 67 permits were issued to landowners during the landowner shooting

permit phase and 19 deer were sampled for CWD (Figure 6). Only 11 (16%) of the landowners that received shooting permits removed at least 1 deer. Through this combined winter surveillance effort, a total of 294 deer were sampled in the CWD surveillance area with no additional positives.

The MNDNR-leased refrigerated trailer was utilized for the duration of both the fall and winter surveillance efforts for deer harvested inside DPA 603. A total of 328 deer were held; 285 and 43 deer during the regular firearms season and special late hunt respectively.

From July 2017 to June 2018, MNDNR collected a total of 41 samples through opportunistic surveillance efforts. This included samples from 6 escaped farmed deer, 5 deer found dead with no apparent injuries, 1 possible escaped farmed mule deer, 1 wild deer that jumped into a farmed deer enclosure, 1 vehicle-killed deer, and 27 free-ranging deer with clinical signs; all tests were confirmed as not detected for CWD.

Surveillance Costs

One of the driving reasons MNDNR wanted to switch from a voluntary to a mandatory CWD sampling framework was to reduce project costs and improve efficiency. Importantly, the comparison of associated costs between the two surveillance formats; however, since this was the first year MNDNR attempted mandatory sampling and it encompassed 3 separate areas of the state, it was not a straight-forward comparison to voluntary sampling in southeast MN in fall 2016.

In fall 2016, CWD surveillance occurred at 30 big game registration stations throughout Zone 3 in southeast MN with a sampling goal of 2,700 deer. This effort involved 65 DNR staff working as station leads and 133 students. Work occurred over all 4 weekends of the 3A and 3B seasons and select stations (n=7) were staffed the entire first week. A total of 2,966 samples were collected and surveillance costs totaled \$364,115. This would equate to \$122.76/sample collected and tested for CWD.

In fall 2017, CWD surveillance occurred at 46 sampling sites, not associated with big game registration stations, in 3 separate areas of the state with a combined sampling goal of 7,200 deer. The effort involved 129 DNR staff working alongside 198 students over just the 2-day opening weekend of firearm season. A total of 11,909 samples were collected and surveillance costs totaled \$886,977. This would equate to \$74.48/sample collected and tested for CWD.

Total expenditures for the CWD sampling effort in 2017 (including the fall hunt, special January hunt, and landowner shooting permit phase) was \$996,990.

After-Action Review

Following the fall hunter-harvest surveillance efforts, an after-action review (AAR) process was initiated. Approximately 1-2 weeks after the mandatory sampling effort, an AAR survey was sent to DNR staff who participated in sampling. The function of this survey was to obtain feedback on a multitude of items including: the adequacy of CWD sampling equipment and supplies, staff preparedness, sampling station logistics, staffing requirements, data entry/proofing, and overall sample organization.

Staff were encouraged to provide other comments relevant to improving the performance and efficiency of the CWD sampling process. Feedback was received from 52 DNR staff participants or about 40% of the DNR staff participants. A few major outcomes thru this process showed needed improvement towards the total number of deer each sampling team could handle in a day; from 60/deer a team per day down to 45/deer a team per day, and more communication and outreach efforts to the public. More energy towards messaging efforts to inform hunters that the CWD test is not a food safety test, and that processing deer as they normally would should not wait until final test results are received. This would alleviate some of the wanton

waste issues that enforcement staff saw for deer harvested outside of the CWD management zone.

Future Surveillance Plans

Mandatory sampling of adult deer (≥ 1 years of age) and restricted whole-carcass movements inside DPA 603 will remain in effect for fall 2018 hunting seasons. Outside of DPA 603, mandatory sampling will occur again in north-central and central areas; however, the surveillance areas have been reduced to focus on the 15mi² area around the CWD-infected deer farms (Figure 7). In southeast Minnesota, surveillance is expanding due to the recent detection of a CWD-positive deer farm in Winona County, a wild deer found positive in Eau Claire County, Wisconsin, and the continued discovery of infected deer within DPA 603. In total, MNDNR plans to sample >4,150 hunter-harvested deer for CWD during the first 2 days of both the A and B firearms seasons through a mandatory sampling framework. Targeted CWD surveillance of deer exhibiting clinical signs of illness will continue statewide.

ACKNOWLEDGMENTS

We would like to thank all the MNDNR Wildlife and Enforcement staff, who assisted with this large surveillance project. Thanks go out to fisheries staff at Lanesboro and forestry staff at Preston for allowing us use of their office sites and facilities. We also wish to thank the students and faculty from the Universities within Minnesota that reached out to support our surveillance efforts. Special thanks to Julie Hines, Chris Scharenbroich, Bruce Anderson, Steve Benson and Bob Wright for tackling our GIS mapping and tablet needs. Our communication efforts would not have been possible without help from Harland Hiemstra, David Schueller, Kristi Coughlon, Dan Ruiter, and Pete Takash for their efforts in communications and internet-related work. We would also like to thank Andrew Herberg for his help during our surveillance efforts. We very much appreciate the support of the USDA-Wildlife Services disease biologist, Tim White.

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Counties with Deer Feeding and Attractants Prohibited

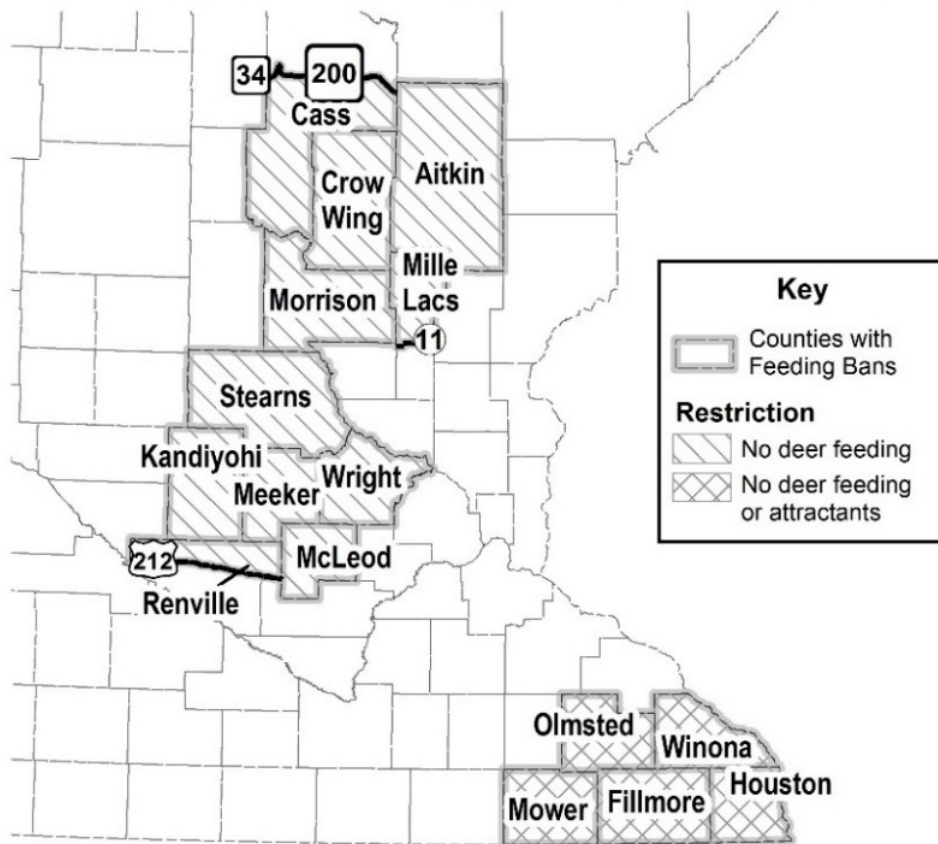


Figure 1. Minnesota counties under a ban on recreational deer feeding or deer feeding and use of attractants, 2017.

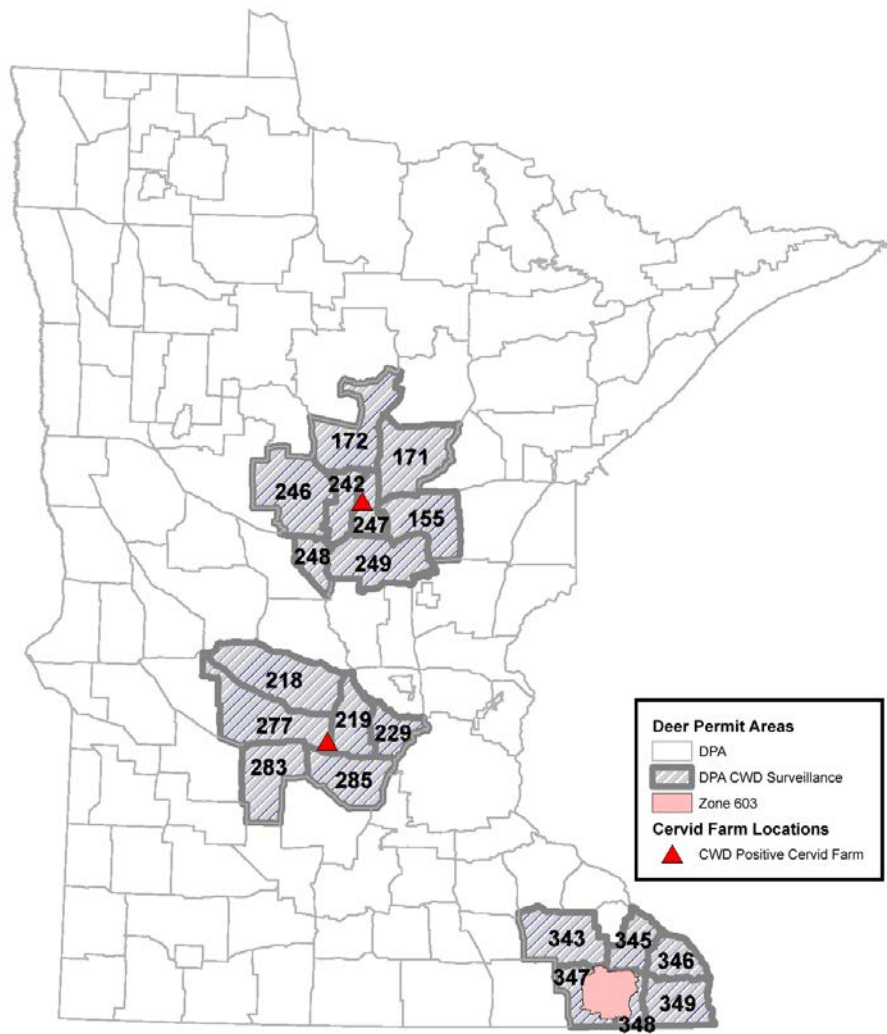


Figure 2. Chronic wasting disease (CWD) surveillance efforts throughout Minnesota, fall 2017.

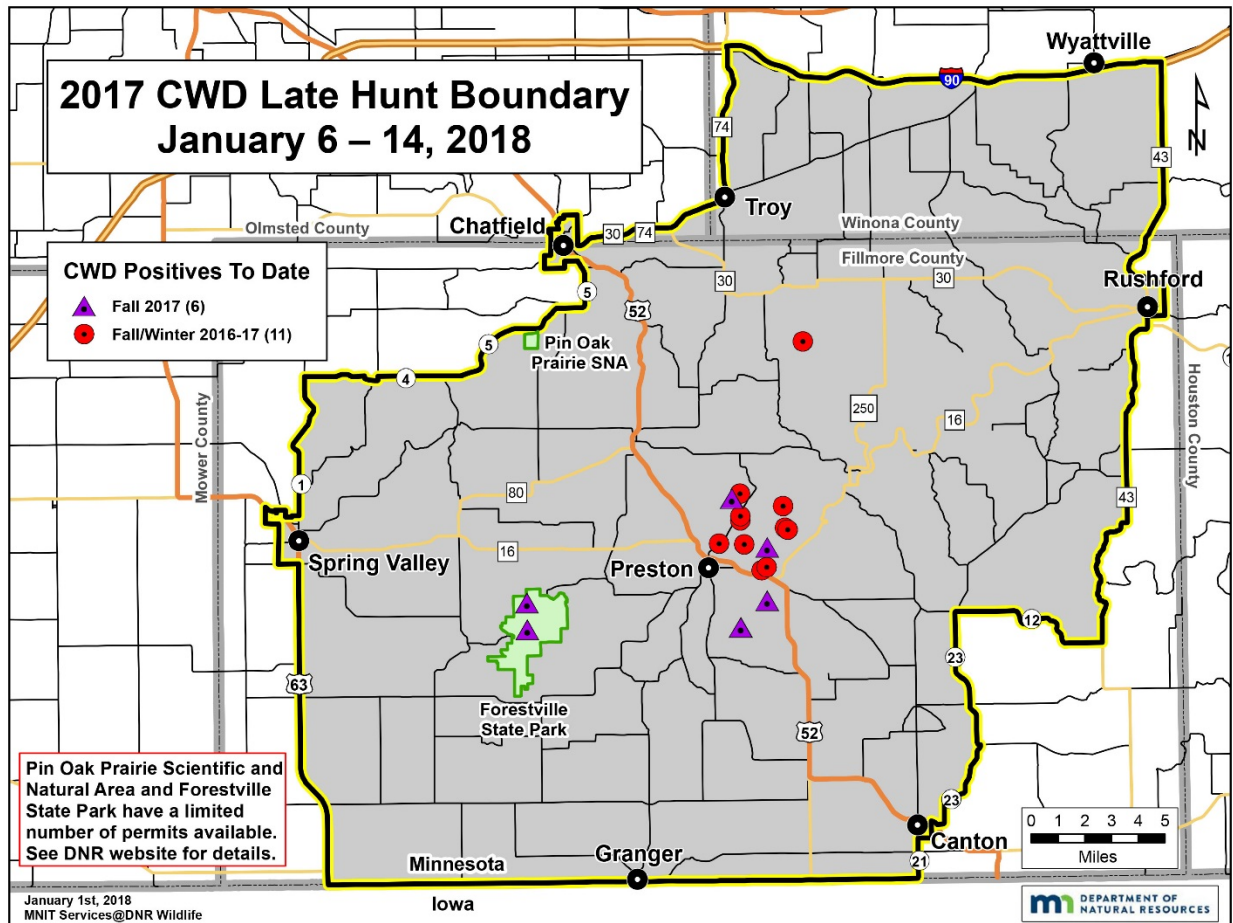


Figure 3. Chronic Wasting Disease (CWD) special late hunt boundary in southeast Minnesota, January 6 – 14, 2018.

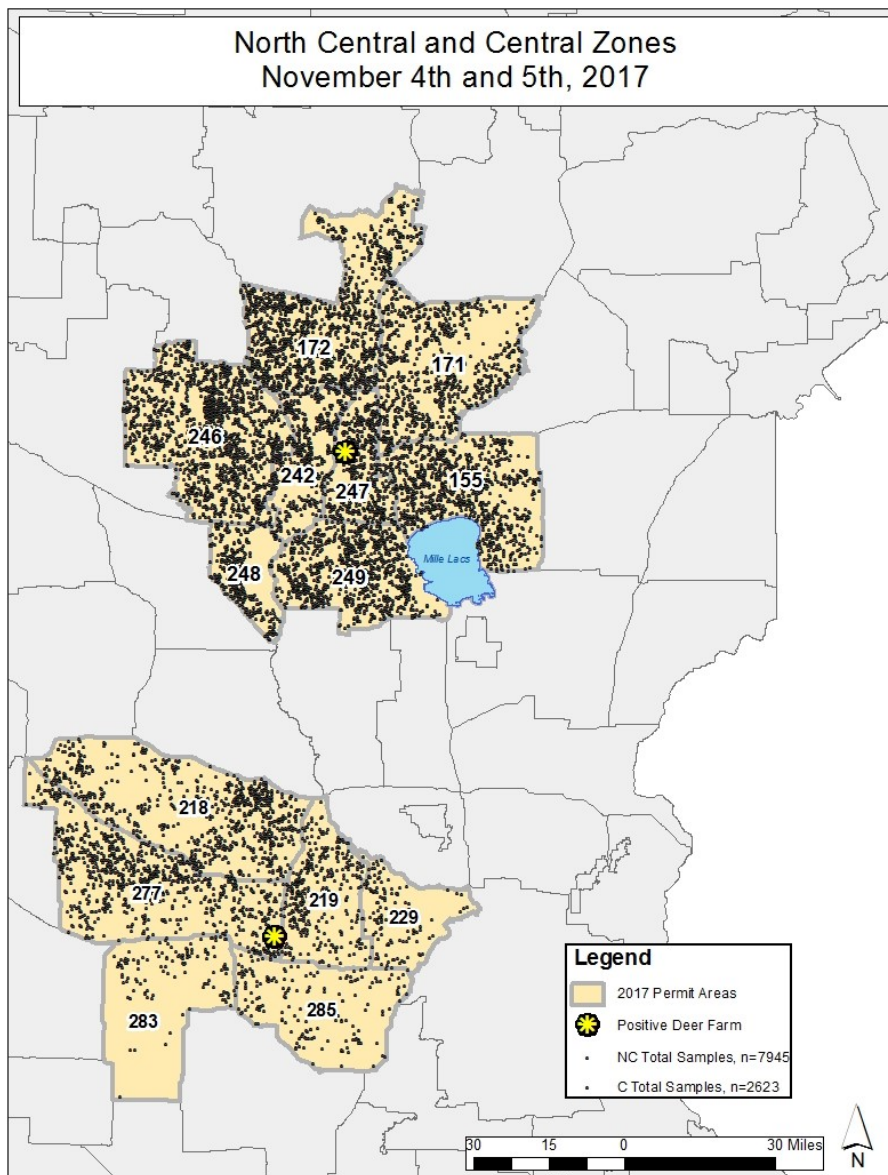


Figure 4. Chronic wasting disease (CWD) samples collected in north-central (n=7,945) and central (n=2,623) surveillance deer permit areas (DPAs) of Minnesota, fall 2017.

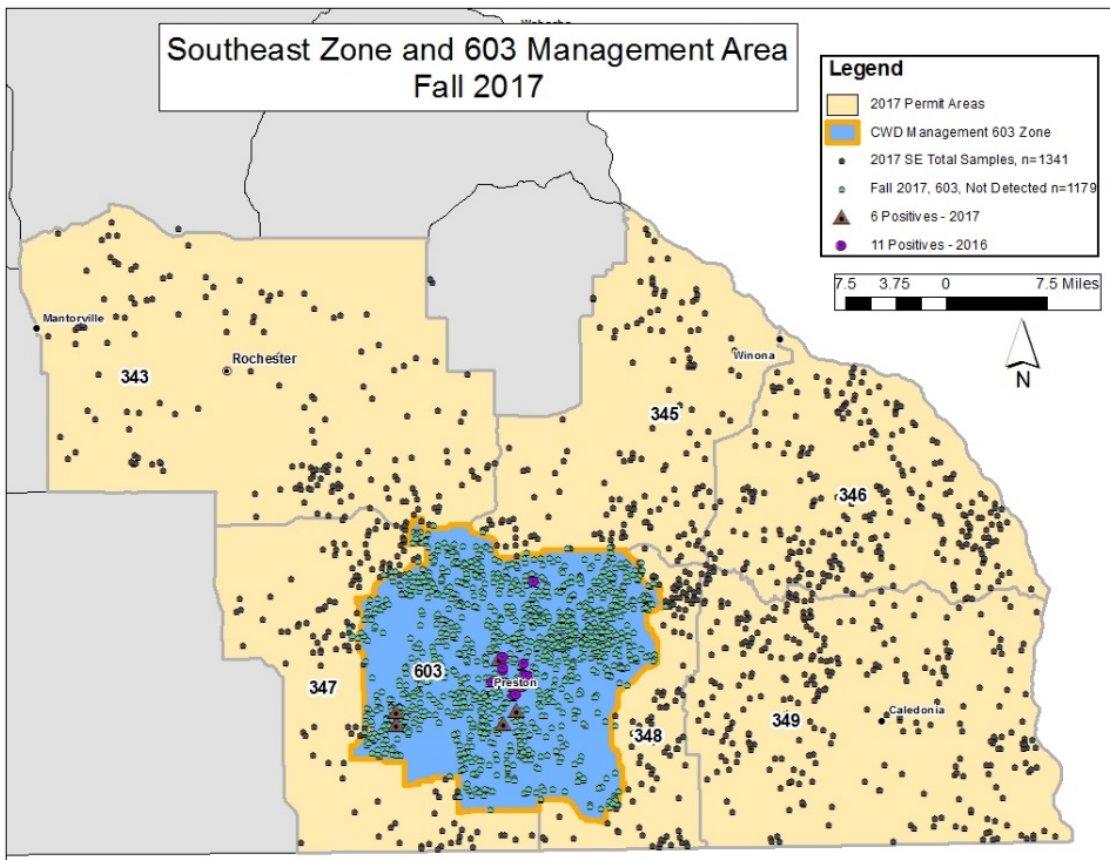


Figure 5. Chronic wasting disease (CWD) samples collected from wild deer in southeast deer permit areas (DPAs) (n=1,341) and DPA 603 (n=1,185) in Minnesota, fall 2017.

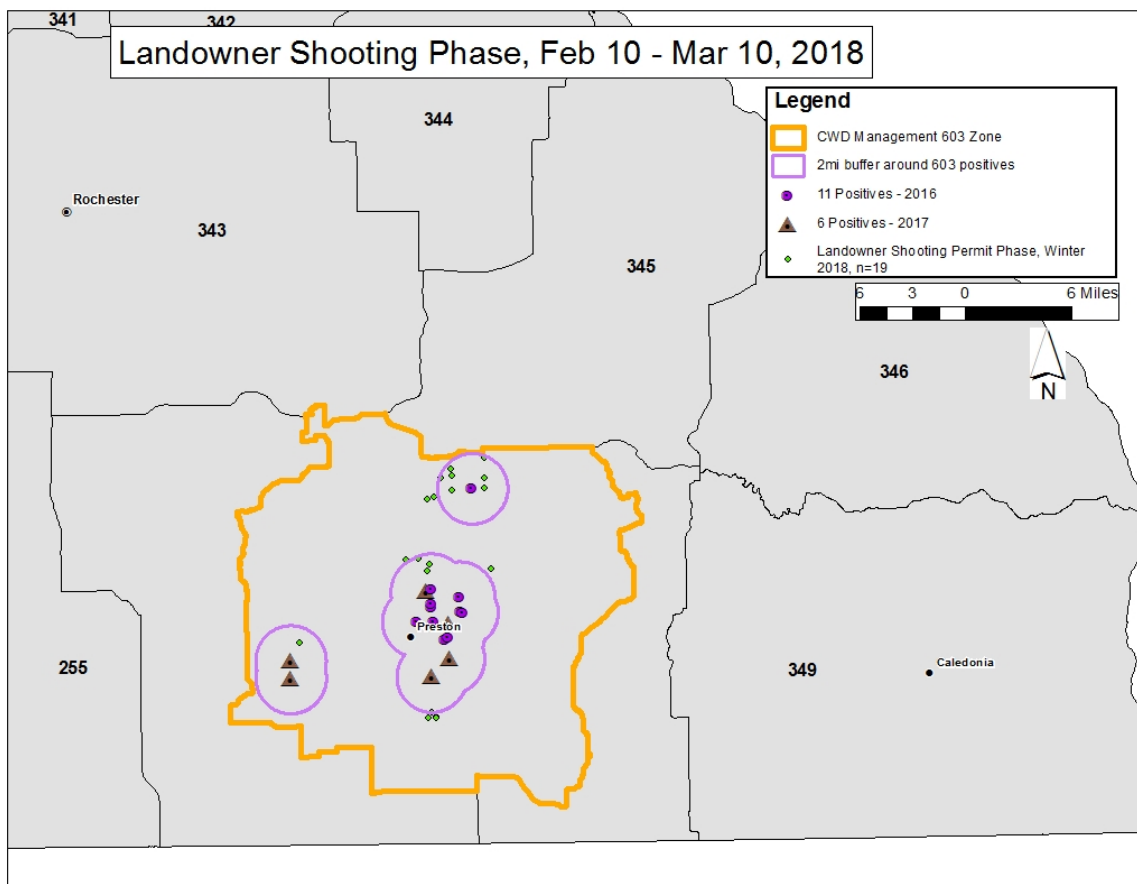


Figure 6. Chronic wasting disease (CWD) sampling efforts during the landowner shooting permit phase (n=19) in deer permit area (DPA) 603 in Minnesota, winter 2018.

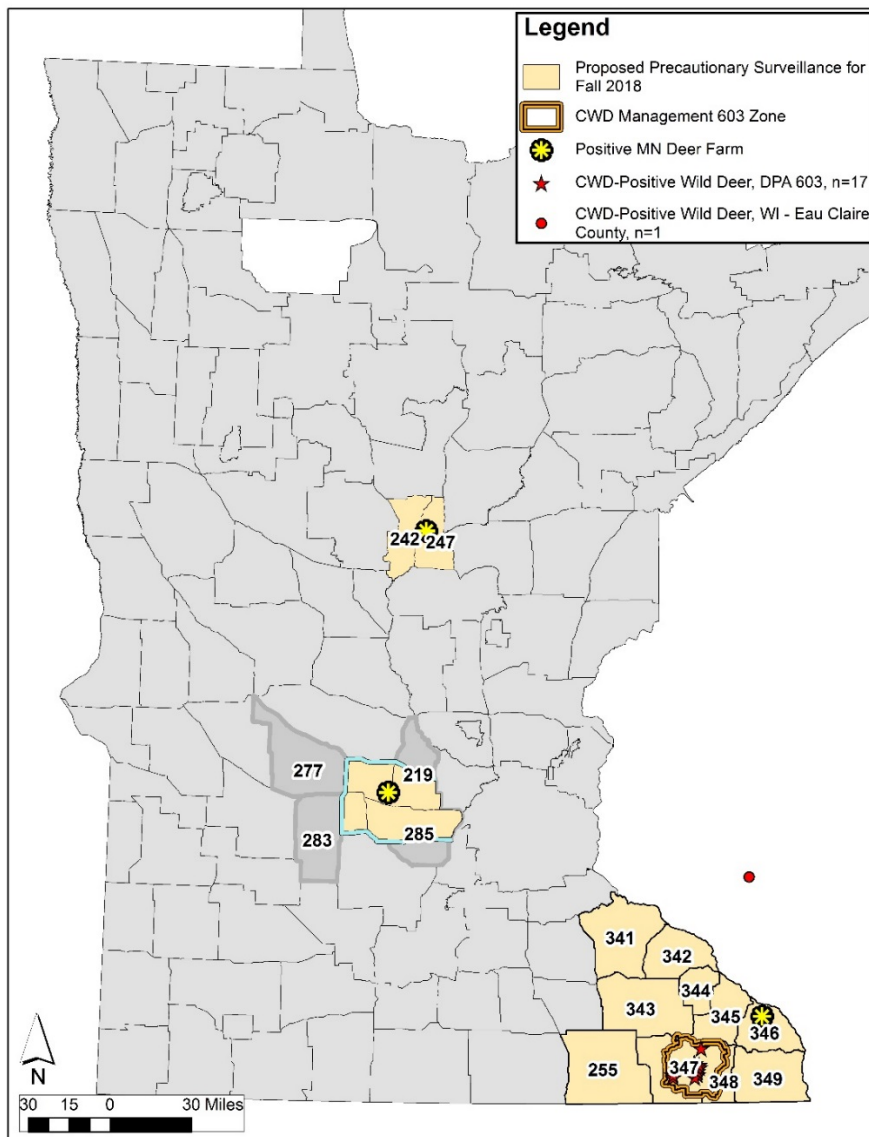


Figure 7. Chronic wasting disease (CWD) surveillance efforts in deer permit areas (DPAs) in Minnesota, fall 2018.



INVESTIGATION OF MOVEMENT DYNAMICS OF WILD DEER IN SOUTHEASTERN MINNESOTA TO UNDERSTAND POTENTIAL SPREAD OF CHRONIC WASTING DISEASE

Chris Jennelle, Andrew Norton, Ryan Tebo, Michelle Carstensen, Erik Hildebrand, Lou Cornicelli, Kelsie LaSharr, Tyler Obermoller, Patrick Hagen, and Margaret Dexter

SUMMARY OF FINDINGS

In November 2016, the Minnesota Department of Natural Resources (MNDNR) discovered chronic wasting disease (CWD) in wild white-tailed deer (*Odocoileus virginianus*) in southeastern Minnesota. To date, 17 positive cases have been detected in 2 spatial clusters approximately 5 miles apart. The apparently early detection of CWD in wild deer motivated research to 1) understand potential pathways of CWD spread on the landscape by movement of deer, and 2) increase our likelihood of managing the outbreak in this and other areas of Minnesota. We received \$449,557 through the Environment and Natural Resource Trust Fund (ENRTF)-Emerging Issues account (M.L. 2015, Chp. 76, Sec. 2, Subd. 10) - to initiate this investigation. In March 2018, we captured and collared 109 white-tailed deer (34 juvenile females, one adult female, 25 adult males, and 49 juvenile males) in southeastern Minnesota to initiate the study and better understand activity ranges and dispersal patterns of juvenile deer in and around our CWD Management Zone, called Deer Permit Area (DPA) 603. As of Aug 5 2018, 12 deer have slipped free of their collars and 10 have died, leaving 87 deer available for satellite tracking, which includes 30 juvenile females, one adult female, 19 adult males, and 37 juvenile males. Prior to the dispersal period between April and July 2018, we estimated the average winter home range size as 1.60 km² for juvenile females, 2.96 km² for adult males, and 2.28 km² for juvenile males. Preliminary assessment of dispersal suggests that dispersal probability of juvenile females (40%, n=20) was slightly greater than juvenile males (28%, n=28) in spring 2018, although this difference was not statistically significant ($p = 0.41$). The average apparent dispersal distance travelled was 30.4 km (n=8) and 14.6 km (n=8) for juvenile females and juvenile males, respectively. Although sample sizes for apparent dispersing animals was small (n=16), more formal analyses of spring dispersal is pending. These valuable data will be informative for understanding potential CWD spread in wild deer in southeastern Minnesota and enable MNDNR to adjust surveillance and management activities more effectively to counter CWD in southeast Minnesota.

INTRODUCTION

Chronic wasting disease (CWD) is a fatal infectious disease first characterized in the late 1960s in Colorado that affects elk (*Cervus canadensis*), mule deer (*O. hemionus*), white-tailed deer, reindeer (*Rangifer tarandus*) and moose (*Alces alces*). It has been detected in wild and captive cervids in 25 states and 2 Canadian provinces in North America, as well as Finland, Norway, and South Korea. Recent work has demonstrated that CWD can cause population declines in deer in the western US, particularly at high prevalence levels in a population (Edmunds et al .2016). In the upper Midwestern US, an ongoing study of CWD in white-tailed deer in Wisconsin has shown that CWD-infected deer die at 3x the rate of uninfected deer (Wisconsin Public Radio, 2018). In the same CWD system, research has shown that deer regularly die from CWD in the wild, although they typically go undetected by people (Samuel and Storm

2016).

During the regular hunting season in 2016, MNDNR detected 3 wild white-tailed deer with CWD in Fillmore County, Minnesota, and established a disease management zone (forming deer permit area 603) approximately 10 miles in radius around the positive detections. Further sampling of wild deer through March 2017 resulted in a total of 11 positives found in two spatial clusters approximately 5 miles apart (Figure 1). Additional sampling during the 2017 regular and special hunting seasons resulted in an additional 6 CWD cases detected in DPA 603 with a new apparent cluster in ForestvilleMystery Cave State Park (Figure 1). It is not clear how CWD was introduced into the area, but potential routes of introduction include movement of infectious deer from neighboring states (e.g., Wisconsin, Iowa, or Illinois), contact between wild deer and prior CWD-positive captive cervid facilities (e.g., Pine Island, MN), or contamination of the environment with infectious cervid carcass material facilitated by out-of-state hunters disposing of butchering remains on their property. In June 2017, the MNDNR was made aware of available funding in the emerging issues account of the Environment and Natural Resources Trust Fund (ENRTF) managed by the Legislative-Citizen Commission on Minnesota Resources (LCCMR). We applied for and received funding (M.L. 2015, Chp. 76, Sec. 2, Subd. 10) which allowed us to execute a research project to 1) understand potential pathways of CWD spread on the Minnesota landscape mediated by movements of wild deer, 2) increase the likelihood of success of managing the CWD outbreak in this and other areas of the state, and 3) quantify sources of mortality for deer in southeast Minnesota and incorporate survivorship estimates into population models. This study aims to better understand deer movement ecology as it relates to potential prion transmission in southeastern Minnesota in and around the newly established CWD management zone, DPA 603. The apparent sample prevalence of CWD in DPA 603 through fall 2017 sampling was 0.4%, which suggests we have discovered the disease in the early stage of the outbreak. This timing offers the best chance of mitigating disease spread and optimizing surveillance and management actions. A growing body of research suggests that in the long-term, CWD causes deer population decline (Monello et al. 2014, Edmunds et al. 2016) and has the potential to cross species barriers (Waddell et al. 2018).

As infected and non-infected deer interact and move across the landscape, they transmit infectious prions through direct contact with other deer or indirectly through environmental deposition (Almberg et al. 2011). Limited information exists about deer contact rates and their relationship to transmission rates, especially in areas recently infected. The presumed main driver of spatial spread among wild deer are natural movements. Currently, there is no research that demonstrates the extent to which potentially infected deer move across the landscape and interact with each other in southeastern Minnesota.

Deer behavior and movements vary by biological and environmental conditions, along with deer population demographics and social structure. Two types of movement likely facilitate disease spread across the landscape, recurrent seasonal movements and one-time dispersal or foray events. The most substantial long-distance movements involve dispersal from birth to adult ranges, most likely to occur in 1-year-old deer. Because deer densities and movement behavior can be altered by management actions, a better understanding of both deer density and movement tendencies related to density will enhance our ability to effectively manage disease risk in the Minnesota deer population. The importance of this research is underscored by the increased risk of disease spread from Wisconsin and Iowa, and our findings will help the MNDNR understand those risk factors as well.

METHODS

Study Area

The study area, approximately 7,250 km², is centered on DPA 603, also referred to as the CWD

management zone, in Fillmore County, Minnesota (Figure 2). The study area limits are flexible and have been established as approximately a 20 mile buffer outside and including DPA 603. We need extensive area around DPA 603 to capture and release GPS-collared deer, so that our collared sample is representative of the deer population inside and surrounding the CWD management zone in southeastern Minnesota. Since some deer were collared inside and may move through DPA 603, we expect some study animals to be exposed to higher harvest pressure during hunting seasons. This tradeoff is necessary, however, to gain understanding of how deer move through the DPA 603 landscape and for estimating survivorship in areas with liberal hunting regulations.

The study area is composed of a matrix of agricultural lands interspersed with deciduous forest upon a landscape of rolling hills and in some cases very steep ridges and valleys. There is considerable heterogeneity in landscape topography and land use, particularly as one moves from east to west. The eastern part of the study area is composed of forested bluffs and steep ridges and moving west and south, the landscape transitions to be flat and dominated by agriculture. More than 90% of the landscape is held in private ownership, and there is significant heterogeneity in deer density due to both habitat heterogeneity and localized refugia.

Since most of the region is in private ownership, our initial efforts focused on securing permission to access private property in the study for our deer capture and collaring efforts. We secured permissions to use 105,473 acres of property, consisting of private (67,924 ac) and public (37,549 ac) lands, for search and capture of white-tailed deer in southeastern Minnesota (Figure 3). We could not have achieved our sampling goals without the enormous outpouring of support from private landowners in the study area (>200). Public properties included state wildlife management areas, state forests, and state natural areas. We do not have a limit on acquiring available properties for future deer capture, and hope to add to our land permission list with time. We focused on properties that are forested (where deer may be flushed) with adjacent open fields (where deer may be captured and a helicopter may safely land).

Sampling Design and Data Collection

Given the breadth of the study area, we divided it into 10 quadrants (Figure 3) from which we established a baseline target goal of capturing 6 juvenile males (\approx 7-9 months old), 3 juvenile females (\approx 7-9 months old), and 2-3 adult males ($>$ 2.5 years old) per quadrant. In total, our intent was to capture and collar 115 deer in the first year of this study; 60 juvenile males, 30 juvenile females, and 25 adult males. For years 2 and 3, we plan to capture and collar an additional approximately 60 juvenile male deer (6 per quadrant). We did not collect biopsy tissue for CWD sampling at the time of capture, but plan to collect retropharyngeal lymph nodes for CWD testing at the time of death for all recovered carcasses.

We contracted with Hells Canyon Helicopter Company (Clarkston, WA) to capture deer by net-gunning from a R44 Raven 2 helicopter. Deer handling consisted of collar placement, collection of auxiliary measurements (body temperature, age class, sex, and body condition), blood sampling for serological screening of diseases, and an ear punch for genetic analysis. Average handling time was approximately the same across all sex and age groups at 13.1 minutes.

Deployed GPS collars (Iridium TL330, Lotek Wireless Inc, Newmarket, Canada) were programmed to collect location coordinates primarily during spring dispersal, fall dispersal, and rut periods. The rate of GPS location fixes was approximately once per hour for the following age-sex cohorts and time periods: all juvenile deer between April 1 and July 15 and September 1 through November 30; and adult males between September 1 and December 31. During all other time periods, collars were scheduled to collect positional data every 2 hours or 12 locations per day. We chose these periods in part based on seasonal movements recorded from yearling males in Wisconsin. To ensure that location data were collected across the entire

24-hour day distribution instead of fixed times per day, we programmed collar GPS fixes on a staggered data collection schedule which changes every day.

All GPS collars housed identical hardware for communication with satellites, carried at the bottom of the collar, underneath the deer's neck. The only difference between collar types consisted of the type of expansion mechanism which allowed the necks of individuals to change in size over time. Specifically, juvenile female collars did not have an expansion mechanism and consisted of a complete loop of inelastic leather as female neck size does not vary appreciably after maturity. On the other hand, the necks of juvenile males expand because of growth associated with aging and seasonal reproductive changes. During the reproductive period (i.e., rut), the necks of male deer expand substantially, and following this period they contract again. Similar dynamics occur in adult male deer. To accommodate the expansion and contraction of neck size depending on season, we fitted our juvenile male deer with a collar that contained an elastic band permitting expansion and contraction. For adult males, we used a magnetic expansion mechanism on collars to accommodate seasonal neck growth and contraction dynamics.

Data Analysis

Objective 1: Document dispersal patterns and estimate movements of juvenile (1-year-old) males and females, and adult males (>2 year old)

Generally, we will follow methods from Kenward et al. (2001), Long et al. (2005), Lutz et al. (2015, 2016), and Peterson et al. (2017). For juveniles, we define dispersal as having occurred if an individual displayed a permanent, 1-way movement from a natal range to a distinct adult range (Kenward et al. 2001, 2002), such that pre-dispersal locations do not overlap post-dispersal locations (Long et al. 2005, Lutz et al. 2015). We will estimate natal and adult home ranges using minimum convex polygons (MCP). We will assume that we captured juveniles on their natal range and model the probability of dispersal from natal to adult range using time-to-event models (Walsh et al. 2015). We will designate the date of dispersal as the first date a location was recorded outside the natal range. These methods accommodate uncertainty in dispersal specification (Walsh et al. 2018). For deer in which we have insufficient location data suggesting dispersal based on Kenward et al. (2001) methods, we will assign a probability that a dispersal event occurred on a case-by-case basis. We will calculate dispersal distance as the straight-line distance between the median x and y natal range coordinates and the median x and y adult coordinates (Kenward et al. 2002). We will designate a dispersal location as the first dispersal location when all subsequent locations do not occur within an MCP containing all previous locations. Similarly, the last location of a dispersal path will be determined by when it was the last location not contained within a natal MCP. We will map dispersal path movements by beginning at the edge of the natal range nearest the first dispersal location and ending at the edge of the adult range nearest the last dispersal location (Karns et al. 2011). We will perform all spatial data analysis using R software and ArcMap 10.2.2 (Environmental Systems Research Institute, Redlands, CA, USA).

We will calculate the direction of dispersal as the azimuth measurement from true north between the median x and y natal range coordinates and the median x and y adult range coordinates. We will use Rao's spacing test (Batschelet 1981) to test for a directional trend in dispersal direction and correlate trends with the orientation of topographical features (e.g., ridges, valleys). We will use Greenwood and Durand's V test to determine if there is a relationship between dispersal directions and mean axial orientation of topographic landscape features. We will estimate dispersal path distance as the sum of distances between the nearest edge of the natal MCP to the first location of the dispersal event, subsequent points of the dispersal event, and the nearest edge of the adult MCP to the last point of the dispersal event.

However, we will consider only sequential movements >250 m (i.e., we retained only the first location of closely spaced sequential locations) to minimize the influence of high location frequencies (Long et al. 2010). We will classify a movement as a foray (or excursion event) if it was a movement >1.5 km from the edge of the natal MCP with a subsequent return to the natal MCP and designate the foray date as the first date a foray location is recorded outside the natal range. We will estimate the distance of a foray as the straight-line distance between the farthest foray location out of the natal range and the nearest edge of the natal range. Likewise, we will estimate foray path distance as the sum of distances between the nearest edge of the natal MCP to the first location of the foray, subsequent locations of the foray event, and the nearest edge of the natal MCP to the last location of the foray before the animal returned to the natal MCP. We will examine seasonal differences in the direction and distance of foray and dispersal movements with linear mixed models assigning direction and distance as continuous response variables; season, sex, natal range deer density, and mean percent forest cover along the linear dispersal path (from National Land Cover Database) as fixed effects; and each deer as a random effect.

For adult males, we will assume animals are already within their adult range and use minimum convex polygons (MCP) to estimate this range. Foray events will be calculated as described for juveniles and we will examine seasonal differences in the direction and distance of foray movements with linear mixed models assigning direction and distance as continuous response variables, season as a fixed effect, and each deer as a random effect.

Following Long et al. (2010), we will consider potential barriers to dispersal to be highways (e.g., interstate highways, U.S. routes, state routes), dense residential or developed areas (as delineated on U.S. Geological Survey 7.5-minute Quadrangle topography maps and confirmed via aerial photographs), and large rivers.

Objective 2: CWD spatial pathways mapping to inform future surveillance and management

Using results from analysis of objective 1, we will map MCPs from collared animals onto GIS layers containing the landcover types and major topographical features (e.g., ridge lines, interstates, large cities, etc.). We will identify movement corridors by examining trends in dispersal path directions and distance by sex. Using Greenwood and Durand's V test to determine if there is a relationship between mean dispersal directions and mean axial orientation of topographic landscape features, we will also characterize the distribution of dispersal distances and directions by sex. This initial effort will entail qualitative spatial pathway risk mapping based on inferences from objective 1. Future work to further characterize spatial pathways of likely CWD spread will entail application and extension of methodology presented in Hefley et al. (2017), which uses a spatio-temporal partial differential equation.

Objective 3: Determination of cause-specific mortality

Each GPS collared deer is designed to transmit a mortality text and email message signaled by a 12-hour window of inactivity. To adhere to the current harvest-based deer population model used by MNDNR, we will attempt to verify cause of death as hunting or non-hunting within 48 hours of deer mortalities by searching for a carcass at the collar location. Although current models used by MNDNR only require these broad categories, we will record additional details about cause-of-death (e.g., deer-vehicle collision, poaching, depredation, and starvation). Collared deer will not be protected from legal harvest during hunting seasons, and we will request via press release that hunters select animals for harvest based on their personal preference regardless of whether the hunter notices a collar on the deer. We will ask hunters that harvested a collared deer to contact DNR and return the collar, as there is valuable GPS location and activity data stored on-board devices. While we receive GPS locations via a web service, there is potentially additional location data stored in memory, which is invaluable for our

movement analyses. In addition, the activity data consists of a numeric index indicating the relative level of movement the collar experienced every 5 minutes, and this can be used as a proxy for relative levels of deer activity. For estimation of cause-specific mortality, we will use the Bayesian hierarchical modeling approach outlined in Walsh et al. (2018). We will estimate the instantaneous hazard $h(t)$ using a conditional survival function (Kalbfleisch and Prentice 2002) using time to death (T_i), time of entry (e_i), time the subject was last known alive (r_i), and the time the subject was first encountered dead (s_i) for each deer classified according to sex and age. We will then assign a probability the fate was associated with a specific cause based on field investigations (Walsh et al. 2015). We will evaluate the best fitting model using deviance information criterion (DIC), and consider model averaging for estimating age, sex, and mortality source survival probabilities.

RESULTS AND DISCUSSION

From March 18-23, 2018, we captured 111 white-tailed deer in our study area (Figure 4). Of these 111 - one juvenile male was able to kick off its collar twice and escaped without being recollared, and 1 adult male accidentally broke its neck upon being captured. Of the 109 deer captured with successfully deployed collars, 3 animals (2 juvenile males and 1 adult male) were able to kick their collars off within the first week, reducing our sample size to 106. Of these 106 remaining collared deer, 10 additional deer had died by August 10, 2018. Nine of these mortalities occurred before April 10, 2018 and appear to be related to some extent to capture based on necropsy results (such as capture myopathy, $n=5$), although coyote predation ($n=2$), suspected vehicle collision ($n=1$), and suspected disease ($n=1$) also played a role. Due to structural failures of the expansion mechanism, we have lost 9 additional juvenile male collars leaving 87 GPS-collared deer available including 30 juvenile females, 1 adult female, 19 adult males, and 37 juvenile males. We have established databases for capturing updated movement and mortalities, and are monitoring all GPS-collared animals daily.

As of August 10, 2018, we have amassed over 120,000 records of deer location data. Prior to the dispersal period between April and July 2018, we estimated the average winter home range size as 1.60 km² for juvenile females, 2.96 km² for adult males, and 2.28 km² for juvenile males (Table 1). These winter home range estimates align with our expectations of deer activity at this time of year. Contrary to our expectations, juvenile female deer had higher apparent dispersal probability (40%, $n=20$) than juvenile males (28%, $n=28$) in spring 2018, although this difference was not statistically significant ($p = 0.41$). The average apparent dispersal distance travelled was 30.4 km ($n=8$) and 14.6 km ($n=8$) for juvenile females and juvenile males, respectively (Table 1). Although sample sizes for apparent dispersing animals was small ($n=16$), more formal analyses of spring dispersal is pending. The data also suggests that only 17% of our adult male sample underwent appreciable foray movements ($n=2$).

We found that as many as 7 deer have traveled to and from Iowa, and it's not clear yet if they have established an adult range in that state. Of these 7 deer, they include 3 juvenile males, 1 juvenile female, and 3 adult males. We do not yet have sufficient mortality data to estimate cause-specific mortality, and we expect that upcoming hunting season mortalities will be able to inform this analysis.

While juvenile male dispersal is typically regarded as the primary force driving potential disease spread (CWD) on the landscape (Gear et al 2006, Oyer et al. 2007), evidence suggests that females orphaned at a young age (Etter et al. 1995) or high underlying deer density (Lutz et al. 2015) can drive juvenile females to disperse. Given the relatively high rate and extent of juvenile female dispersal and high deer densities in the farmland-forest transition zone of our study area (Norton and Giudice 2017), we hypothesize that this phenomenon is playing out in southeastern Minnesota. This highly productive landscape favors high deer survival and

fecundity, given extensive food resources, winter cover, and relatively mild winters. Future capture and GPS collaring efforts in the study area should include representative cohorts of juvenile female and male deer to monitor the rate and extent of dispersal movements, as it relates to potential spread of CWD prions on the Minnesota landscape.

We made considerable efforts to provide outreach materials both for landowners that have provided us with permission to use their properties for deer capture and for the general public. We have established a structure to contact participating landowners quarterly with map updates on all of the collared deer in the study, and provide big picture messages about periodic study findings and expectations for future work. Similarly, we have created a website dedicated to this research project at <https://www.dnr.state.mn.us/cwd/deer-movement-study.html>. This site provides information about the purposes of the study, periodic updated findings, and information about how readers can assist and contribute to our efforts. We encourage the public to provide us with trail camera photos of collared deer they may encounter, and with their permission, we make these pictures available on our website. There have also been over a dozen popular press articles covering this study in various media outlets. We seek to continually improve how we communicate science to the public, and provide transparency in all of the work that we conduct.

Future Capture and GPS-Collaring Efforts

Between January and February 2019, we plan to capture and GPS-collar approximately 60 juvenile white-tailed deer in the study area to maintain a sample size of about 100 deer for location monitoring at any given time. The exact distribution of juvenile male and female deer for capture has not been decided upon yet, but we expect to conduct capture and collaring operations for at least an additional 2 years in the study area.

ACKNOWLEDGMENTS

We extend warm thanks to all of the participating landowners in southeastern Minnesota that gave us permission to access and conduct capture operations on their properties. We thank all the MNDNR Wildlife and Enforcement staff, who assisted in contacting landowners and fielding questions about the study to the public including Don Ramsden, Mike Tenney, and Mitch Boyum. Special thanks to Jason Jensen for flying our spotter aircraft during capture operations; John Giudice for flying in the spotter aircraft as an observer; Julie Hines and Bob Wright for their great work assisting us with our GIS mapping needs; Mike Kallok, Chris Scharenbroich, and Pete Takash for their efforts with setting up a webpage dedicated to this project on the DNR website; Larissa Minicucci for being available for darting efforts had we needed them; Rushford Municipal Airport (Airport Manager Mike Thurn) and Fillmore County Airport (Airport Manager Isaac Deters) in Preston, Minnesota for use of their facilities; Jamie Gangaware, Louise Spiczka, Dan Stark, John Wollenberg, Scott Noland, and Marshall Deters for their willingness to assist as aircraft observers. We appreciate the continued support and assistance of the USDA-Wildlife Services disease biologist Tim White. Without the support of these and many more people behind the scenes, this project would not be possible. We also thank LCCMR – the Environment and Natural Resource Trust Fund (ENRTF) - Emerging Issues account (M.L. 2015, Chp. 76, Sec. 2, Subd. 10) that we received grant funding from providing the needed funds to get this project off the ground. Additional funding was provided in part by the Wildlife Restoration (Pittman-Robertson) Program.

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Table 1. Preliminary mean estimate (and 95% confidence interval) of winter home range (HR - km²), apparent spring dispersal probability (Pr. Dispersal), and apparent spring dispersal distance (Distance – km) of white-tailed in southeastern Minnesota collared in March 2018. The distance estimates do not account for non-linear pathways traveled, forward and backwards movements along pathways, and only describe straight-line distances. Please note the differences in sample sizes used in the analysis of winter home range (n-HR) and apparent dispersal (n-Dispersal).

Cohort	n-HR	HR (95% C.I.)	n-Dispersal	Pr. Dispersal (95% C.I.)	Distance (95% C.I.)
Juvenile Female	31	1.60 (1.19, 2.00)	20	0.40 (0.20, 0.64)	30.42 (1.35, 52.40)
Juvenile Male	39	2.28 (1.65, 2.91)	29	0.28 (0.13, 0.47)	14.65 (7.64, 19.60)
Adult Male	18	2.96 (2.12, 3.79)	12	0.17 (0.03, 0.49)	18.90 (8.80, 29.00)
TOTAL	88		61		

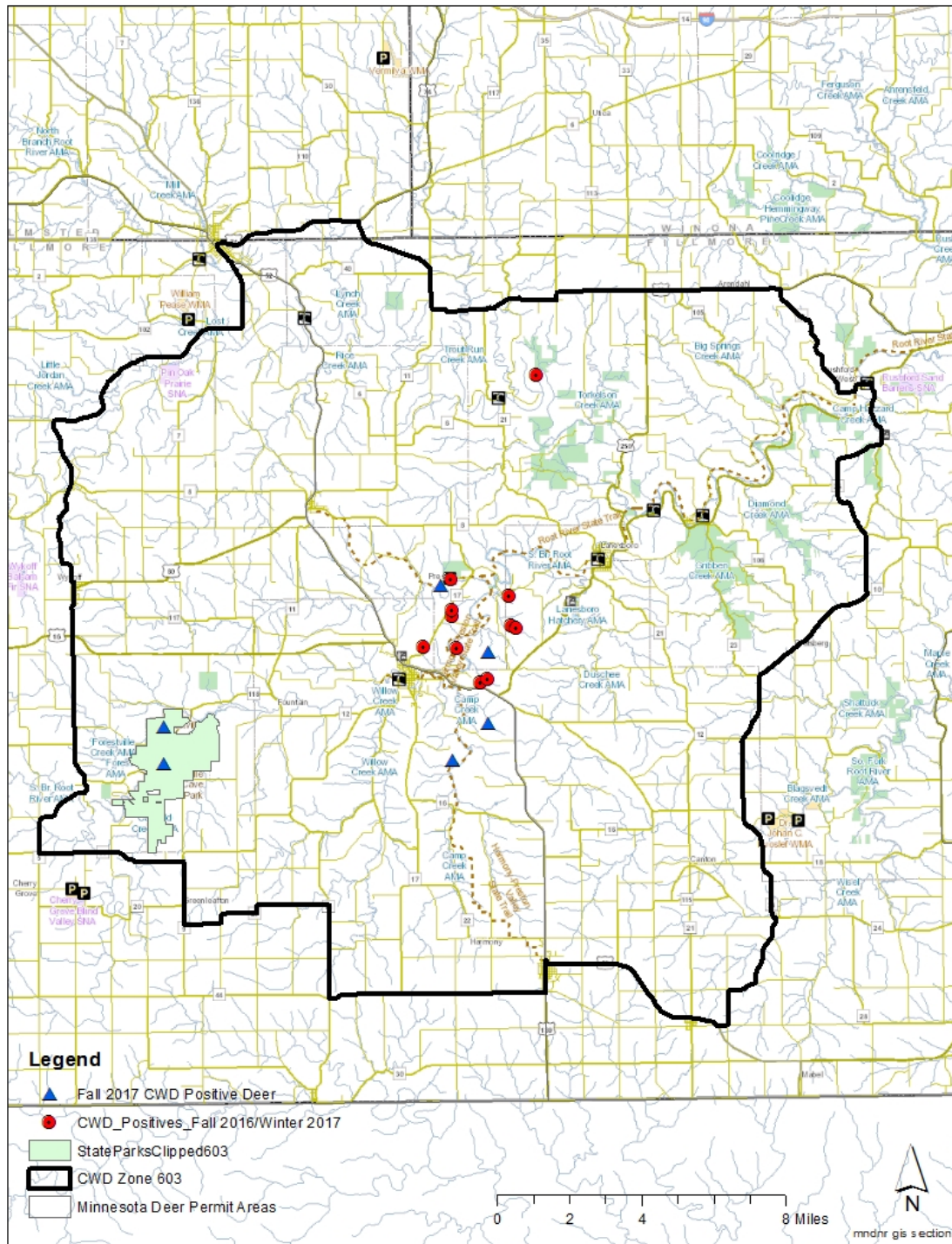


Figure 1. Spatial distribution of wild white-tailed deer confirmed with CWD infection in DPA 603 in Minnesota as of 08/10/18. In the 2016-17 season, there were 11 confirmed detections, and in 2017-18 there were an additional 6 confirmed detections.

Study Area

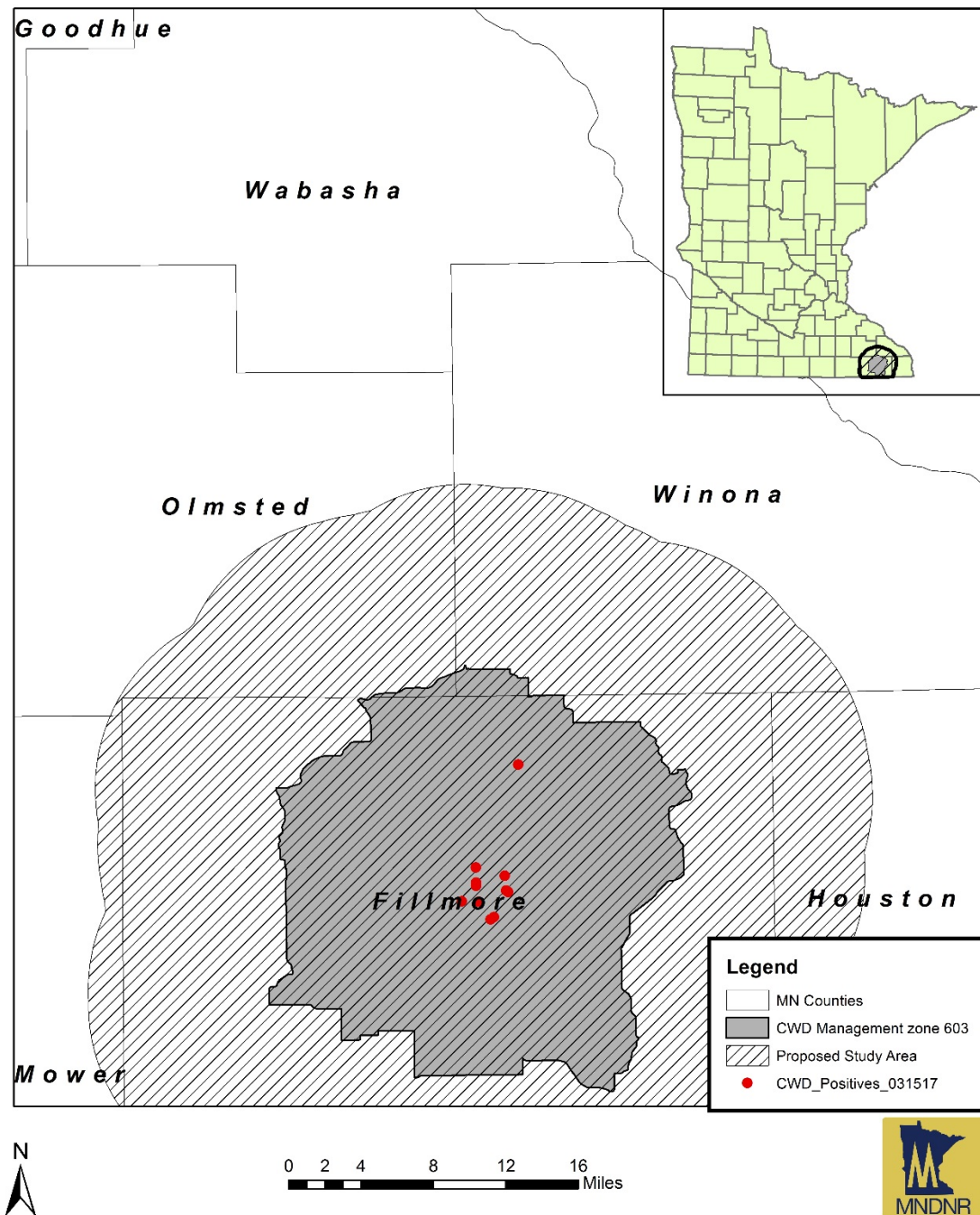


Figure 2. Approximate study area boundaries in and around the chronic wasting disease management zone (Deer Permit Area 603) in Minnesota. This area is largely private land, so the final disposition of sampling locations for GPS collaring deer will depend on permissions we receive from cooperating landowners, weather patterns, and local scale landscape characteristics that facilitate helicopter capture of wild white-tailed deer.

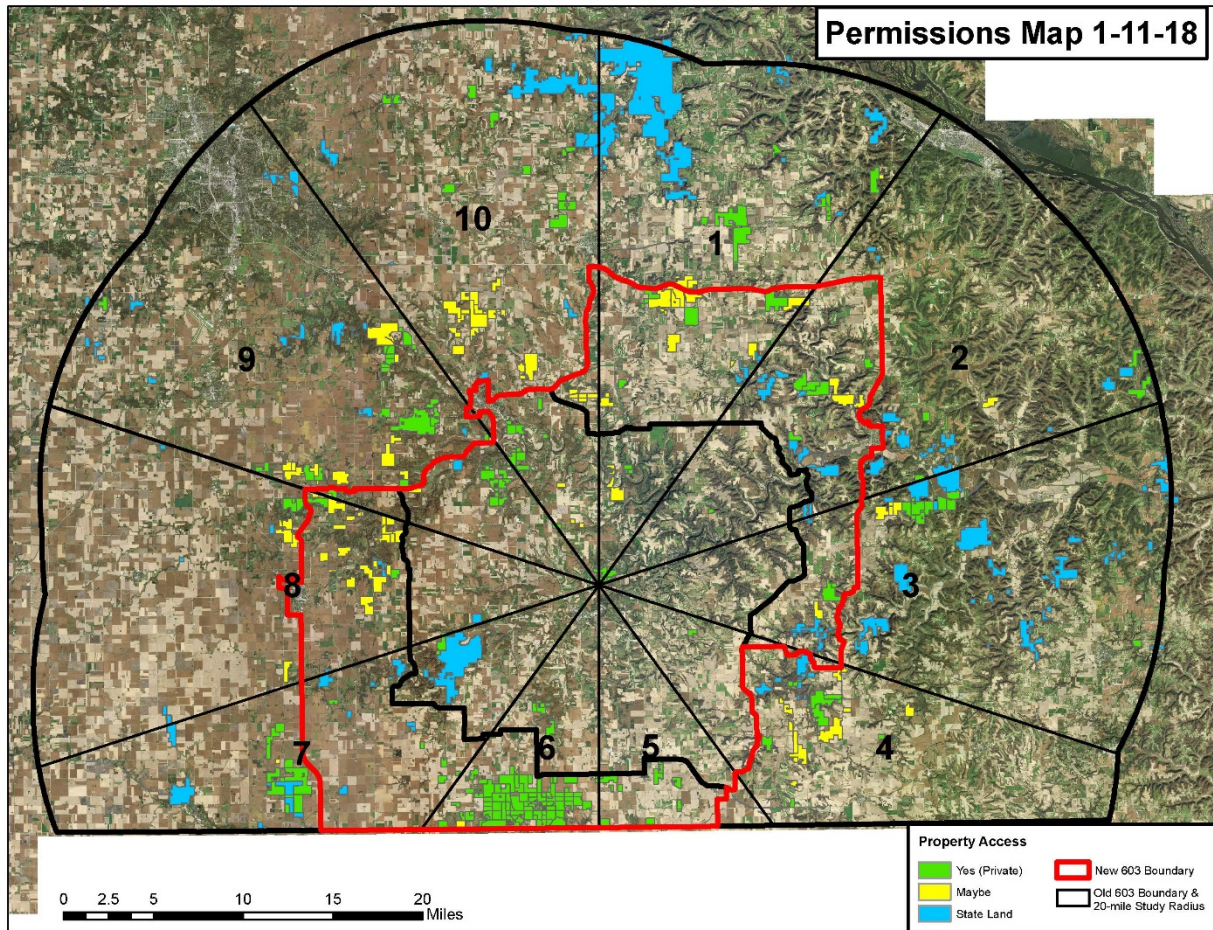


Figure 3. Spatial distribution of study area capture quadrants used as a basis for establishing Minnesota's 2018 deer capture goals. The target optimal capture distribution was established as six juvenile male, three juvenile female, and 2-3 adult male deer captured per quadrant.

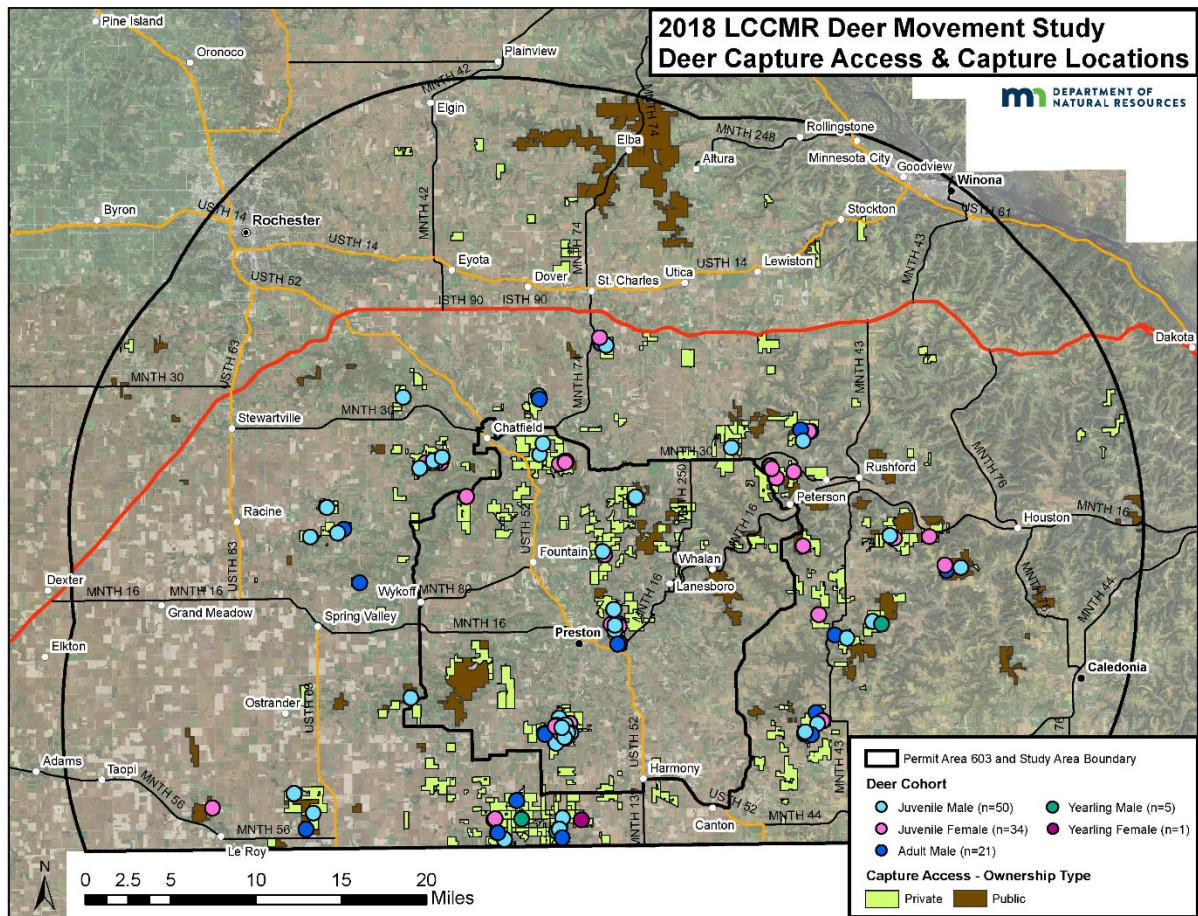


Figure 4. Spatial distribution of private (67,924 acres) and public (37,549 acres) properties secured in the study area for the 2018 deer capture season. Points represent the locations where white-tailed deer were captured, collared with GPS units, and released in the study area centered on CWD management zone 603 in Fillmore County, Minnesota, between 03/18/2018 and 03/23/2018.

CAUSES OF NON-HUNTING MORTALITY OF ADULT MOOSE IN MINNESOTA, 2013 – 2017

Michelle Carstensen, Erik C. Hildebrand, Dawn Plattner, Margaret Dexter, Arno Wünschmann¹, and Anibal Armien¹

SUMMARY OF FINDINGS

Minnesota's moose (*Alces alces*) are dying at rates much higher than elsewhere in North America. Moose have been nearly extirpated from the northwestern part of the state and aerial surveys indicate the northeastern population has declined 55% over the past decade. In 2013, a new study began to determine cause-specific mortality of adult moose in northeastern Minnesota by using GPS-satellite collars to get rapid notification of mortality events and recover carcasses within 24 hours of death. A total of 173 moose were collared over 3 years with annual non-hunting mortality rates of 19%, 12%, 15%, 13% and 14% in 2013-2017, respectively, and an overall mean of 14.4%. In total, 57 moose have died from non-hunting sources of mortality and 3 moose were legally harvested. Response times from mortality notification to arrival at the carcass were within 24 hours for 65% of death events. Most causes of mortality were health-related (65%), which included parasites (30%), bacterial infections (20%), accidents (3%), calving (2%) and other undetermined health issues (10%). The remainder was wolf-related (30%), with predisposing health conditions identified in nearly half of these moose. Legal harvest accounted for 5% of moose deaths. During the same time period, we also necropsied anecdotal moose deaths ($n=91$) across northern Minnesota, which included vehicle or train collisions, sick, and found dead animals. *Parelaphostrongylus tenuis* was confirmed in 42% of these cases, which is nearly twice the rate of detection of this parasite as in the collared moose studied during the same time period.

INTRODUCTION

Until recently, 2 geographically distinct moose (*Alces alces*) populations occurred in Minnesota (MN), one in the northwestern (NW) and the other in the northeastern (NE) part of the state. Since the mid-1980s the NW population has decreased from an estimated 4,000 to less than 100 moose, and since 2006 the NE population has declined 66% from an estimated 8,840 to 3,030 moose (DelGiudice 2018). However, there is some evidence that the moose population in the NE may be stabilizing over the last 7 years (2012-2018) at approximately 4,000 animals. Mean annual mortality rates of adults have been similarly high (21%) in both regions (Murray et al. 2006, Lenarz et al. 2009).

Parasites, including liver flukes (*Fascioloides magna*) and brainworm (*Parelaphostrongylus tenuis*) and other non-specific health-related issues have been documented in the majority of moose deaths through these past research efforts (Murray et al. 2006, Lenarz et al. 2010, Wünschmann et al. 2015). Climate change has also been implicated as an underlying factor in both population declines. Recent study of moose calf survival documented survival rates between 29-40% from 2013-2016, with predation by wolves accounting for over two-thirds of mortalities (Severud 2017).

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This 5-year study was designed to document causes of non-hunting mortality of adult moose in NE MN by deploying satellite-linked collars and by preparing an extensive network of responders trained in conducting field necropsies. Moose mortalities were thoroughly investigated within 24 hours of death to identify the proximate cause of mortality and to examine the influence of potential contributing factors. Further, efforts to investigate reports of non-collared sick and dead moose were intensified to provide additional anecdotal information on moose population health during the same time period. Once causes of death and major influential factors are identified, appropriate management actions may be taken to address the population decline.

METHODS

Moose (>1 year of age) were captured within the 3,732.8 km² study area located between 47°12'N and 47°95'N latitude and 90°33'W and 91°72'W in NE MN from 2013 to 2015, as described previously (Butler et al. 2013; Carstensen et al. 2014, 2015, 2016). All moose were fitted with GPS-Iridium satellite collars (Vectronic Aerospace GmbH; Berlin, Germany). Mortality implant transmitters (Vectronic Aerospace GmbH) were placed orally into a subset of the captured moose and provided immediate notification of mortality and recorded internal body temperature (Minicucci et al. 2018, Herberg et al. 2018).

Moose mortality response teams have 8 primary team leaders that have undergone 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 respond to a moose mortality event within 24 hours of notification and 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. If a moose was found to be alive, but obviously ill, it was euthanized (via gunshot to the neck). If carcass extraction was not possible, a field necropsy was performed. Samples were submitted to the UMN VDL for diagnostic evaluation (Carstensen et al. 2014, 2015, 2016).

From 2013 to 2017, efforts were enhanced to respond to reports of sick or dead non-collared moose from anywhere in MN, from hereafter referred to as “anecdotal moose.” This included sick moose that were still alive at the time of the initial report, recently found dead animals, and vehicle or train collisions. MNDNR biologists and conservation officers responded immediately to these reports and samples or whole carcasses were collected in a similar manner to the collared moose study, with the exception of salvage permits provided for the meat of vehicle-killed moose.

Moose age was determined by cementum annuli analysis of incisor teeth removed at time of capture and we used one-way analysis of variance to compare age among years. Annual (Jan-Dec) survival rates were estimated using Kaplan-Meier to allow for staggered entry design. Moose were censored from the analysis on the date their collar stopped transmitting data, regardless of their survival state beyond that time, if known. Censored animals included those that died <2 weeks post-capture (presumed to be capture-related mortalities), slipped collars, remotely-release collars through a built-in blow off mechanism, hunter-harvested moose, and collars that stopped transmitting location data due to collar malfunction.

RESULTS AND DISCUSSION

Annual Survival and Cause-Specific Mortality of Collared Moose

From 2013–2015 a total of 173 adult moose (123 females, 50 males) were captured and collared. Mean age at capture was 6.1 (± 0.3 , $n=163$) years for all moose; range was 1 to 16 years. Age of moose at capture was similar [F -stat=1.65, $p=0.19$] among years (6.0 years in 2013, $n=101$; 5.8 years in 2014, $n=32$; and 7.2 years in 2015, $n=30$). Annual (January–

December) survival rates were 81%, 88%, 85%, 87% and 86% in 2013 through 2017, respectively (Figure 1); the overall 5-year mean for non-hunting mortality was 14.4%. This is lower than the average non-hunting mortality rate of 21%, reported previously in MN, but higher than the 8-12% rates of North American moose populations (Mytton and Keith 1981, Larsen et al. 1989, Ballard et al. 1991, Stenhouse et al. 1995, Modafferi and Becker 1997). A total of 60 collared moose (41 females, 19 males) have died since this study began; which excludes 12 capture-related mortalities that are censored from subsequent survival analyses. Most collared moose mortalities (96%) occurred within the current moose range in northeast MN (Figure 2). Overall proximate causes of death included: wolf predation ($n=18$, 30%), parasitic infections ($n=18$, 30%), bacterial infections ($n=12$, 20%), accidents ($n=2$, 3%), hunter-harvest ($n=3$, 5%), calving complication (dystocia) ($n=1$, 2%), and undetermined health issues ($n=6$, 10%; Figure 3). Health-related causes were attributed to 68% of total deaths, with the remaining 32% being predator-related.

Eight (44%) of the wolf-killed moose had significant health conditions that likely predisposed them to predation, including encephalitis and meningitis, *P. tenuis* infections, winter tick (*Dermacentor albipictus*) infestations, calving, and pneumonia in the lungs (Figure 4a). Unfortunately, diagnostics were limited in 10 of the wolf-killed moose due to the degree of carcass consumption prior to the mortality team's arrival to the scene. It is possible that health issues may have compromised some of these moose as well.

Parasitic infections were a leading cause of moose deaths (Figure 4b). *P. tenuis* directly led to the death of 8 moose in this study; however, this parasite was also implicated in 5 wolf-caused deaths and 1 bacterial infection. Overall 23% of the moose that died during this study have been impacted by *P. tenuis* and this is likely an underestimate, as not all dead moose could be evaluated for this parasite. Winter tick infestations were primarily seen in spring 2013 (attributed to 3 moose deaths), as the severe and prolonged winters in 2012-13 and 2013-14 likely reduced tick survival. However, the past 4 winters have been extremely mild and it's likely that winter tick loads have recently increased on moose. In spring 2016, one moose in the study died from winter ticks; however, significant tick infestations were observed in other moose as well. We had expected that moose surviving into spring 2017 would experience a significant winter tick burden and this would result in an increase in tick-related mortalities; however, none of the collared moose died from winter tick burdens in 2017 but the sample size has markedly declined to only 37 animals left to monitor at the beginning of this year. Most moose in this study had livers that were damaged by liver flukes (*F. magna*), the severity of which varied from mild cases to severe infections that directly caused the death of 3 moose. Similarly, the majority of moose in this study had hydatid cysts in the lungs or liver, caused by *Echinococcus granulosus*, but only 2 moose had severe enough infections with this parasite to cause mortality. We also observed one moose with an extensive cysticercus (*Taenia krabbei*) infection in various skeletal muscles and heart, which likely resulted in death due to reduced cardiac function.

Bacterial infections were the third leading cause of moose deaths (Figure 4c). Four moose were attacked by a predator, most likely a wolf or wolves, and survived the initial encounter, but the wounds became infected and led to their death days to several weeks later. Prior to this study, scant evidence in the literature points to secondary bacterial infections caused by a predator attack as a major cause of moose mortality. Other trauma, including one case consistent with conspecific fighting of antlered males, resulted in puncture wounds that provided a route for bacteria to enter the body and cause systemic infection and septicemia. The exact circumstances that led to some of these trauma-induced injuries were unknown.

The remainder of moose deaths were caused by accidents (1 vehicle collision and 1 fall through the ice), hunting (3 moose was legally harvested during tribal hunts), calving complications or

dystocia (1 moose had twin calves stuck in the birth canal while being expelled simultaneously), and undetermined health-related deaths (6 moose).

There were 18 moose remaining in the study with active collars at the start of 2018. Unfortunately, collar failure rates have been high (causes unknown, assumed to be battery or transmission failures), with 79 collars that failed at varying times throughout the study (Table 1). Luckily, we were able to recover 40 of these failed collars (51%) from live moose via remote release mechanisms and obtain the data stored on the collar (e.g. MIT, activity, GPS location). Three moose had their collars slip off their necks, presumably due to an excessively loose fit, and were recovered in the field. Of the 18 collared moose that remained active into 2018, we attempted to remotely blow off all of these collars over 3 separate flights in January and February, and successfully recovered 16 of those collars. The satellite service for the 2 remaining collars was turned off at the end of March.

Anecdotal Moose Mortality

From 2013–2015 a total of 91 anecdotal sick/dead moose (46 females, 43 males, and 2 unknown sex) reports were investigated throughout Minnesota (Figure 2). This included 62 adults (mean age was 4.5 ± 0.5 years, $n=48$), 11 yearlings (>1 and <2 years of age), and 18 calves (<1 year of age). The majority (52%) of reports involved vehicle-killed moose, followed by found dead (24%), sick and euthanized (17%), and train-killed (7%) animals (Figure 5). The majority of these cases reported during the fall season (35%), where moose are moving more due to the breeding season and as a result, are more vulnerable to both vehicle and train collisions (Figure 6). Further, we had the most reports of found dead moose in the fall season, likely due to hunters afield pursuing other big game and upland birds. Nearly half of all vehicle-killed moose cases occurred in the summer when tourist season peaks in the northeast and moose may be moving more in response to insect harassment.

Trauma was the cause of death for moose hit by either vehicles ($n=47$) or trains ($n=6$); however, examination of their internal organs confirmed *P. tenuis* infection in 8 moose, brain lesions of unknown cause in 2 moose, and marked liver fluke-induced hepatitis in one moose. Decomposition was a confounding factor in determining the cause of death for half of the 22 moose found dead by members of the public; however, *P. tenuis* infection ($n=9$), winter tick-associated anemia ($n=1$), marked liver fluke-induced hepatitis ($n=1$), and bacterial infection ($n=1$) were confirmed in the remainder. Interestingly, *P. tenuis* infection was determined to be the cause of 15 of the 16 sick moose reports where the animals had to be euthanized. One of these moose was suffering from grain overload and brainworm simultaneously; both conditions likely contributed to its death. The only sick moose that didn't have *P. tenuis* infection was an old bull that was injured by conspecific fighting and was dying from a bacterial infection from its wounds.

Our findings of parasitic loads of anecdotal moose from 2013-2017 were very similar to those reported by Wünschmann et al. (2015) for 62 anecdotal moose cases investigated between 2003 and 2013 in Minnesota. Those authors reported 45% of moose had *P. tenuis* infections, 60% had evidence of liver flukes, and 23% had noticeable winter tick loads. Similarly, we found 42% of moose had *P. tenuis* infections, 76% had evidence of liver flukes (18 marked, 13 moderate, and 20 mild infections of 76 cases evaluated), and 21% had noticeable winter tick loads (4 marked, 5 moderate, and 2 mild infestations of 52 cases evaluated).

Parelaphostrongylus tenuis infections occurred in anecdotal moose at nearly twice the rate of collared moose during the same 5-year time period in this study. This is likely due to a sightability bias for *P. tenuis*-exposed moose, as the infection causes animals to seek open areas (roads, train tracks, fields, pastures, logging openings) for prolonged periods of time, which greatly enhances opportunities for humans to see them and report sick moose. In some

cases, these brainworm-infected moose appeared to be stuck in the mud or stranded on ice-covered lakes and local wildlife staff would “save” these moose from their dire predicaments. Celebrations were often short-lived as these animals soon returned to compromising situations again and would be euthanized due to public safety concerns. In the collared moose study, it's likely *P. tenuis* infections were underestimated due to limited diagnostics in cases where carcasses were heavily scavenged or decomposition was too advanced. Thus, the true impact of *P. tenuis* on Minnesota's moose likely lies between 23-42%, and is clearly playing a key role in the population decline.

ACKNOWLEDGMENTS

This project was very demanding and would not have been possible without the assistance of the following groups and individuals: the Environment and Natural Resources Trust Fund and the Minnesota Department of Natural Resources for funding this project, Mike Schrage (Fond du Lac Natural Resources) and Andy Edwards (1854 Treaty Authority) for their assistance in the field and during captures, Richard Gerhold and Caroline Grunenwald (University of Tennessee) for assisting with the identification of microfilaria and *P. tenuis*, Ulrike Munderloh (University of MN, Department of Entomology) for testing samples for tick-borne illness, J. P. Dubey (USDA, ARS) for neospora and toxoplasma testing, our team of primary responders (Dave Pauly, Nancy Hansen, Dave Ingebrigtsen, Jessica Holmes, Bailey Petersen, and John Giudice; MNDNR), our team of secondary responders (Bob Fashingbauer, Bob Kirsch, Bryan Lueth, Carolin Humpal, Jim LaBarre, Leslie McInenly, Lindsey Shartell, Meadow Kouffeld-Hansen, Steve Piepgras, Tim Pharis, Tom Rusch, Ted Dick, Penny Backman, Marshall Deters, and Jeff Hines; MNDNR), Dan Ryan and Dave Grosshuesch (US Forest Service), Brandon Seitz (Grand Portage National Monument), EJ Issac and Seth Moore (Grand Portage Band), Lance Overland (Fond du Lac Resource Management Division), Nick Bogyo (1854 Treaty Authority), Bill Severud and Tyler Obermoller (UMN) for their assistance in the field, and the MNDNR enforcement pilots (Jason Jensen, John Heineman, Tom Buker, Chris Lofstuen, and Bob Geving) for their assistance during captures and collar blow-offs, USDA-Wildlife Services (Paul Wolf) for use of their necropsy trailer, and Kaytee Firnett, Jeanna Lodel, Beth Martin, Amanda McGraw, and Amy Kingsley for assistance with data management and gearing-up for captures. Rob Fasteland (MNDNR Forestry) and the Lake & Cook County Highway Department staff for snow plowing and maintaining helispots used during capture events. Special thanks to special operations staff for remote hook/sling and radio training, including Bill Schuster, Lee Kessler, Mike McLaughlin, Dustin Nelson and Pat Coughlin. This project was funded in part by the Wildlife Restoration (Pittman-Robertson) Program.

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Table 1. Sample size of active, added, dead, and censored moose annually, throughout the 5-year study, 2013-2017.

	2013	2014	2015	2016	2017
Active collars at start of year (n)	0	79	81	72	37
New collars added (n)	111	31	31	0	0
Non-hunting related deaths (n)	20	12	13	6	3
Censored moose (n):					
• Capture-related deaths	4	3	5	0	0
• Hunting-related deaths	0	0	1	1	1
• Slipped collars	1	1	1	0	0
• Transmission failures/missing animals	7	13	16	28	15
Active collars at end of year (n)	79	81	72	37	18

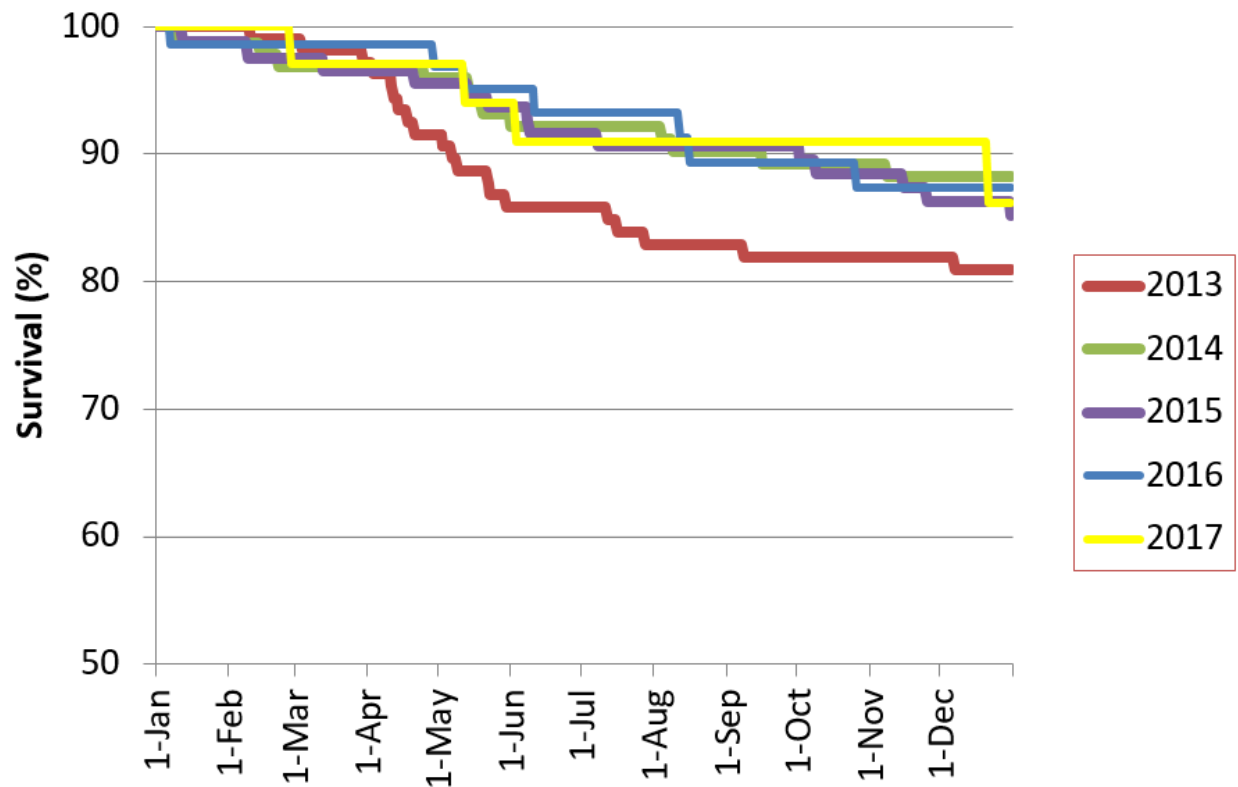


Figure 1. Annual survival of collared, adult moose ($n=173$) captured from 2013-2017, northeast Minnesota.

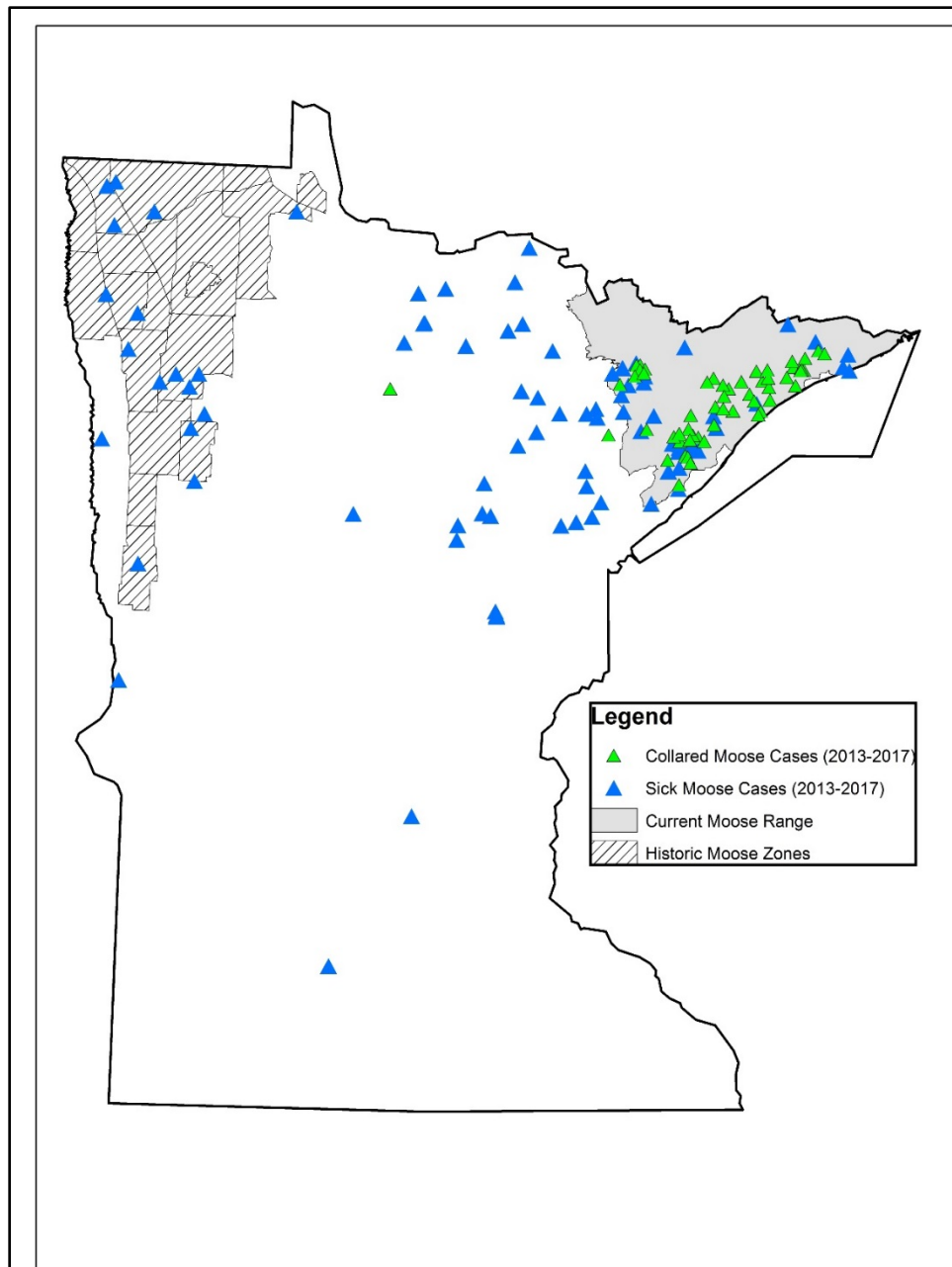


Figure 2. Location where mortalities were investigated for collared ($n=60$) and anecdotal moose ($n=91$) in Minnesota, 2013-2017.

Proximate Causes of Adult Moose Mortalities Feb 2013-Feb 2018 ($n=60$)

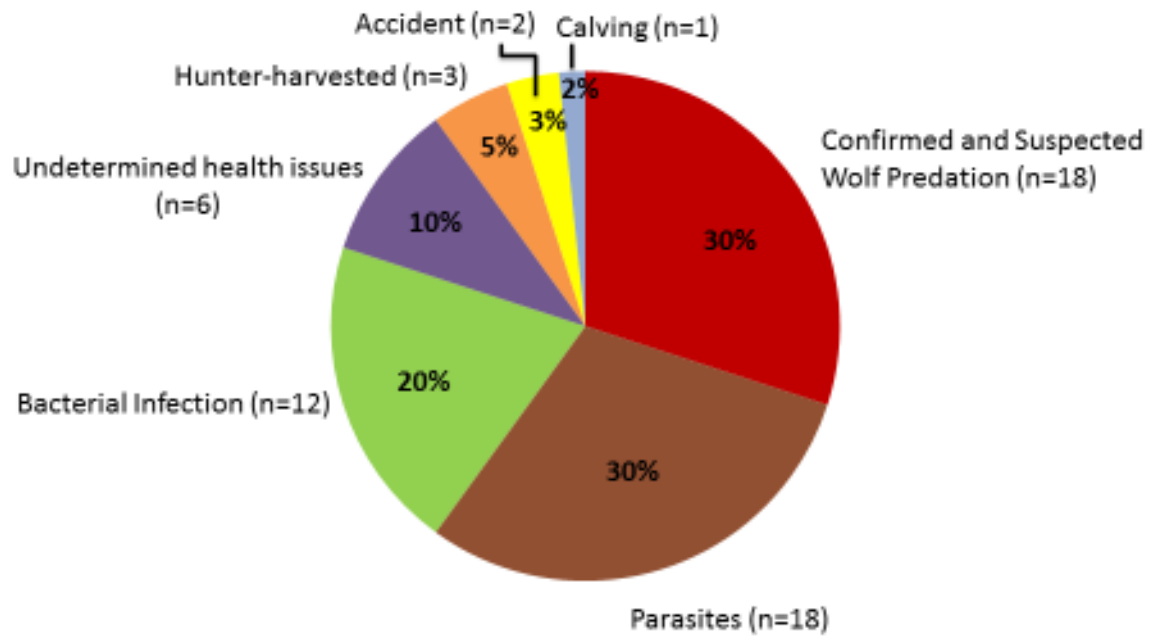


Figure 3. Cause-specific mortality of collared, adult moose ($n=60$) from 2013–2017, northeast Minnesota.

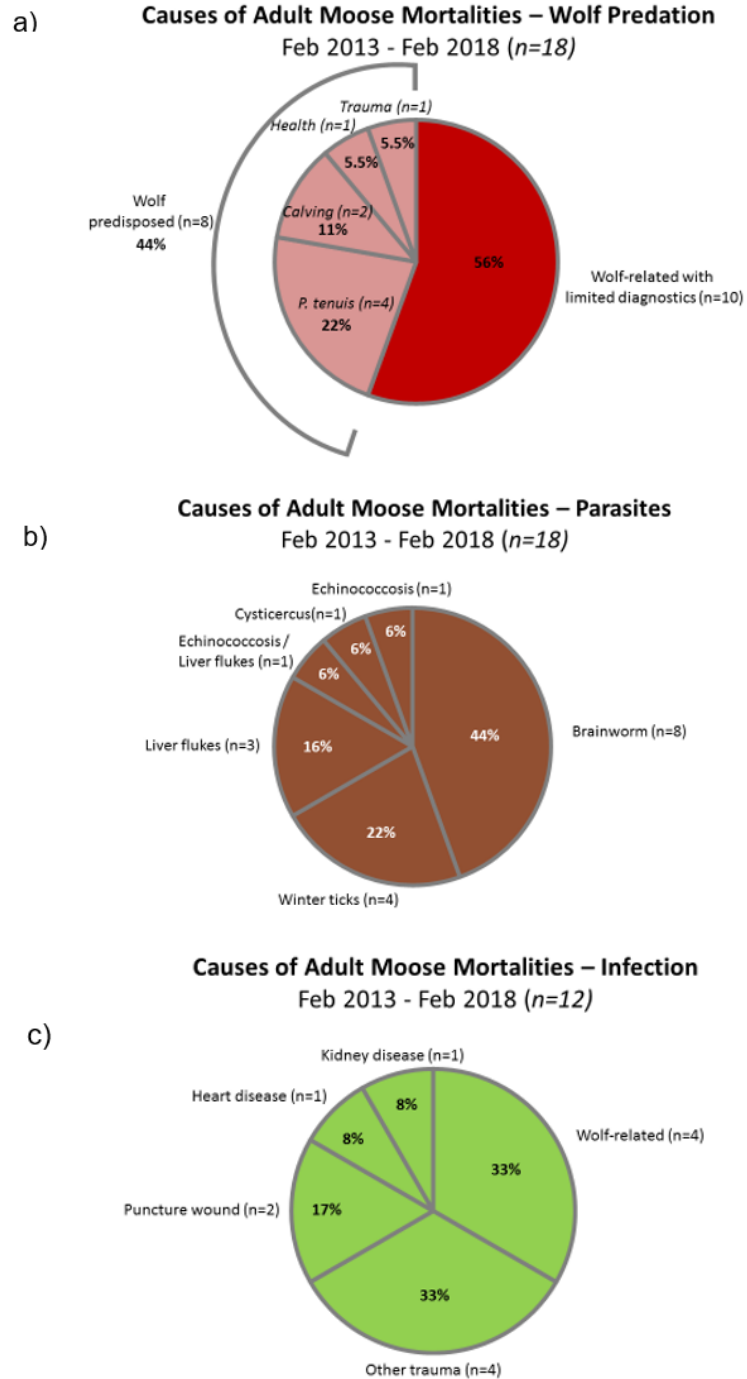


Figure 4. Breakdown of adult moose mortalities caused by wolf predation (a), parasites (b), and bacterial infections (c), 2013–2017, northeast Minnesota.

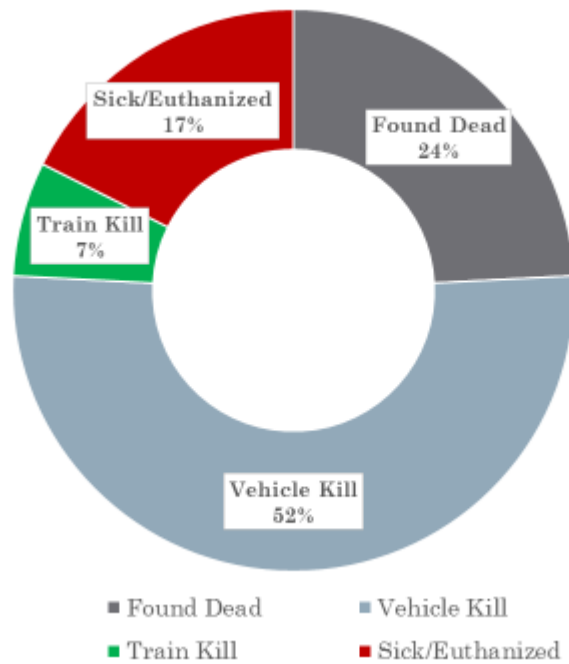


Figure 5. Causes of anecdotal moose ($n=91$) from 2013–2017 in Minnesota.

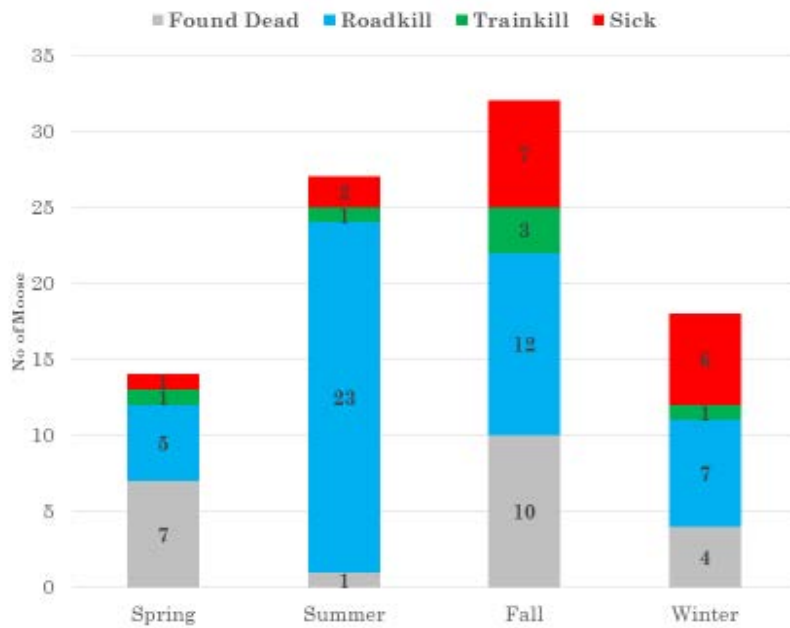


Figure 6. Seasonal variation in the causes of anecdotal moose deaths ($n=91$) in Minnesota from 2013–2017.

SURVEILLANCE FOR HIGHLY PATHOGENIC AVIAN INFLUENZA IN MINNESOTA'S WILD BIRDS IN 2017-18

Chris Jennelle¹, Tim White², Kelsie LaSharr, Patrick Hagen, Erik Hildebrand, Tom Cooper³, and Michelle Carstensen

SUMMARY OF FINDINGS

Surveillance for highly pathogenic avian influenza (HPAI) virus in wild birds is a national priority in the United States. The HPAI outbreak of 2015 resulted in culling of over 50 million domestic turkeys and chickens and had an economic cost on the order of a billion dollars. Outbreaks of HPAI continue to occur in domestic poultry, wild birds, and people around the globe, and there is continued concern for the introduction of HPAI viruses into North America. Minnesota Department of Natural Resources (MNDNR) has partnered with the United States Department of Agriculture's Wildlife Services (USDA-WS) since 2007 to conduct HPAI surveillance in wild birds, and in 2015 a highly pathogenic strain of H5N2 was detected in Minnesota. The H5N2 HPAI virus strain discovered in Minnesota was a combination of the highly pathogenic Eurasian H5 and low pathogenic North American H2 subtypes. Since the 2015 HPAI outbreak, which affected 110 poultry facilities and led to the euthanasia of more than 9 million turkeys and chickens in Minnesota, MNDNR extended its partnership with the United States Geological Survey's National Wildlife Health Center (USGS), the United States Fish and Wildlife Service (USFWS), the Michigan State University Veterinary Diagnostic Lab (MSU), and the University of Minnesota (UMN) to conduct surveillance for HPAI virus subtypes in Minnesota wild birds. Only one wild bird, a Cooper's hawk (*Accipiter cooperii*), was confirmed with the HPAI H5N2 strain in 2015 and was likely infected as a spillover from the poultry infections in the area. Since June 2015, there have been no detections of HPAI in MN poultry facilities or in wild birds sampled in Minnesota. As part of the USDA National Avian Influenza Surveillance plan, from May 2017 through March 2018, the MNDNR and partners collected cloacal and oropharyngeal swab samples (combined) from 1,130 dabbling ducks across 6 watersheds; AI viral material was detected in 15.4% of these samples. Only 1.5% and 0.2% of all samples contained detectable H5 and H7 viral material, respectively. The highest apparent prevalence of low pathogenicity avian influenza (LPAI) was in the St. Croix watershed at 20%, and the lowest was 1% in the Red watershed. Only 5 successful viral isolates of LPAI virus were recoverable and included H5N2 (Mississippi Headwaters watershed), H7N3 (Minnesota watershed), H3 (St. Croix watershed), N2 (St. Croix watershed), and N8 (St. Croix watershed). No HPAI positive cases were detected. In addition, the MNDNR partnered with UMN, USFWS, and USDA-WS in a study led by the UMN to investigate avian influenza dynamics in ring-billed gulls (*Larus delawarensis*) across Minnesota.

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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 of 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 860 humans around the world, resulting in 454 reported deaths (World Health Organization 2018). Another strain of HPAI, H7N9, emerged in 2013 and has been confirmed in 1,625 people (mostly in eastern Asia) with 623 deaths (Food and Agriculture Organization 2018). As of February 28 2018, there were 8 countries and territories reporting HPAI outbreaks in wild birds; these included Denmark, Hong Kong, India, Ireland, Netherlands, South Africa, Sweden, and the United Kingdom reporting strains of the subtypes H5N1, H5N6, and H5N8 (OIE 2018). There were 37 outbreaks of 6 HPAI subtypes in domestic poultry operations around the world (World Organisation for Animal Health 2018). There have been no HPAI outbreaks in the US since March 2017, when an American strain of H7N9 was confirmed in domestic poultry facilities in Tennessee (USDA 2017). These results highlight that HPAI viruses continue to be active around the world and pose a threat to both wild birds and domestic poultry. The diversity of active highly pathogenic subtypes, coupled with the ability of avian influenza strains to mutate quickly underscores the pandemic risk from these viruses. As such, there is an urgent need to understand transmission dynamics, host-species susceptibility, the role of wild birds in transmission, and the role of the environment in AI dynamics.

The migratory movements of waterfowl and other shorebirds and subsequent mixing of birds from Asia and North America in the northern latitude breeding grounds likely facilitated the mixing of LPAI and HPAI strains in 2014 (Pasick et al. 2015). Such mixing resulted in discovery of 3 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 in 2014.

In August and December of 2016, HPAI H5N2 was again detected in wild waterfowl in Alaska and Montana, respectively. In addition, there were several detections of HPAI H7N9 (distinct from Asian strain) that affected domestic poultry facilities in the Mississippi flyway. Since July 2015, there have been 112,734 wild waterfowl tested for avian influenza in the United States as part of national surveillance efforts and only four positive HPAI detections have occurred. Since the first Minnesota detection of HPAI H5N2 in a domestic poultry farm in March 2015, the MNDNR has collected over 8,600 samples from wild birds and the environment for AI testing with only one positive detection (Cooper's hawk with H5N2 in 2015). Our efforts to detect HPAI in wild birds, if present, have included live-bird and hunter-harvest sampling of waterfowl, environmental sampling, and the continued monitoring of morbidity and mortality events. These efforts permit the estimation of temporal and spatial detection limits for AI on the Minnesota landscape, which leads to development of specific hypotheses that can help us understand AI dynamics in wild birds. From a broader perspective, our efforts also help to address the role that wild birds may play in HPAI dynamics.

METHODS

We collected samples for AI testing from 3 sources: public- or agency-reported morbid or dead wild birds (i.e., morbidity and mortality events), live-captured and released ducks through banding programs, and hunter-harvested ducks. Dabbling ducks were primarily sampled, including mallard (*Anas platyrhynchos*), blue-winged teal (*A. discors*), American green-winged

teal (*A. crecca*), American wigeon (*A. americana*), gadwall (*A. strepera*), American black duck (*A. rubripes*), northern pintail (*A. acuta*), northern shoveler (*A. clypeata*), and wood duck (*Aix sponsa*). Morbidity and mortality samples were collected statewide but depended on opportunistic circumstances and public willingness to report or submit dead birds. Sampling live wild ducks and hunter-harvested ducks afforded more control over sampling design elements; both spatial and temporal dimensions were within our design control.

USDA National Plan Sampling

As part of the 2017 USDA National Surveillance Plan, which called for 1,140 oropharyngeal/tracheal and cloacal swab samples from dabbling ducks in MN, the MNDNR partnered with USFWS, USDA-WS, and MSU to achieve the sample goal between summer and winter 2017. The samples collected were broken down by watershed (Minnesota, Mississippi Headwaters, Red, St. Croix, Upper Mississippi – Black Root, and Western Lake Superior) and season (summer, fall, and winter). The source of samples was from live waterfowl or hunter-harvested waterfowl. We collected swab samples from the oropharyngeal cavity or trachea (depending on sampling live or dead birds) and cloacal cavities of each bird in order to test for viral shedding. Both swab samples from an individual bird were placed in the same brain-heart infusion (BHI) media, and kept cool in a portable cooler with ice packs or a refrigerator. Samples were shipped overnight to the MSU for avian influenza virus (AIV) testing using a real time reverse transcription polymerase chain reaction (rRT-PCR) matrix test, which tests for type-A influenza virus RNA. Material from positive matrix tests were further tested with an H5 and H7 assay. If either H5 or H7 assay were positive, the remaining sample material was sent to the National Veterinary Services Laboratories in Ames, IA for confirmation and strain-typing.

Morbidity and Mortality Sampling

Through outreach on the MNDNR and Minnesota Board of Animal Health websites and official press releases, we solicited the public and agency staff to report any wild birds exhibiting neurological symptoms consistent with AIV infection anywhere in the state. We investigated reports of dead ducks if circumstances of mortality were unclear and if individuals showed neurologic signs. 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 and swabs), we either collected whole carcasses (double-bagged and frozen) or swabs from the trachea and cloaca of dead birds. Both swab samples from a morbidity/mortality sample bird were placed in the same BHI media, and kept cool in a portable cooler with ice packs or a refrigerator. Whole carcasses were shipped overnight to the USGS National Wildlife Health Center or the University of Minnesota's Veterinary Diagnostic Laboratory for necropsy and AIV testing using real time reverse transcription polymerase chain reaction (rRT-PCR) test. Swab samples were submitted to the USDA National Wildlife Disease Laboratory in Fort Collins, CO. If initial screening of samples via the matrix PCR test were AIV positive at any lab, these samples were forwarded to the National Veterinary Services Laboratories in Ames, IA for confirmation and strain-typing. We had no fixed sample goal for this surveillance effort due to the opportunistic nature of public discovery and reporting of sick or dead birds. We used these data as an auxiliary source of information in our surveillance efforts.

RESULTS AND DISCUSSION

From May 2017 through March 2018, the MNDNR in partnership with USDA-WS and the USFWS collected 1,130 oropharyngeal/tracheal and cloacal samples from dabbling ducks across 6 watersheds of Minnesota (Figure 1). We were short 24 samples for the St. Croix watershed, 33 samples for the Western Lake Superior watershed, and 7 samples for the Mississippi Headwaters (Table 1). However, we exceeded the watershed quota by 31 for the

Minnesota watershed, 21 for the Upper-Mississippi Black Root watershed, and 2 samples for the Red watershed (Table 1). We sampled 577 mallards, 369 wood ducks, 153 blue-winged teal, 20 American green-winged teal, 5 gadwall, 3 northern pintail, 1 American wigeon, 1 northern shoveler, and 1 mallard/black duck hybrid. While about 15.4% of all samples tested positive for LPAI, this aligns with expectations of type-A avian influenza prevalence in waterfowl (Webster et al. 1992). Only 5 LPAI subtypes were isolated (2.9% of all positive samples), and they included H5N2, H7N3, H3, N8, and N2. These results underscore the difficulty of acquiring enough viral material in swab samples to successfully identify AI subtypes. We did not detect HPAI virus in any samples. Of particular note is the apparent prevalence of H5 and H7 LPAI subtypes across all samples at 1.5% and 0.2% - these subtypes are typically considered to be at highest risk for becoming highly pathogenic.

From June 16, 2017 through June 14, 2018, we collected 16 morbidity and mortality samples from wild birds. Of these submissions (2 Canada geese, 8 mallards, 4 ring-billed gulls, and 2 trumpeter swans), none tested positive for HPAI (Table 2).

Since the outbreak of HPAI began in Minnesota poultry in March 2015, the MNDNR and partners have collected and tested over 8,600 wild bird and environmental samples for HPAI, which included waterfowl feces (Jennelle et al. 2016), reported wild bird mortalities, hunter-harvested waterfowl, live waterfowl, and hunter-harvested wild turkeys (Jennelle et al. 2017). To date, there has been only one confirmed HPAI H5N2 positive result, a likely spillover species – Cooper's hawk (predator of small birds) (Jennelle et al. 2016). The positive hawk was discovered in 2015, 12 miles from an infected poultry facility. The final report on the 2014-2015 HPAI outbreak in the U.S., the largest outbreak in the U.S. to date, indicated that 7.4 million domestic turkeys and 43 million egg-layers/pullet chickens were impacted, costing nearly a billion dollars for the response, indemnity, and future preparedness actions (USDA 2016b). The report highlights poultry facility biosecurity as a major concern and likely contributor to the spread and broad impact of the outbreak (USDA 2016b).

Current Projects and Future Surveillance

The MNDNR collaborated on a Legislative-Citizen Commission on Minnesota Resources (LCCMR) funded project, led by Dr. Marie Culhane of UMN, to investigate AI prevalence, exposure, and potential health effects on ring-billed (*Larus delawarensis*) and herring (*L. argentatus*) gulls across Minnesota. Other partners in this effort included the USFWS, USDA-WS, and MN Turkey Growers Association. The study began in fall 2016 with data collection through fall 2017 and led by Master's degree student Todd Froberg. Field sampling efforts focused on capture and sampling of ring-billed gulls at landfills and breeding colonies across MN. The research team collected oropharyngeal, cloacal, and blood samples from over 1,346 ring-billed gulls across the study area. The results suggest significant seasonal, spatial, and age-specific heterogeneity in type-A influenza apparent prevalence (Froberg 2018). Furthermore, the results show that there is significant variation in apparent prevalence between samples collected from the oropharyngeal and cloacal cavities.

At this point in time, there are no plans to continue the USDA national surveillance effort in 2018 to detect HPAI. In the future, if there is sufficient cause and available funding for AI surveillance efforts, Minnesota will participate in sampling dabbling ducks according to protocols outlined by the USDA.

The MNDNR sampling and testing of morbidity and mortality events is ongoing. We continue to apply a risk-based approach to AIV surveillance in wild birds designed to respond to new detection events in a rapid and efficient manner. Three triggers initiate intensive, spatially and temporally designed AI surveillance efforts if HPAI virus is detected in (1) wild, migratory birds in

Minnesota through ongoing morbidity and mortality surveillance, (2) wild migratory birds in the Mississippi flyway, or (3) commercial or backyard poultry in Minnesota.

ACKNOWLEDGEMENTS

These efforts would not have been possible without the valuable contribution of the Wetland Wildlife Population and Research Group including J. Lawrence, and B. Davis. MNDNR management and research staff were invaluable in providing guidance for identifying sampling locations and capture/sampling assistance. We recognize our USDA-WS partners B. Welinski, D. Pauly, and assistants; USFWS partners M. Stefanski, and J. Schmit; MSU-VDL partner Suzanne Mason, and USGS partners B. Bodenstein and H. Ip for their assistance in diagnostic testing needs. We recognize our UMN collaborators T. Froberg, F. Cuthbert, B. Rasmussen, and M. Culhane. We also thank all of waterfowl 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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http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/OIE_AI_situation_report/OIE_SituationReport_AI_February2018.pdf

Table 1. Avian influenza swab results ($n = 1,130$) from Minnesota participation in the 2017 USDA National Avian Influenza Surveillance plan *.

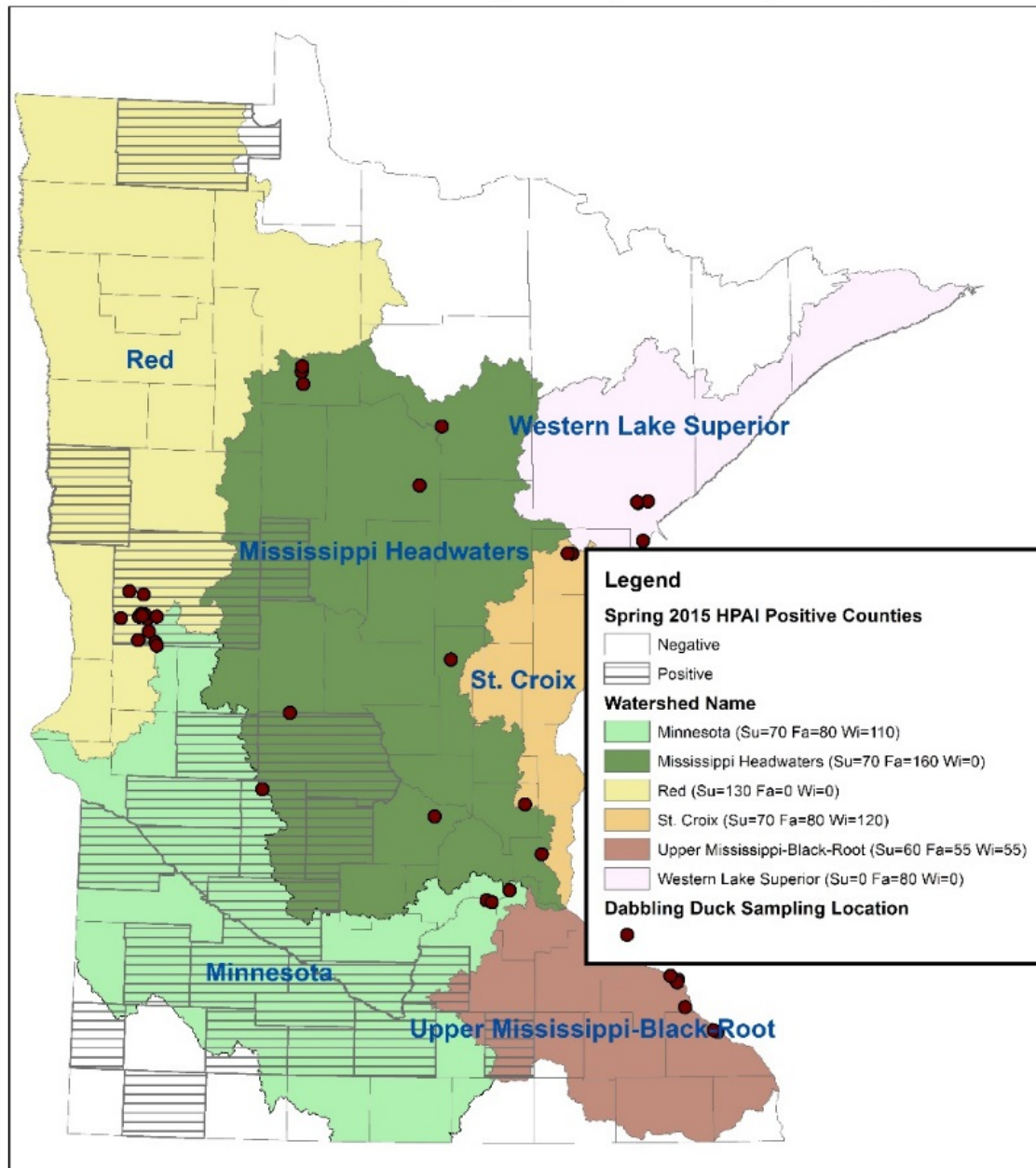
Watershed	n	Type-A	LPAI %	95% C.I.
Minnesota	291	46	15.8	11.8, 20.5
Mississippi Headwaters	223	37	16.6	12.0, 22.1
Red	132	1	0.8	0.1, 4.1
St. Croix	246	48	19.5	14.8, 25.0
Upper Mississippi–Black Root	191	35	18.3	13.1, 24.6
Western Lake Superior	47	7	14.9	6.2, 28.3
TOTAL	1,130	174	15.4	13.3, 17.6

*There were only five successful isolations of type-A influenza completed from these samples H5N2, H7N3, H3, N2, and N8.

Table 2. Species and count of wild bird morbidity & mortality samples ($n = 16$) submitted by the Minnesota Department of Natural Resources for avian influenza testing from June 16, 2017 to June 14, 2018. No birds tested positive for HPAI.

Agency	Species sampled	n
MNDNR	Canada goose (<i>Branta canadensis</i>)	2
	Mallard (<i>Anas platyrhynchos</i>)	8
	Ring-billed gull (<i>Larus delawarensis</i>)	4
	Trumpeter swan (<i>Cygnus buccinator</i>)	2
Total		16

2017 USDA Avian Influenza Surveillance: Summer (n=400) Fall (n=455) Winter (n=285)



Date: 4/20/2018

0 15 30 60 90 120 Miles

mn DEPARTMENT OF
NATURAL RESOURCES

Figure 1. The United States Department of Agriculture (USDA) allocation of targeted Minnesota watersheds for avian influenza sampling (n=1,140) for summer, fall, and winter 2017. The three sample sizes noted beside watersheds in the legend are the quotas requested by USDA for summer, fall, and winter sampling, respectively. Points represent locations of sampling.

AVIAN INFLUENZA PREVALENCE AND VIRAL SHEDDING ROUTES IN MINNESOTA RING-BILLED GULLS (*LARUS DELAWARENSIS*)¹

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ABSTRACT

Birds within the orders Charadriiformes (shorebirds; gulls) and Anseriformes (waterfowl) are reservoir hosts for avian influenza (AI) viruses, but their role in the transmission dynamics of AI viruses is unclear. To date, waterfowl have been the predominant focal species for most surveillance and epidemiological studies, yet gulls, in particular, have been shown to harbor reassortant AI viruses of both North American and Eurasian lineages and are underrepresented in North American surveillance efforts. To address this gap in surveillance, 1346 ring-billed gulls (*Larus delawarensis*) were sampled during spring and fall migrations and at three breeding sites in 2017 across Minnesota. Results indicate noticeable age-cohort dynamics in AI virus apparent prevalence within ring-billed gulls in Minnesota. Immunologically naïve juveniles represented the cohort with the highest prevalence rate (57.8%). Regardless of age, more gulls had AI virus detected in oropharyngeal (OP) swabs than in cloacal (CL) swabs. The high AI virus prevalence within ring-billed gulls, particularly in immunologically naïve birds, warrant further targeted surveillance efforts of ring-billed gulls and other closely related species.

¹ Avian Diseases. 2018. Accepted article.

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EMERGENCE OF THE ARTERIAL WORM *ELAEOPHORA SCHNEIDERI* IN MOOSE (*ALCES ALCES*) AND TABANID FLY VECTORS IN NORTHEASTERN MINNESOTA, USA.¹

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ABSTRACT

Moose (*Alces alces*) are a culturally and economically valued species in Minnesota. However, the moose population has experienced a sudden, marked decline in their range including extirpation in the northwest and a 58% decline in the last decade in the northeast portions of the state. Although the exact cause of this decline is unclear, parasitic metastrongylid and filarioid nematode infections are known causes of morbidity and mortality in moose across North America. To determine if these parasitic nematodes could be contributing to the Minnesota moose population decline, we molecularly tested for their presence in tissues obtained from moose that died of known and unknown causes. Extracted brain DNA of 34 individual moose was amplified utilizing primers targeting the 18S rRNA gene and internal transcribed spacer regions of nematodes. DNA sequencing revealed that PCR products obtained from 15 (44.1%) of the moose were 99% identical to *Parelaphostrongylus tenuis*, a metastrongylid known to cause neurological disease and death. Additionally, brain tissue from 20 (58.8%) individuals yielded sequences that most closely aligned with *Elaeophora schneideri*, a parasite associated with neurological impairment but previously unreported in Minnesota. *Setaria yehi*, a common filarioid parasite of deer, was also detected in the brain tissue from 5 (14.7%) moose. Molecular screening of 618 captured tabanid flies from four trapping sites revealed *E. schneideri* was present (6%) in the Minnesota environment and transmission could occur locally. Prevalence rates among the flies ranged between 0-100% per trapping site, with *Chrysops* spp. and *Hybomitra* spp. implicated as the vectors. Together, these data demonstrate the pathogens *P. tenuis* and *E. schneideri* are present in the Minnesota population and suggest these nematodes could be contributing to morbidity and mortality in moose.

¹Parasites and Vectors. 2018. Accepted Article

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APPLYING A BAYESIAN WEIGHTED SURVEILLANCE APPROACH TO DETECT CHRONIC WASTING DISEASE IN WHITE-TAILED DEER¹

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ABSTRACT

Surveillance is critical for early detection of emerging and re-emerging infectious diseases. Weighted surveillance leverages heterogeneity in infection risk to increase sampling efficiency. Here we apply a Bayesian approach to estimate weights for 16 surveillance classes of white-tailed deer in Wisconsin, USA, relative to hunter-harvested yearling males. We used these weights to conduct a surveillance program for detecting chronic wasting disease (CWD) in white-tailed deer at Shenandoah National Park (SHEN) in Virginia, USA. Generally, for surveillance, risk of infection increased with age and was greater in males. Clinical suspect deer had the highest risk, with weight estimates of 33.33 and 9.09 for community-reported and hunter-reported suspect deer, respectively. Fawns had the lowest risk with an estimated weight of 0.001. We used surveillance weights for Wisconsin deer to determine sampling effort required to detect a CWD-positive case in SHEN if prevalence in yearling males ≥ 0.025 . The sampling required to detect CWD was 37–91 adult deer, depending on the adult male:female ratio in the surveillance stream. We collected rectal biopsies from 49 female and 21 male adult deer, and 10 additional samples from vehicle-killed deer. CWD was not detected and we concluded with 95% probability that prevalence in the reference population (yearling males) was between 0.0 to 3.6%. *Synthesis and applications.* Our approach allows managers to estimate relative surveillance weights for different host classes and quantify limits of disease detection in real time when only a sample of animals from a population can be tested, resulting in considerable cost savings for agencies performing wildlife disease detection surveillance. Additionally, it provides a rigorous means of estimating prevalence limits when a disease/pathogen is not detected in a sample set. It is therefore applicable to other wildlife, domestic animal and human disease systems, which can be characterized by surveillance classes with heterogeneous probability of infection. This methodology is also extendable to other disciplines such as invasive species, environmental toxicology, and generally any ecological question seeking to efficiently use scarce financial and human resources to maximize the detection probability of a rare event.

¹ Journal of Applied Ecology. 2018. Early View: <https://doi.org/10.1111/1365-2664.13178>

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CHRONIC WASTING DISEASE DETECTION AND MORTALITY SOURCES IN A SEMI-PROTECTED DEER POPULATION¹

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ABSTRACT

Surveillance for wildlife diseases is essential for assessing population dynamics of ungulates, especially in free-ranging populations where infected animals are difficult to sample. Chronic wasting disease (CWD) is an emerging infectious disease of concern because of the potential for substantial negative effects on populations of cervids. Variability in the likelihood that CWD is detected could invalidate traditional estimators for prevalence. In some instances, deer located after death cannot be tested for infectious diseases, including CWD, because of lack of availability or condition of appropriate tissues. We used various methods to detect infectious diseases that could cause mortality for deer (*Odocoileus* spp.) residing in Wind Cave National Park, South Dakota, USA, and we report survival estimates for animals in this population. We included 34 monthly encounters of deer resightings and 67 mortalities. We tested live deer by tonsillar biopsy for CWD and estimated pooled prevalence (mean \pm SE) at $5.6 \pm 3.0\%$ over the three-year study. Live deer potentially had exposure to several infectious diseases, including bluetongue, epizootic hemorrhagic disease, bovine viral diarrhea, West Nile virus, and malignant catarrhal fever, but no apparent morbidity or mortality from those diseases. We tested survival and influence of covariates, including age and sex, using known-fate analysis in Program MARK. Those data best supported a model with time-invariant encounter probability and an annual survival of 72.8%. Even without direct pressure from hunting within the park, average life expectancy in this population was 3.2 years. Only 68% of mortalities contained sufficient material for CWD sampling (because of predation and scavenger activity) and >42% of these were CWD-positive. These findings underscore the possible biases in postmortem surveillance estimates of disease prevalence because of potential for subclinical infected animals to be removed by predators and not tested.

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A TECHNIQUE FOR DEPLOYMENT OF RUMEN BOLUS TRANSMITTERS IN FREE-RANGING MOOSE (*ALCES ALCES*)¹

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ABSTRACT

Recent uses for rumen boluses, such as mortality implant transmitters (MITs), in wildlife have made it necessary to adapt deployment techniques developed for livestock. In 29 and 30 attempts to place MITs in Minnesota free-ranging moose (*Alces alces*) in 2013 and 2014, respectively, success was achieved 83% and 63% of the time. In 2014, new methods for MIT deployment were evaluated in captive moose in Alaska. Mandible measurements provided guidance for selection of an appropriate-sized bolus applicator. A Schulze mouth gag was used to aid insertion of the applicator, and canola oil was used to lubricate the bolus to facilitate swallowing. Time to first swallow and time to continuous swallow following sedative reversal was measured to gauge appropriate timing for bolus administration. Using the adapted technique with trained personnel, success rates for MIT deployment were 100% (10/10) for captive moose and 88% (21/24) for free-ranging moose in Minnesota in 2015.

¹ Journal of Zoo and Wildlife Medicine. 2018. 49(1): 227-230

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EXPANDING GIS ANALYSES TO MONITOR AND ASSESS NORTH AMERICAN MOOSE DISTRIBUTION AND DENSITY¹

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ABSTRACT

Development of long-term geographic information system (GIS) databases of species densities and distributions, combined with biological, ecological, and management-related metrics, can help guide research and management strategies. Here we summarize 3 decades of North American moose (*Alces alces*) population and harvest densities collected at the management unit scale for the years 1980, 1990, 2000, and 2010. A summary analysis of these data indicates that moose have both expanded and contracted along their southern range boundary in recent decades - including the Prairie Provinces and states, and much of the northeastern United States. A narrow band of relatively stable and high-density moose populations extends from central Alaska across the Prairie Provinces, and east to the Maritime Provinces and upper New England states. Distributions in 2010 indicate that moose now occupy an area >9,492,000 km² in North America. We also identified that a core range of boreal habitat, only 30% of the occupied range across the continent, supports 89% of the estimated 1 million moose in North America. Time-series analyses can offer a simple and cost-effective approach to monitor the status of moose populations in North America, and might be particularly insightful given the current and predicted future influences of climate change on moose. Other analyses might address population dynamics, habitat, environmental constraints, and harvest management, among other issues. We encourage jurisdictions to cooperate strategically in implementing and coordinating GIS analyses to monitor, assess, and manage the North American moose population.

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CALIBRATION OF A RUMEN BOLUS TO MEASURE CONTINUOUS INTERNAL BODY TEMPERATURE IN MOOSE¹

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ABSTRACT

Mortality implant transmitters (MITs), a device that can record continuous rumen temperature, have been deployed in wild moose (*Alces alces*) in Minnesota to understand physiological and behavioral responses of moose to increasing ambient temperatures. We compared temperatures collected using MITs to temperatures collected using vaginal implant transmitters in 10 captive female moose (>2 years old) at the Kenai Moose Research Center in Alaska. Both devices collected continuous body temperature measurements at 5 min intervals for 1 year. We directly observed moose behavior for a total of 384 hours during four, two-week windows distributed seasonally within the sampling period, to assess potential effects of behaviors on MIT-recorded temperatures. We documented a decrease in MIT-recorded temperatures following water intake and developed an approach for censoring these observations. After removing these observations, MIT-based temperatures were, on average, 0.03°C (95% CI = -0.57–0.55°C; \bar{x} = 38.14°C) lower than VIT-based temperatures (\bar{x} = 38.17°C; n = 760,439). We fit linear mixed-effects models to explore the relationship between MIT and VIT-based temperatures across seasons and individuals. On average, the difference between predicted and observed temperatures was 0.05°C (95% PI = -0.19–0.29°C) and 0.33°C (95% PI = 0.01–0.63°C) for winter and summer seasons, respectively. We conclude that minimally invasive MITs can accurately record internal body temperature in moose, and thus provide a tool for understanding the physiological and behavioral responses of moose to environmental stressors.

¹Wildlife Society Bulletin. 2018. 42(2): 328-337.

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FALL MOVEMENTS OF MALLARDS MARKED IN MINNESOTA

Bruce E. Davis

SUMMARY OF FINDINGS

During August-September of 2016, I marked 119 mallards (*Anas platyrhynchos*) with tracking units. I obtained GPS locations from dataloggers recovered by hunters or uploaded through the Argos satellite system to yield 3506 tracking locations. Locations within the state of Minnesota ($n=2848$) were used to examine habitat use. Marked birds were retained in Minnesota longer than expected; freeze up dates were later than average in 2016. When marked birds did leave the state, movements upon departure tended to be long with a mean distance 434 km between a bird's last known location in Minnesota and its first known location outside of Minnesota. Marked birds used open water and emergent herbaceous wetland habitats for combined 55-80% of the time. Crop habitats were used most frequently at night (30% of proportional use) and more frequently for birds marked in the south hunting zone than for birds marked in the north hunting zone. Sample sizes were sufficient to detect differences in use of habitats among capture zones and time of day, but I did not detect differences in emigration rates between zones; given the late onset of winter, rates of emigration may have been similar between zones. For birds marked in 2016, biologically relevant differences were detectable when present.

During August-September of 2017, I marked 90 mallards with tracking units. Seventy-nine of these units were GPS units that transmitted through the Argos system; the other 11 units were GPS dataloggers. Herein, I present summaries of preliminary analyses for birds marked in 2016, but have not yet completed analyses for birds marked in 2017.

INTRODUCTION

Distribution of waterfowl during fall migration and concurrent hunting seasons is affected by numerous factors. Wildlife managers are tasked with setting season dates, bag limits, shooting hours, and further restrictions on harvest. Availability of waterfowl throughout the hunting season (retention) is important to Minnesota waterfowl hunters. Understanding the chronology of immigration and emigration events and the factors affecting those events is imperative.

Many factors may impact emigration rates and use of habitats. Weather plays an important role in the timing of migration by waterfowl during fall; as winter weather severity increases, the probability of southward waterfowl migration also increases (Schummer et al. 2010). Repeated exposures to disturbance associated with hunting have been found to alter the distribution and habitat use and cause increased movements of wintering waterfowl (Dooley et al. 2010, Pease et al. 2005), but the effects of disturbance have not been investigated for waterfowl nearer their breeding habitats. Importantly, the effects of weather and anthropological disturbance are likely confounded; hunting seasons often coincide with changing weather patterns. In the presence of elevated human disturbance to waterfowl habitats that occurs during hunting seasons, it may be difficult to detect causes of temporal or spatial changes to a bird's natural migration pattern. Numerous studies have been implemented to understand aspects of breeding waterfowl and some information is available on wintering waterfowl, but little work has been completed on

waterfowl during migration periods. Due to their transient nature, waterfowl are inherently difficult to study during the migration periods. Thus, few studies have been undertaken to investigate patterns of fall migration.

In an effort to provide habitat to local and migrating waterfowl, retain waterfowl on the landscape throughout the duration of the season, provide hunting opportunities for its constituents, and to control waterfowl harvest, the Minnesota Department of Natural Resources has implemented numerous restrictions on duck harvest and disturbance to wetlands. Restrictions include establishment of waterfowl refuges, a 4 PM closure to duck hunting for the earliest portion of the duck season, designation of feeding and resting areas which restrict the use of motorized boats, a statewide ban on motorized decoys for the earliest portion of the season, and a ban on motorized decoys on state owned Wildlife Management Areas (WMA) for the entire season.

The importance of the hunting regulations implemented in Minnesota to provide secure areas for ducks is unclear because fall emigration and factors affecting the chronology of fall migration are poorly understood. Restrictions on afternoon shooting hours unilaterally in Minnesota did result in 3-4% lower recovery rates (a proxy for harvest rates) than when sunset closures occurred, but the researchers were unable to detect a difference in annual survival rates (Kirby et al. 1983). Restrictions on shooting hours that are more restrictive than what is allowed in the federal framework have been in place since 1973 but their importance is unknown. Assessment of the effects of shooting hour restrictions and other hunting regulations on movement patterns warrants investigation. Better understanding of movement patterns gained from this work will allow managers to better set season dates and alter restrictions on harvest.

OBJECTIVES

Overall study objectives were to:

1. Better understand emigration chronology for mallards in Minnesota.
2. Estimate distances and directions moved by mallards in Minnesota.
3. Identify migration stopovers used by mallards in Minnesota.
4. Estimate use of habitats for birds while in Minnesota.

More specifically, during the pilot-year of this study, we sought to inform subsequent years of data collection by addressing these specific objectives:

5. Estimate variability in emigration, movement, and habitat use data within and among hunting zones.
6. Estimate rate of sample size reduction throughout the tracking period.
7. Evaluate alternative tracking units in terms of data quantity and quality.

STUDY AREA

Currently, Minnesota utilizes 3 zones to manage duck hunting seasons (Figure 1). Timing of seasons and restrictions on shooting hours differ among the zones. I attempted to mark equal numbers of birds in each hunting zone, but was unable to mark birds in the central zone in 2016. In 2017, I marked 59, 20, and 11 birds in the north, central, and south zones, respectively.

METHODS

Marking

In 2016, I attached 39 GPS-Argos backpack units (Lotek Wireless Inc., Newmarket, Ontario, Canada) to adult female mallards; these units logged GPS data and then transmitted that data back to the Argos system upon completion of their duty cycle. These units were 15 g and able to record about 100 GPS fixes and transmit those fixes to Argos satellites before exhausting their

battery life. In 2016, I also marked 80 hatch year male mallards with GPS-archival backpack units (Lotek Wireless Inc.). These units record GPS location data at a user specified interval, but must be recovered to acquire data. These units weighed 11 g and were configured as backpack type transmitters. I attached these units to hatch-year males because they have the highest recovery rate of any mallard age-sex cohort. Apparent direct (within first hunting year after marking) recovery of hatch year male mallards banded in Minnesota based on band returns was predicted to be 18% and an additional 6% were expected to be recovered in the 2nd hunting season after deployment (USGS, Gamebirds data set).

GPS-logger or GPS-Argos backpack transmitter units receive satellite signals to estimate highly accurate locations; precision of locations is accurate to within a few meters. Of all available options, these units were deemed best suited for estimating detailed parameters associated with habitat use, use of refuge areas, local movements, and migration events. Birds were marked in conjunction with our current banding effort. We paid a \$50 incentive for hunters returning tracking units.

After preliminary analyses for birds marked in 2016, we determined that slightly more data per unit cost were attained for birds marked with GPS-Argos units than GPS-logger units. Further, the manufacturer was able to change the firmware and programming of these tracking units for 2017 so that they would consume less battery and collect more data than the 2016 units. Thus, we elected to purchase only GPS-Argos units for use in 2017. In 2017, I was also able to reuse 11 GPS-logger units and 8 GPS-Argos units that had been deployed in 2016, recovered by hunters, and refurbished with new harness material.

Tracking

In 2016, GPS-logger units were configured to attain location data every 11.5 hours; GPS-Argos units were configured to attain fixes every 22.5 hours and the units were set to begin this cycle at differing times. This allowed locations throughout the day and locations on each individual bird to shift over days and attain day and night fixes accordingly. In 2017, advances in firmware and programming of the tracking units allowed more frequent tracking; GPS-Argos units were set to attain location data every 11.5 hours in 2017.

Movement Data

For birds marked in 2016, estimated point locations were determined to be inside or outside the state of Minnesota. A bird was determined to have emigrated upon its permanent exit from the state. Movement direction was measured as the azimuth between the birds marking location and its first location outside the state upon permanent emigration.

Use of Habitats

For birds marked in 2016, estimated point locations were overlaid on the 2011 National Land Cover Data layer and habitats were determined based on estimated point locations. Similarly, it was determined whether locations were on refuge or non-refuge locations and WMA or non-WMA locations based on appropriate GIS data layers.

Data Analyses - Movement Data

For birds marked in 2016, I determined date of permanent departure from the state (emigration) for each bird based on its location data. I used proportional hazards regression (Allison 1995) to examine variation in emigration rates due to the effects of the bird's age and sex or its location of marking. I present product-limit emigration estimates (Kaplan and Meier 1958) for the marked sample. Further, I present a plot latitude of location data over time and a plot of the array of movement direction upon emigration from Minnesota.

Data Analyses - Use of Habitats

For birds marked in 2016, I divided the tracking period into 3 time periods based on hunting seasons: PREHUNT (the period before regular duck season was opened in Minnesota), HUNT (the period when regular hunting season was open anywhere in Minnesota), and POST (the period after regular duck season had closed anywhere in Minnesota). I divided location data in portions of the day as diurnal (sunrise to sunset) or nocturnal. I collapsed habitats to 5 basic categories for analysis including open water, forested or developed habitats, pasture habitats, crop habitats, or emergent marsh habitats. I determined use of habitats within the state of Minnesota based on 2848 location estimates from 44 birds using compositional analyses (Aebischer et al. 1993). I determined diurnal and nocturnal proportional use of each bird in every habitat during each time period, I replaced zero values with 0.002 (an order of magnitude lower than the lowest nonzero proportion of a habitat used by any bird in a combination of any time period and portion of day). To remove the unit sum constraint, I constructed log ratios by dividing proportional use of each habitat by proportional use of emergent marsh habitat and used Napierian logarithms of these ratios as response variables. I used split-plot, repeated measures multivariate analysis of variance to test for overall effects of season (PREHUNT, HUNT, POST), portion of day (day or night), cohort of marked bird, or zone of capture. I fit a full model containing all 4 of these explanatory factors as well as a term for repeated measures among birds.

Models were fit using backwards-stepwise procedures. I present estimates of proportional use of each habitat, averaged across birds, from the untransformed data within levels of significant ($P \leq 0.050$) explanatory variables from the final fitted model.

Similarly, I examined use of refuge areas (areas closed to waterfowl hunting by statute or regulation) and use of WMAs using analysis of variance after constructing proportions as outlined above. I present proportional use of these habitats below.

RESULTS

Movement Data

For birds marked in 2016, I did not detect differences in rates of emigration among cohorts or zones of capture ($P_s > 0.018$). Retention rates of marked birds in Minnesota remained $> 80\%$ until early November then declined to about 45% by mid-November, remaining birds left the state in early-December (Figure 2). Latitudes of marked birds declined throughout the season (Figure 3) and vectors of emigration flights were mostly long and southeasterly (Figure 4).

Use of Habitats

For birds marked in 2016, I did not detect differences among proportional use of habitats by seasons or cohorts ($P_s > 0.090$), but proportional use of habitats differed among zones of capture ($P = 0.018$) and portion of day ($P < 0.0001$). Use of crop habitats were higher for birds marked in the south capture zone than for birds marked in the north capture zone (Figure 5). Use of open water habitats were highest during the day; use of crop habitats were highest during the night (Figure 6).

Use of areas closed to hunting varied by zone of marking and season ($P_s < 0.0029$). Proportional use of areas closed to hunting was 45% in the north hunting zone, whereas use of refuge areas was only 8% in the south hunt zone. Use of refuge areas was highest (44.9%) during the preseason period, but decreased to 22.6% and 27.3% during the hunting season and post-hunting periods, respectively.

Use of WMAs was 56% , 37% , and 27% during the preseason, hunting season, and post-hunting seasons, respectively. Use of WMAs during night was 39% , but 53% during the day.

DISCUSSION

Emigration rates were similar between zones of capture and cohorts; given the late onset of winter that occurred in 2016, this was not surprising. Temperatures were above normal through early December in northern Minnesota. When freeze up did occur in the north hunting zone it also occurred in much of the southern portion of the state shortly thereafter. I speculate that the extended retention time of the marked sample within Minnesota was likely due to the late onset of winter.

Use of open water and emergent wetland habitats was high; these estimates were based on the National Land Cover Database data currently available. More refined analyses of habitat use could be conducted if more informative and accurate GIS data layers are available. Further analyses including data from birds marked in 2017 will be conducted in 2018. An additional sample of 65 Mallards are scheduled to be marked in August-September 2018 using GPS-Argos type tracking units.

ACKNOWLEDGMENTS

This project was funded in part by the Wildlife Restoration (Pittman-Robertson) Program.

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REGULAR SEASON DUCK AND GOOSE ZONES

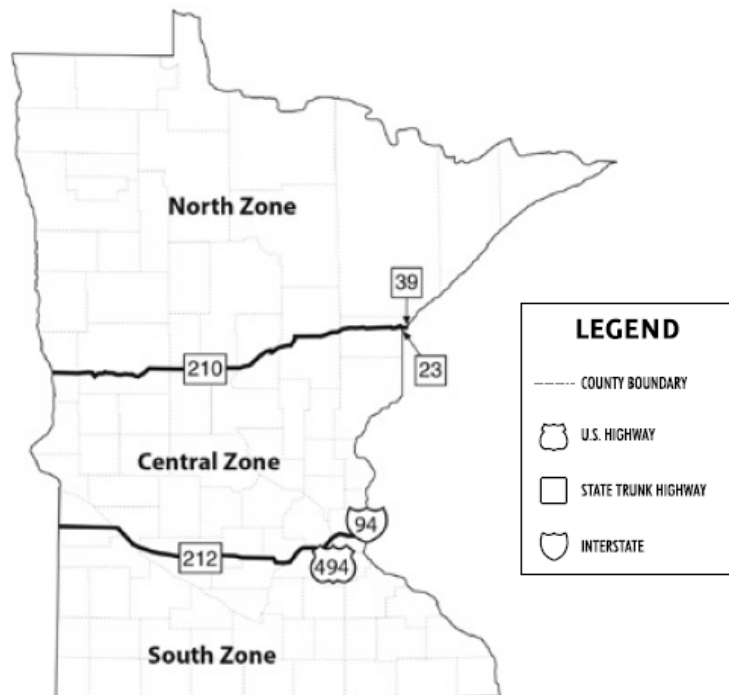


Figure 1. Minnesota waterfowl hunt zones boundaries, 2016.

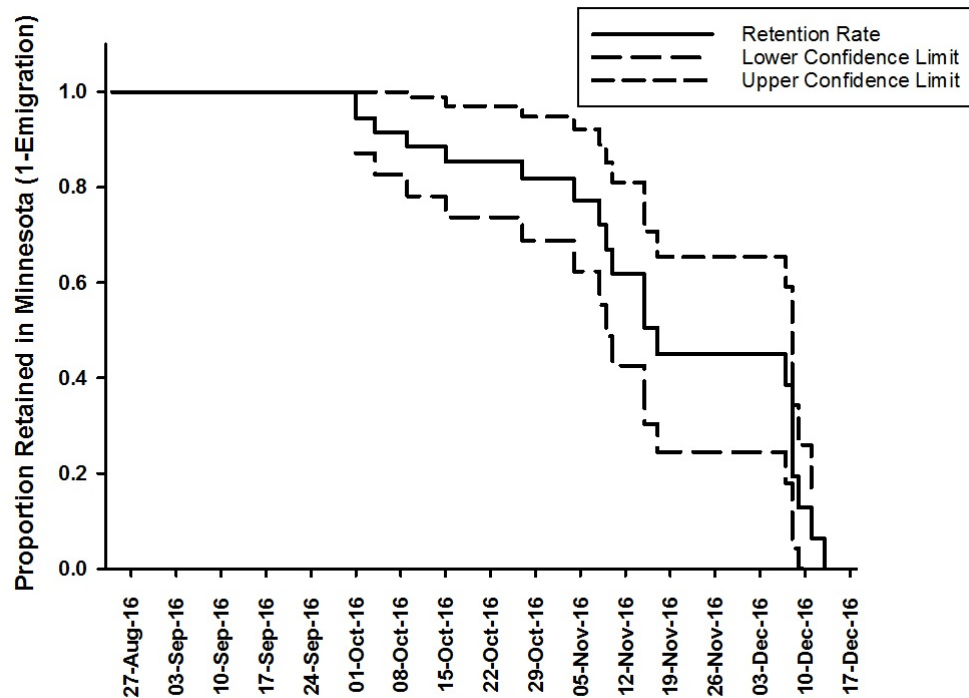


Figure 2. Retention curve for mallards marked with tracking units in Minnesota, 2016.

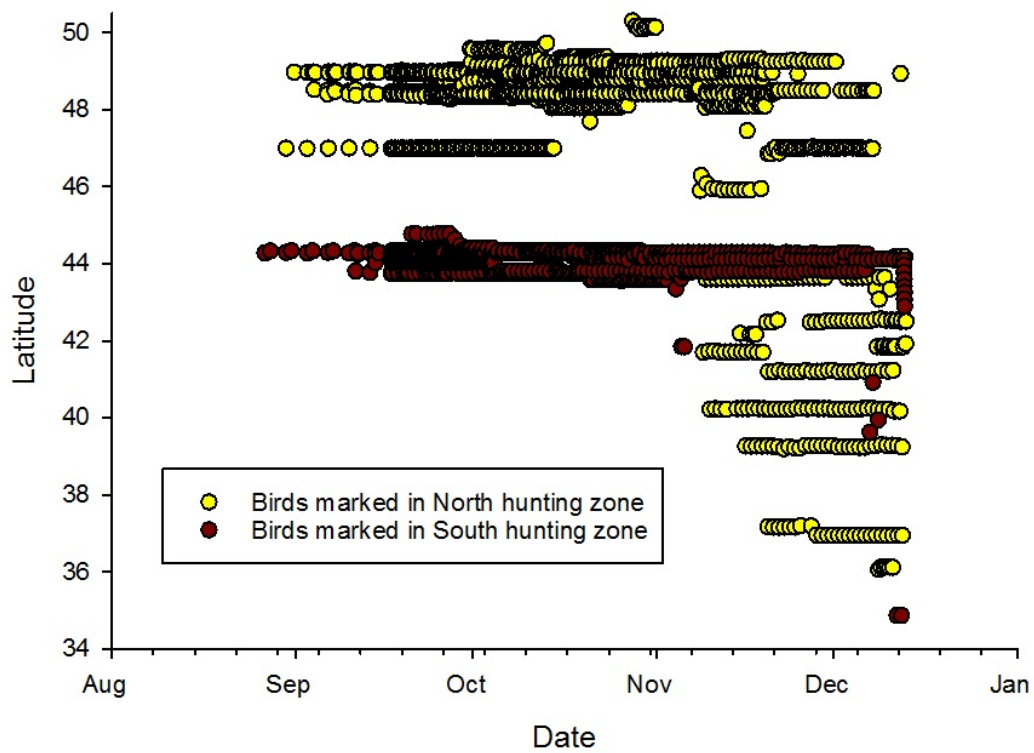


Figure 3. Scatter plot of location latitudes by date for mallards marked with tracking units in Minnesota, 2016.

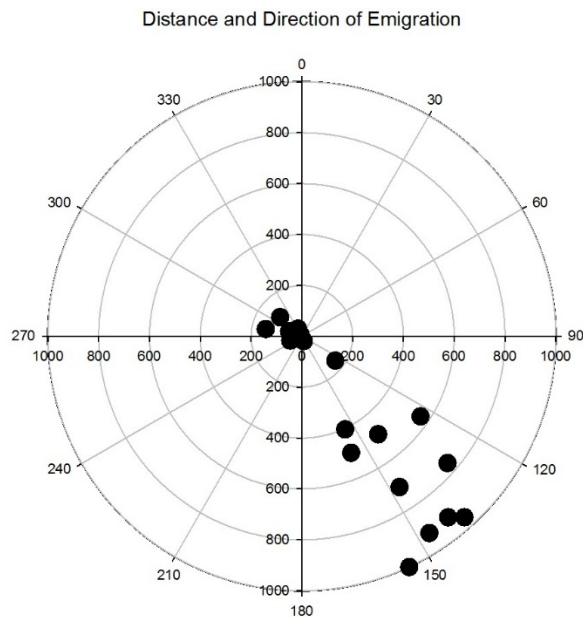


Figure 4. Polar plot of distance and direction of movement on permanent emigration (black dots) for mallards marked with tracking units in Minnesota, 2016. Concentric rings represent distances (km); azimuth (degrees) of movements are indicated on the outermost ring of the plot.

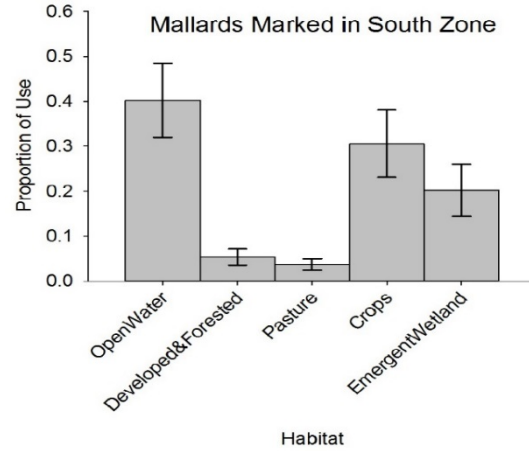
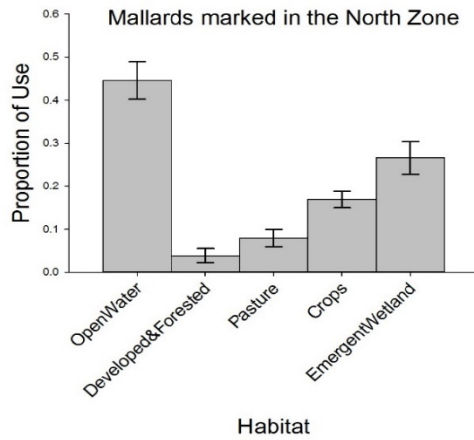


Figure 5. Proportional use of habitats by mallards marked in the Minnesota's north and south hunting zones, 2016. Proportions are expressed as an average across birds.

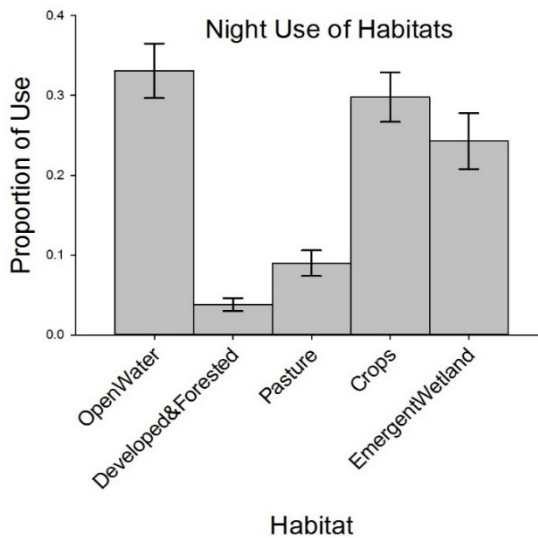
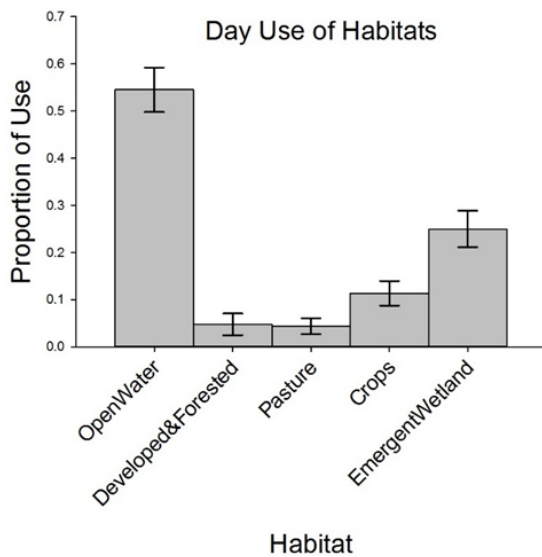


Figure 6. Proportional day or night use of habitats by mallards marked in the Minnesota's north and south hunting zones, 2016. Proportions are expressed as an average across birds.



DEVELOPING METHODOLOGIES FOR PREDICTING THE LOCATIONS OF WOOD DUCK BREEDING HABITAT COMPONENTS IN MINNESOTA

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SUMMARY OF FINDINGS

There have been alterations to both aquatic and terrestrial habitats used by wood duck (*Aix sponsa*) hens and broods in Minnesota and the Upper Midwest during recent decades. We initiated this study to develop methodologies to predict the locations and monitor spatiotemporal changes in the areal extent of wood duck breeding complexes. Specifically, we want to use Light Detecting and Ranging (LiDAR) data to identify multiple habitat components and to monitor future changes in these components. We will provide better historical context regarding spatiotemporal changes in nesting habitat by analyzing Forest Inventory and Analysis (FIA) data with a quantitative method currently being developed to accurately estimate the population variance of stems that may have suitable nesting cavities. Our specific objectives are to (1) develop and evaluate spatial predictive models of habitat components that are important to breeding wood ducks (i.e., tree species [alternatively deciduous v. coniferous], diameter-at-breast height [DBH], tree canopy density, stand type, wetland type, water depth) based on LiDAR-generated metrics or other sources of spatial data [e.g., National Wetland Inventory (NWI), existing Geographic Information System (GIS) layers, aerial photographs], (2) ascertain the optimal pulse density of LiDAR needed to accurately measure or classify each habitat component of importance to wood ducks (3) determine the generalizability of the LiDAR method for predicting the locations of habitat components by applying algorithms developed from data collected in the main study area (Cass County, Forest Ecological Province) to other sites in the Forest, Prairie, and/or Transition Provinces at which adequate LiDAR data have been obtained, (4) estimate the species- and DBH-specific proportions of trees with suitable cavities and detection probability of suitable cavities from empirical field data, and (5) determine whether there has been a change in the number of potential nest trees since the 1970s based on changes in FIA data.

We conducted vegetation surveys at 677 wetland plots during Summer 2016 and 2017, and forest plots during Fall 2016, Spring 2017, and Fall 2017. We assigned a habitat classification to 14 types of dominant emergent cover and 6 types of loafing structures during wetland surveys, and 12 cover types to forest plots during nesting habitat surveys, and measured several other habitat variables in each survey. We examined 7,357 trees during forest surveys, and classified 162 cavities as suitable and 88 as marginally suitable for nesting wood ducks. Because data were sparse for relatively large DBH trees of multiple species (≥ 40 cm for early and mid-successional species, ≥ 50 cm for late successional species), we will survey more forest plots with the intent of obtaining sufficient data to more reliably estimate the proportion of large-DBH trees with suitable cavities.

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Flights to collect LiDAR data originally scheduled to occur during Fall 2016 were postponed until Fall 2017. Thus, we cannot begin associating ground-level aquatic and forest vegetation measurements to LiDAR data until Summer 2018.

We will begin analyzing Forest Inventory and Analysis data to ascertain the abundance and trend of trees in several species-, DBH-, and perhaps health-status classes in our study area from 1977 until the current time. We will use this trend information and empirical knowledge of the proportion of trees in each of these classes with suitable nesting cavities to make inferences about the temporal change in abundance of suitable nesting cavities.

INTRODUCTION

Some terrestrial and aquatic habitats used by wood duck hens and broods during the pre-nesting, nesting, and brood-rearing life-cycle phases have been altered substantially in Minnesota and the Upper Midwest during recent decades. For example, there were decreases in the areal extent of some classes of aquatic habitats in northcentral Minnesota (Radomski 2006) and in the number of beaver impoundments in the forested portion of Minnesota between the early 1990s and 2002 (Dexter 2002, p. 52), both of which were used by wood duck broods (see McGilvery 1968, Bellrose and Holm 1994). Although the number of potential nesting trees for wood ducks was projected to increase both in Minnesota (Jaakko Pöyry Consulting, Inc. 1994) and the Upper Midwest (Denton et al. 2012*b*), there has been recent concern among Minnesota Department of Natural Resources (MNDNR) managers that harvesting relatively large-DBH trees of economically valuable species [e.g., aspen (*Populus* spp.)] in northern Minnesota will reduce the availability of cavity trees frequently used for nesting by some waterfowl (R. A. Norrgard and D. P. Rave, MNDNR, personal communication).

Thus, there is a need to develop methodologies that can be used to predict the locations of the habitat components that compose wood duck breeding complexes (i.e., important habitats used during the pre-breeding to brood-rearing life cycle phases). These methodologies should have the (A) flexibility to identify both forested and non-forested habitat components that occur at different spatial scales, (B) accuracy and precision to reliably quantify spatiotemporal changes in the characteristics (e.g., areal extent) of habitat components, and (C) efficiency to collect habitat data over large spatial scales. It also would be beneficial to develop such methodologies so that long-term trends in habitat characteristics could be analyzed in the future.

It is unlikely that all of these needs can be met with a single methodology or existing dataset. Consequently, we will develop 2 methodologies for obtaining better knowledge regarding spatiotemporal changes in wood duck breeding-habitat components. We propose to develop LiDAR methodology to identify multiple habitat components and to monitor changes in these components from the contemporary period forward. This methodology also could be used to provide habitat trend information that can be used in MNDNR administrative efforts [e.g., subsection planning] and research (e.g., estimating habitat availability in resource selection studies; see Aebischer et al. (1993)).

We also propose to provide better historical context regarding spatiotemporal changes in nesting habitat by analyzing FIA data with a quantitative method currently being developed. Reliable FIA surveys have been conducted in Minnesota since the 1970s. We propose to conduct analyses of FIA data to identify spatiotemporal changes in nesting habitat components not characterized by LiDAR, at spatial scales smaller than those of previous investigations, and over a greater time period (i.e., since the 1970s). This methodology also will provide database queries that can be used in future monitoring efforts, and an insight of whether the predicted trend in the abundance of tree cavities (e.g., Denton et al. 2012*b*) is accurate.

GOALS AND OBJECTIVES

The ultimate goal of this project is to develop methodologies that can be used to predict the locations and monitor spatiotemporal changes in the areal extent of wood duck breeding complexes (i.e., important habitats during the pre-breeding to brood-rearing life cycle phases) and perhaps other species that use similar habitat components. Meeting this goal will require that we (1) identify the location and areal extent of breeding-habitat components in the main study area, (2) validate the predicted locations of wood duck breeding complexes with independent, empirical data from other sites, and (3) quantify the spatiotemporal trends in potential nesting trees in Minnesota over the long term. We will meet this goal using multiple sources of data (e.g., empirical field data, FIA, LiDAR, and associated remote sensing imagery). Our specific objectives are to:

- 1) Develop and evaluate spatial predictive models of habitat components that are important to breeding wood ducks [i.e., tree species (alternatively deciduous v. coniferous), DBH, tree canopy density, stand type, wetland type, water depth] based on LiDAR-generated metrics or other sources of spatial data [e.g., NWI, existing Geographic Information System (GIS)] layers, aerial photographs). This evaluation will include determining the accuracy with which each component can be predicted with LiDAR data.
- 2) Ascertain the optimal pulse density of LiDAR needed to accurately measure or classify each habitat component of importance to wood ducks.
- 3) Determine the generalizability of the LiDAR method for predicting the locations of habitat components by applying algorithms developed from data collected in the main study area (Cass County, Forest Ecological Province) to other sites in the Forest, Prairie, and/or Transition Provinces at which adequate LiDAR-cloud data have been obtained (e.g., J. Erb's study areas, MNDNR statewide elevation measurement project).
- 4) Estimate the species- and DBH-specific proportions of trees with suitable cavities and detection probability of suitable cavities from empirical field data.
- 5) Determine whether there has been a change in the number of potential nest trees since the 1970s based on changes in FIA data.

METHODS

Study Area

The primary study area encompasses 254,051 ha in northeastern Cass County, Minnesota (Figure 1). Parts of Chippewa Plains, Pine Moraines-Outwash Plains, and St. Louis Moraine Ecological Subsections (Hanson and Hargrave 1996) occur within this area. This study area occurs in BCR 12.

Wetland Surveys

In 2016, we used the available wetland spatial data from NWI (Cowardin et al. 1979, MNDNR 2009) to select 260 sampling plots in the study area. We stratified wetlands contained in the NWI GIS layer by NWI system, subsystem, and class (hereafter, wetland types). Unfortunately, information about NWI subclasses was not available for many wetland types. We calculated the proportion of the wetlands in the study area composed of 9 major wetland types: Lacustrine-Littoral-Emergent Vegetation (0.004), Palustrine-Emergent Vegetation (0.102), Lacustrine-Limnetic-Unconsolidated Bottom (0.522), Lacustrine-Littoral-Unconsolidated Bottom (0.020), Palustrine-Forested (0.191), Palustrine-Shrub Scrub (0.130), Palustrine-Unconsolidated Bottom (0.026), Riverine-Upper Perennial-Unconsolidated Bottom (0.003), and Riverine-Lower Perennial-Unconsolidated Bottom (0.002). We then randomly selected 260 2- X 2-m plots from these wetland types: 60 plots from both the Lacustrine-Littoral-Emergent Vegetation and Palustrine-Emergent Vegetation types, and 20 plots each from the remaining types. We

selected more plots from the first 2 wetland types because we surmised that these habitats were more likely to be used by wood duck broods (e.g., Grice and Rogers 1965), and that there was a greater likelihood that these habitats would be structurally diverse and thus more difficult to identify from LiDAR signatures. We also specified that plots had to be ≥ 100 m apart to reduce the likelihood of non-independence among these sampling units (i.e., sampling plots with similar vegetation structure).

Many relatively small, isolated wetlands were not delineated in the NWI GIS layer, so we later selected 50 additional plots in these habitats from the MNDNR Hydrography GIS layer (MNDNR 2015). We randomly selected 1 plot per selected wetland if it was 0.81–8.09 ha, ≤ 402 m from a road, and adjacent to public land. After initially selecting plots from both layers, we examined aerial photos to assess the accessibility of these locations. We attempted to sample plots that initially appeared accessible.

We changed our approach to selecting wetland and plot locations for the 2017 field season to reduce number of plots located in wetland habitats not likely to be used by wood duck broods and to increase sampling efficiency. Specifically, we selected wetlands classified as either inundation or intermittent water; lake, pond or reservoir; river or stream; shallow water; or wetland from the MNDNR Hydrography GIS layer (MNDNR 2015) that either (1) had a public boat access site or (2) were on public lands and ≤ 100 m from both a public road and water feature. From sites that met these criteria, we then randomly selected ≤ 5 sampling locations per wetland that were ≥ 4.05 ha, with these points ≥ 100 m apart.

Because potential loafing sites were encountered infrequently at randomly selected plots during 2016, we chose to nonrandomly select and measure a variety of these structures as encountered so that we could observe the LiDAR signature for each. We also documented and measured these structures at randomly selected points during 2017.

We navigated to the approximate location of each plot center using a Garmin Montana Global Positioning System (GPS) unit, and established a plot center. If the plot center was difficult to access (e.g., because of soft bottom substrate that could not be traversed on foot, dense vegetation that could not be penetrated via boat) or on or near an ecotone, we moved the plot location to a site that was as close as possible to the initial location, accessible, and in the interior of a somewhat homogeneous vegetation patch. Moving plots away from ecotones reduced the likelihood of misclassifying habitats (i.e., habitat misclassifications are more likely to occur near ecotones because the exact location of a sampled plot is difficult to determine with somewhat imprecise GPS units). We also moved some plots located in open water to the nearest vegetated location within the wetland because the former habitat is simple and easily identified with LiDAR data. Instead, we chose to dedicate the greatest sampling effort to vegetated plots.

For each plot, we recorded the date, start time, observers, plot number, whether wood ducks were observed within 100 m of the plot, and if so, provided a count of individuals in each cohort (male, female, brood, unknown). We did not adjust wood duck counts for detectability. We ascertained whether the NWI classification (system, subsystem, class) available on our GIS layer was correct at each plot (i.e., some wetlands may have changed since the original classification or the original classification may have been incorrect), and recorded the appropriate NWI wetland classification to the level of subclass. We classified the types of wood duck loafing structures present within the plot (7 classes: none, rock, log or stump, muskrat lodge, beaver lodge or dam, small island or tussock, barely or lightly vegetated shoreline), as well as the type of beaver modification, if any that had some influence on the plot (6 classes: none, water level, runs, tree removal, dam or lodge, food cache). We also obtained location data for each plot center using a Geneq Sx Blue II GPS unit (15–20 cm accuracy in open

habitats when data were obtained at 1 reading / second for 1 minute), and recorded the specific GPS unit used.

At each plot, we placed a 2- X 2-m Daubenmire square (Daubenmire 1959, Gilmore et al. 2008) so its center was located at plot center, and measured several habitat variables within the device. This square had 0.2 m delineations, which facilitated the measurement of several habitat variables. Specifically, we used these delineations to estimate the % coverage (5% increments) of 5 habitat classes [emergent, floating leaf, ground, open water, shrub (woody vegetation ≤ 1.37 m tall)] that were present at or above the water surface, and of submergent plants, when possible to make reliable observations (i.e., at locations in which water turbidity or sun glare did not substantially hinder observability). Within the Daubenmire square, we also documented the dominant emergent cover type (14 classes: none, alder [*Alnus spp.*], Canada bluejoint grass [*Calamagrostis canadensis*], giant bur-reed [*Sparganium eurycarpum*], cattail [*Typha spp.*], ericaceous shrub, floating-leaf, giant reed grass [*Phragmites spp.*], rush [*Scirpus spp.*], reed canary grass [*Phalaris arundinacea*], sedge [*Carex spp.*], willow [*Salix spp.*], wild rice [*Zizania aquatica*], other), and measured the minimum depth of submergent vegetation and the height of emergent vegetation and shrubs (0.1 m increments) with a 3-m ruler, tree canopy height (0.1 m increments for woody vegetation ≥ 1.37 m tall) with a Suunto clinometer or with a 3-m ruler, mean tree canopy closure with a spherical densiometer, and water depth with either a 3-m measuring pole (0.1 m increments) at relatively shallow plots or an Eagle FishEasy 245DS depth finder (0.03 m increments) at deeper locations.

Within the Daubenmire square, we also estimated vertical vegetation cover and structure using a round Robel pole (Robel et al. 1970) that had alternating 0.1-m white and black bands and narrow, vertical, and contrasting marks at the midpoint of each band. Because it was not possible for personnel to stand at plots in relatively deep water or where the soil substrate was soft, it was necessary to adapt this device so that it could be used by 2 people in a boat. This adaptation consisted of attaching a long wooden pole to the Robel pole in a perpendicular manner. One crew member extended the Robel pole to the corner of the Daubenmire square opposite the other crew member, and oriented this device upright to the water surface. The other crew member placed their sighting eye 0.8 and 1.6 m above the water surface with the aid of the 3-m ruler, and recorded the lowest decimeter or 0.5 dm mark that could be observed from diagonally across the Daubenmire square (2.8 m). Crew members switched assignments and took readings from across the opposite diagonal of the square. This approach generated 2 measurements from each observation height, all of which were averaged together.

Forest Surveys

We first obtained forest spatial data (e.g., forest cover type, stand age and location) of public forest lands from Cass County, State of Minnesota, and United States Department of Agriculture (USDA) Forest Service databases. There were slight differences in the manner that these agencies classified forest cover types, so we aggregated appropriate stands (i.e., likely to be used by nesting wood ducks) from each database into 5 basic cover types: aspen-birch, lowland hardwoods, mixed conifer-hardwood, northern hardwoods, and oak. We identified stands on public lands that were likely old enough to have developed cavities suitable for use by nesting wood ducks (i.e., aspen-birch ≥ 50 years, all other stand types ≥ 80 years), and constrained the potential sample to stands of these ages or greater. We then stratified stands by cover type and randomly selected 300 forest stands (60 stands of each of the 5 types) to be surveyed.

We then selected plots within these stands with the stipulations that (1) plot centers must be both ≥ 50 m apart and ≥ 30 m from the nearest stand boundary and (2) ≤ 2 plots per stand could be established. We used these selection criteria to increase the likelihood that plots adequately represented the diversity of vegetation structure of each forest type, thus facilitating the

development of biologically realistic LiDAR models. We then randomly selected $n=563$ plots to be surveyed. It was necessary to remove 19 plots from the sample because of nearby heritage sites or scheduled timber harvests (i.e., interpretation of habitat characteristics would be confounded if harvesting occurred between the times forest surveys were conducted and LiDAR data were collected).

We navigated to the selected plot centers using a Garmin Montana GPS, and established 20-m radius circular plots (0.126 ha) around those points. Plots located near ecotones not indicated on available GIS layers were moved sufficiently into the stand interior as to avoid potential edge effects on vegetation structure. We first recorded the plot identification number, date, start and end times of survey, visit number to the plot (first or second), observers, proportion of visible sky obscured by cloud cover (0.1 increments), and proportion of tree boles covered by snow or obscured by leaf-out (0, 0.01–0.10, 0.11–0.33, 0.34–0.66, 0.67–1.00). We obtained location data for each plot center using Geneq Sx Blue II (0.9–1.8 m accuracy under closed forest canopy when obtaining 1 reading / 5 seconds for approximately 15 min) and Geneq Sx Blue II + GNSS (0.5–0.9 m accuracy under closed forest canopy when obtaining 1 reading / 5 seconds for approximately 15 min) GPS units, and recorded the GPS make, model, and unit number used at each plot. We classified the stand structure following USDA Forest Service methodology (2014; 5 classes: single story, two-storied, multi-storied, mosaic, unknown/unassessable). We assigned all plots to 1 of the 5 general forest cover types (Table 2) and to an Eyre (1980) cover type.

We then examined and measured individual tree stems within each plot following an established protocol (USDA Forest Service 2014), with some exceptions. Specifically, we surveyed only trees large enough to have cavities used by nesting wood ducks [i.e., ≥ 22.0 cm DBH (Haramis 1975)] and tall enough for the DBH to be measured (i.e., ≥ 1.37 m). Starting at the 0° azimuth within each plot, we proceeded clockwise, numbering each suitable tree stem, and recording the following data for each stem: species, DBH (0.1 cm increments), distance (0.1 m increments) and direction (1° increments that were not adjusted for declination) from plot center, health status (following Thomas 1979, Appendix 1), and crown class (5 classes: remnant, dominant, codominant, intermediate, overtopped; USDA Forest Service 2014).

All field crew members then used binoculars to conduct a preliminary search of each tree ≥ 22.0 cm DBH in the plot to identify cavities that potentially were suitable for nesting by wood ducks. During the initial search, personnel ascertained whether the entrance dimensions likely were sufficient to permit a wood duck to pass through (i.e., 6 x 6 cm; Zwicker 1999, cited in Denton et al. 2012b) and the bottom of cavity entrance was high enough to be used by nesting wood ducks [i.e., ≥ 0.6 m above ground level (Strom 1969)]. When a potentially suitable cavity was encountered, we used a Pyle Model PLCM22IR remote camera attached via a stiff, braided wire to a 15.2 m Crain CMR Series Measuring Ruler (*sensu* Waldstein 2012) to perform a more careful examination of the entrance and interior of the cavity. We first determined whether cavity entrance dimensions were suitable by attempting to pass a cardboard cut-out of the minimum usable dimensions (i.e., 6 x 6 cm) through the cavity opening. This cut-out was placed on the wire connecting the camera to the measuring ruler. We then examined cavity interiors with the camera to ascertain whether the following conditions had been met: horizontal depth (approximately 10 cm from inner edge of the entrance opening toward the back of the cavity) appeared large enough for hens to move from the entrance to the interior of the cavity, vertical depth (from the bottom of the cavity to the bottom of the entrance) was ≥ 10.2 cm to 4.5 m; (Bellrose and Holm 1994 p. 176) and not hollow to the ground (Robb 1986, cited in Bellrose and Holm 1994, p. 178), nest platform dimensions were ≥ 14 x 15 cm (Boyer 1974, Haramis 1975, Denton et al. 2012a), and the cavity did not contain standing water or excess debris (Sousa and Farmer 1983).

Field personnel used this information to classify the suitability of each examined cavity for wood duck nesting (4 levels: suitable, marginal, unsuitable, unknown). We considered a cavity to be suitable if all these conditions were met. A cavity was classified as marginal if it was unclear whether all dimensional requirements were met (i.e., ≥ 1 dimensional measurement appeared to be close to some minimum or maximum value). Cavities typically were classified as unknown/unobservable if personnel were unable to completely observe the cavity, either because of cavity height or some structural attribute did not permit observation with the camera system. We considered a cavity to be unsuitable if any dimensional measurement was not met or if there was standing water or excess debris in the cavity. Field personnel also provided a cause for unsuitability (7 classes: entrance dimensions too small, insufficient horizontal depth, insufficient vertical depth, insufficient platform dimensions, too deep or hollow to the ground, standing water in the cavity, excessive debris in the cavity). We classified the reason that a cavity was unsuitable based on the order that structural restrictions would have been encountered as a wood duck entered a cavity (i.e., entrance dimensions, followed by horizontal depth, vertical depth, and finally, dimensions and other characteristics of the platform). Our assessment of the suitability of interior characteristics required some subjectivity because direct measurements could not be made with our camera system.

For each cavity inspected, we recorded tree number, cavity entrance type (3 classes: opening on the top, side, combination of top and side openings which are joined on the exterior of the tree), primary and secondary sources of cavity formation (11 classes: split, broken limb, broken top, woodpecker, fire, lightning, insect, logging wound, decay/rot, other, unknown), evidence of animal use (9 classes: eggshell/ membrane, nesting materials, hive or other insect structure, animal present, scratching at entrance, pecking at entrance, other, unknown, none), and animal taxa. We also measured cavity height with either a 15.24 m measuring ruler (± 0.1 m), Leupold RX-800i rangefinder (± 0.1 m), or Suunto clinometer (± 0.5 m).

LiDAR Data Collection

The MNDNR Resource Assessment Program (RAP) originally planned to have LiDAR and associated remote sensing data collected during aerial flights conducted by a vendor during Fall 2016, but these efforts did not occur until Fall 2017. We anticipate the data will be available for analyses in Summer 2018.

Forest Inventory and Analysis (FIA)

We plan to conduct an analysis of FIA data to gain an understanding of temporal changes in the potential number of nest trees of common tree species (American basswood, bigtooth aspen, northern red oak, paper birch, red maple, quaking aspen, and sugar maple) that are common in our study area and the Minnesota portion of the Bird Conservation Region (BCR) 12 (Boreal Hardwood Transition, North American Bird Conservation Initiative 2018) from 1977 until the present. We will use this trend information and empirical knowledge of the proportion of trees in each species-, DBH-, and perhaps health-status class with suitable nesting cavities to make inferences about the temporal change in abundance of suitable nesting cavities.

STUDY AREA

The primary study area encompasses 254,051 ha in northeastern Cass County, Minnesota (Figure 1). Parts of Chippewa Plains, Pine Moraines-Outwash Plains, and St. Louis Moraine Ecological Subsections (Hanson and Hargrave 1996) occur within this area. This study area occurs in BCR 12.

RESULTS

Wetland Surveys

We conducted surveys at 677 randomly selected wetland plots during the late summer and early fall of 2016 and 2017 (Table 1, Figure 2). We classified the dominant emergent cover as alder (0.7%), blue joint grass (0.6%), bur reed (0.3%), cattail *spp* (6.9%), ericaceous shrub (2.2%), floating leaf (18.0%), phragmites *spp* (2.5%), rush *spp* (20.7%), reed canary grass (2.2%), sedge *spp* (8.3%), willow (0.4%), wild rice (31.3%), other vegetation (0.9%), and none (4.9%). We also documented trees at 10 plots (1.5%), with canopy coverage ranging from 0.05 to 0.85. We observed that 12.3% of randomly selected plots were modified by beaver, wood ducks were present ≤ 100 m of 9.6% plots, and 4.4% of plots had potential wood duck loafing sites.

The potential loafing structures identified in randomly selected plots were 2 beaver lodges, 6 floating vegetation mats, 4 small islands or tussocks, 14 patches of bare or lightly vegetated shore, 5 logs or stumps, and 1 muskrat house in the randomly selected plots. We observed 6 beaver lodges, 2 logs or stumps, and 1 muskrat house in the 15 non-randomly selected plots.

Forest Surveys

We surveyed 213 forest plots during Fall 2016, Spring 2017, and Fall 2017 (Figure 3). The percentages of these plots located on United States Forest Service (USFS) Chippewa National Forest, Cass County, and State of Minnesota lands were 75%, 15%, and 10%, respectively. When using the Eyre (1980) approach to classify the forest cover types of surveyed plots, we observed that these units primarily were sugar maple-basswood, aspen, and northern red oak (Table 2). When assigning the more general forest-cover type to plots, the most commonly surveyed types were aspen-birch, northern hardwoods, and oak (Table 2). Interestingly, our classifications of general forest type differed from that of land-management agency classifications on 37% of plots.

A total of 7,357 trees of 27 species were measured and inspected for cavities (Table 3). We more closely examined 969 total cavities in 727 of these trees with the remote camera-system (i.e., many trees had multiple cavities). The majority of these cavities were classified as unsuitable for nesting by wood ducks (66%), and the remainder were classified as suitable (17%), marginally suitable (9%), or of unknown suitability (9%). The reasons many cavities were considered unsuitable were: insufficient vertical depth (44%), entrance dimensions too small (21%), insufficient horizontal depth (19%), insufficient platform dimensions (13%), excessive debris (3%), and too deep or hollow to the ground (1%). The primary sources of cavity creation of those structures considered suitable were: broken limb (40%), split (20%), broken top (17%), woodpecker (15%), decay or rot (2%), other (4%), and unknown (1%).

Preliminary results suggest that the proportion of trees with suitable cavities varied by species, DBH, and health status (Tables 4 and 5). It appears that the greatest proportion of suitable cavities were present in sugar maple (*Acer saccharum*), northern red oak (*Quercus rubra*) and American basswood (*Tilia americana*, Table 4). The proportion of trees with suitable cavities appeared to be generally greater in larger DBH classes (Table 3). More specifically, the average DBH of all trees sampled was 33.1 cm (range: 22.0–94.3 cm), but that of trees with suitable cavities was 42.5 cm (range: 22.8–73.6 cm). Generally, there was a lower proportion of individual trees with suitable cavities in live, healthy trees (health status 1) than in live, health impacted trees (health status 2) and dead trees (health status 3–7, Table 5). Specifically, the percentage of surveyed trees in each health status class (see Appendix 1 for criteria) was as follows: 1=64%, 2=23%, 3=1%, 4=1%, 5=1%, 6=7%, and 7=3%. In contrast, the percentage of

trees with suitable cavities in each of these classes was: 1=21%, 2=42%, 3=2%, 4=2%, 5=5%, 6=19%, and 7=9%. The mean height of suitable cavities was 7.5 m (range: 1.0–15.0 m).

LiDAR Data Collection

Aerial single-photon LiDAR data and associated remote sensing imagery were collected during Fall 2017. These data were collected during peak fall color, usually at about 30 return pulses / m² (minimum of 12, up to 40–50; J. Corcoran, MNDNR, unpublished data). The quality of green LiDAR data was not as good as anticipated. Thus, identifying the presence/absence and density of submergent vegetation and depth of water in relatively shallow locations likely will not be discernable.

FIA Analysis

We have not yet analyzed FIA data, but plan to complete this portion of the project during the upcoming fiscal year.

DISCUSSION

Wetland Surveys

Initially, we randomly selected wetlands for sampling to obtain an adequate sample size for each NWI class, with special emphasis placed on those classes that are most likely to have diverse vegetation structure. However, these efforts were confounded in-part by limitations of the existing NWI spatial data. Specifically, we observed during field-data collection that NWI classifications of some plots were incorrect, which we attribute to a combination of misclassification of wetland habitats, habitat changes since the original classification, and projection error. Further, the currently available NWI GIS layer often classifies wetlands only to the level of class, which provides little information regarding vegetation type or structure. Thus, it was not possible to select plots based on subclass or vegetation type and structure. Such limitations of available data contributed to an allocation of sampling locations that were not balanced among the 14 types of emergent covers observed. It is likely, however, that the emergent covers sampled were representative of those available in the study area.

Fortunately, we were able to collect data for a substantial number of plots (1) with structurally similar vegetation types that are difficult to distinguish from aerial photographs (i.e., wild rice v. rush *spp.*; D. Dustin, MNDNR Fisheries, personal communication), (2) dominated by the types of aquatic vegetation that should begin to subside and thus change structure (e.g., floating-leaf plants, wild rice) approximately when LiDAR imagery was obtained (i.e., late September and October), (3) with vegetation types that may be sparse, and (4) with vegetation types that frequently occur in a mix of other types of vegetation (e.g., floating-leaf plants). We anticipate that a substantial amount of data will be needed to develop reliable LiDAR signatures of such sites. Presumably, wetland habitats with no surface vegetation should have a rather simple and readily identifiable LiDAR signature.

Although identifying potential loafing sites for wood ducks using LiDAR imagery was a secondary objective, we were able to locate 6 types of these structures in randomly selected plots and 3 in non-randomly selected plots. These structures likely are a somewhat important habitat component to wood ducks (McGilvery 1968).

Forest Surveys

Although sugar maple, northern red oak, and basswood have relatively high proportions of suitable cavities, aspen species (*Populus* spp.) also may be an important source of this type of structure, given the large number of stems in the contemporary landscape (Minnesota Forest Resources Council 2017) and relatively intermediate proportion of stems with suitable cavities in these species (Table 3). Interestingly, quaking aspen (*Populus tremuloides*), American elm

(*Ulmus americana*), and sugar maple were important species for nesting wood ducks in north-central Minnesota, but American basswood also was used (Gilmer et al. 1978).

Wood ducks select nesting trees that on average have a larger diameter than that observed in our study area. We observed that trees with suitable cavities had a mean DBH of 42.5 cm (range: 22.8–73.6 cm), but that of nesting trees across 12 studies conducted in eastern North America was 58.5 cm (Soulliere 1990) and 47 cm in north-central Minnesota (Gilmer et al. 1978). We plan to compare our proportions of suitable cavities in each tree species-, DBH-, and health status-class to those of other published investigations, but anticipate that there will be differences in the same classes among these studies. This variation may be attributable in-part to spatial differences in those variables (e.g., disease, insects, animal populations, soil conditions, weather patterns) that contribute to tree damage and eventually cavity formation (Morin et al. 2016).

With the exception of Lowney and Hill (1989), we have examined more trees for cavities suitable for nesting wood ducks than any other published studies of which we are aware (see Soulliere 1990, Bellrose and Holm 1994, Denton et al. 2012a, b). Despite the large sample size of stems examined, we observed relatively few large-diameter trees of all species in our plots. Such sparse data will limit our ability to develop models that reliably predict the proportion of large diameter trees with suitable cavities. It is important to obtain a sufficient sample size of large diameter trees so that models can be developed to accurately predict the proportions of such trees with suitable nesting cavities, given that wood ducks frequently use large diameter trees for nesting (e.g., Gilmer et al. 1978, Soulliere 1990, Bellrose and Holm 1994). Therefore, we will explore ways to select and survey more plots that are likely to contain relatively large diameter trees during Spring 2018.

Beyond developing or training this predictive model, we also would like to generate an additional dataset to test the predictive ability of the initial model (*sensu* Fortmann-Roe 2012). We are seeking additional funds to conduct further field surveys during Fall 2018 that will be used for the model-testing dataset.

It is possible that the forest and cavity properties (e.g., species composition, mean age, cavity density) we observed on the public lands that we surveyed were different than those on private land, perhaps because of differences in management practices and site characteristics. However, the use of LiDAR data, remote sensing imagery, and FIA data should permit us to discern whether such ownership-based differences in forest and cavity characteristics exist. Alternatively, we could obtain permission to conduct forest surveys on private lands within the study area to determine whether forest and cavity characteristics are similar to those on public lands, but it probably would be time consuming to obtain enough data to detect significant differences between the 2 forest ownership classes.

Forest Inventory and Analysis (FIA)

Our field crews and the databases of natural resource agencies differed in the classification of general forest type of 37% of our plots. This discrepancy may be attributed to misclassification, or changes to these stands caused by natural disturbance, logging, and forest succession that had occurred since the time of classification. Regardless, substantial misclassification of stand type in existing databases could confound our ability to estimate of the number of *suitable cavities* across the landscape (i.e., the density of cavities appears to be associated with stand type). Intuitively, a reliable estimate of the abundance of suitable nesting cavities is more likely to be generated using empirical cavity data and either FIA data or LiDAR data and associated remote imagery.

ACKNOWLEDGMENTS

T. Kaebish, S. Hillard, and D. Kepler (MNDNR RAP) provided insight regarding the potential uses of LiDAR data, and facilitated the use of their data in our research. We thank S. Mortenson (Leech Lake DRM), J. Watts (Cass County MIS), S. Dunham, J. Rickers, and T. Tisler (U.S. Department of Agriculture Forest Service) for logistical support. We thank C. Scharenbroich and R. Wright for technical assistance. J. Giudice and V. St.-Louis provided suggestions regarding study design, sampling regime, and data analysis. B. Coleman, T. Duquette, C. McCarty, H. North, T. Roerick, and E. Spry provided valuable assistance with field data collection. J. Lawrence for provided reviews of research proposals and B. Davis reviewed an earlier draft of this report. This project was funded in part by the Wildlife Restoration (Pittman-Robertson) Program.

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Table 1. The National Wetland Inventory classification and sample size of plots surveyed in Cass County, Minnesota, USA during 2016–2017.

National Wetland Inventory system, subsystem, class and subclass of sampled plots ^{a, b}	Number of plots surveyed
Lacustrine limnetic unconsolidated bottom unknown	1
Lacustrine limnetic unconsolidated bottom sand	3
Lacustrine limnetic aquatic bed rooted vascular	1
Lacustrine littoral aquatic bed unknown	1
Lacustrine littoral aquatic bed rooted vascular	60
Lacustrine littoral aquatic bed floating vascular	5
Lacustrine littoral emergent nonpersistent	233
Lacustrine littoral unconsolidated bottom unknown	12
Lacustrine littoral unconsolidated bottom sand	1
Lacustrine littoral unconsolidated shore unknown	1
Palustrine aquatic bed floating vascular	13
Palustrine aquatic bed rooted vascular	43
Palustrine emergent nonpersistent	130
Palustrine emergent persistent	93
Palustrine emergent <i>Phragmites australis</i>	9
Palustrine forested broad-leaved deciduous	1
Palustrine scrub-shrub broad-leaved deciduous	20
Palustrine scrub-shrub broad-leaved evergreen	1
Palustrine unconsolidated bottom sand	3
Palustrine unconsolidated shore organic	1
Palustrine unconsolidated shore sand	5
Riverine lower perennial unconsolidated bottom unknown	2
Riverine lower perennial unconsolidated bottom mud	3
Riverine lower perennial rock bottom unknown	1
Riverine lower perennial emergent nonpersistent	28
Riverine upper perennial aquatic bed rooted vascular	2
Riverine upper perennial emergent nonpersistent	4

^a Wetlands in the palustrine system are not assigned a subsystem classification in the National Wetland Inventory classification scheme.

^b The National Wetland Inventory subclasses of some plots were classified as unknown if distinguishing characteristics were not discernable in the field.

Table 2. Crosswalk between the Forest Cover Types of Eyre (1980) and the more general forest types used to classify stands from GIS databases, and sample size of forest plots surveyed by Eyre (1980) cover types in Cass County, Minnesota, USA during 2016–2017.

General forest type	Eyre (1980) forest cover type	Number of plots surveyed
Aspen-birch	Aspen (16)	45
	Paper Birch (18)	20
Mixed conifer	Balsam fir (5)	1
	Eastern white pine (21)	3
	Red pine (15)	12
	White pine–northern red oak–red maple (20)	1
Northern hardwood	Sugar maple (27)	2
	Sugar maple–basswood (26)	59
Oak	Bur oak (42)	11
	Northern red oak (55)	32
Lowland hardwood	Black ash–American elm–red maple (39)	19
	Red maple (108)	8

Table 3. The number of stems counted in each tree species and diameter-at-breast-height (DBH, in centimeters) class within forest plots located in Cass County, Minnesota, USA during 2016–2017. In parentheses are the proportion of those trees with suitable cavities followed by the associated standard error. Dashed lines indicate that no trees were sampled or standard errors were not estimable for a tree species-DBH class.

Tree species	DBH class (cm)						
	22–29	30–39	40–49	50–59	60–69	70–79	≥80
American basswood (<i>Tilia americana</i>)	522 (0.006, 0.003)	381 (0.016, 0.006)	168 (0.048, 0.016)	51 (0.196, 0.056)	19 (0.105, 0.070)	1 (0,–)	1 (0,–)
American elm (<i>Ulmus americana</i>)	16 (0,–)	2 (0,–)	1 (0,–)	–	–	–	–
Balsam fir (<i>Abies balsamea</i>)	105 (0,–)	18 (0,–)	3 (0,–)	–	–	–	–
Balsam poplar (<i>Populus balsamifera</i>)	7 (0,–)	17 (0,–)	6 (0,–)	1 (0,–)	–	–	–
Bigtooth aspen (<i>Populus grandidentata</i>)	182 (0,–)	154 (0.013, 0.009)	65 (0.031, 0.021)	23 (0.043, 0.042)	11 (0.091, 0.087)	3 (0,–)	–
Black ash (<i>Fraxinus nigra</i>)	214 (0,–)	55 (0,–)	14 (0,–)	3 (0,–)	1 (0,–)	–	–
Black cherry (<i>Prunus serotina</i>)	1 (0,–)	–	–	–	–	–	–
Black spruce (<i>Picea mariana</i>)	1 (0,–)	–	–	–	–	–	–
Box elder (<i>Acer negundo</i>)	3 (0,–)	1 (0,–)	1 (0,–)	–	–	–	–
Bur oak (<i>Quercus macrocarpa</i>)	162 (0.006, 0.006)	91 (0,–)	25 (0,–)	10 (0,–)	8 (0.250, 0.153)	–	–
Eastern cottonwood (<i>Populus deltoides</i>)	1 (0,–)	–	–	–	–	–	–
Eastern hophornbeam (<i>Ostrya virginiana</i>)	1 (0,–)	–	–	–	–	–	–
Eastern larch (<i>Larix laricina</i>)	–	1 (0,–)	1 (0,–)	–	–	–	–
Eastern white pine (<i>Pinus strobus</i>)	18 (0,–)	31 (0,–)	34 (0,–)	8 (0.056, 0.054)	14 (0,–)	4 (0.250, 0.217)	5 (0,–)
Green ash (<i>Fraxinus pennsylvanica</i>)	129 (0.008, 0.008)	63 (0,–)	27 (0,–)	8 (0,–)	1 (0,–)	–	–
Hackberry (<i>Celtis occidentalis</i>)	3 (0,–)	1 (0,–)	–	–	–	–	–
Jack pine (<i>Pinus banksiana</i>)	12 (0,–)	10 (0,–)	3 (0,–)	–	–	–	–
Northern pin oak (<i>Quercus ellipsoidalis</i>)	6 (0,–)	7 (0,–)	–	–	–	–	–
Northern red oak (<i>Quercus rubra</i>)	278 (0.007, 0.005)	315 (0.041, 0.011)	153 (0.039, 0.016)	31 (0.097, 0.053)	14 (0.143, 0.094)	1 (0,–)	–
Northern white-cedar (<i>Thuja occidentalis</i>)	14 (0,–)	15 (0,–)	4 (0,–)	2 (0,–)	–	–	–
Paper birch (<i>Betula papyrifera</i>)	444 (0.005, 0.003)	288 (0.010, 0.006)	46 (0,–)	2 (0,–)	1 (0,–)	–	–
Quaking aspen (<i>Populus tremuloides</i>)	371 (0,–)	447 (0.016, 0.006)	252 (0.060, 0.015)	51 (0.098, 0.042)	6 (0,–)	–	–
Red maple (<i>Acer rubrum</i>)	353 (0.008, 0.005)	167 (0.024, 0.012)	22 (0.091, 0.061)	3 (0.333, 0.272)	3 (0.667, 0.272)	–	–
Red pine (<i>Pinus resinosa</i>)	90 (0,–)	181 (0,–)	106 (0,–)	42 (0,–)	14 (0,–)	3 (0,–)	1 (0,–)
Sugar maple (<i>Acer saccharum</i>)	393 (0.010, 0.005)	218 (0.055, 0.015)	103 (0.175, 0.037)	35 (0.143, 0.059)	7 (0.571, 0.187)	–	1 (0,–)

White spruce (<i>Picea glauca</i>)	12 (0,-)	9 (0,-)	2 (0,-)	-	-	-	-
Yellow birch (<i>Betula alleghaniensis</i>)	20 (0.050, 0.049)	12 (0.083, 0.080)	16 (0.062, 0.060)	3 (0,-)	-	1 (0,-)	-
Unidentified ash spp (<i>Fraxinus spp</i>)	5 (0,-)	-	1 (0,-)	-	-	-	-
Unidentified pine spp (<i>Pinus spp</i>)	1 (0,-)	3 (0.333, 0.272)	-	-	-	-	-
Unidentified aspen spp (<i>Populus spp</i>)	7 (0,-)	16 (0.125, 0.083)	10 (0, -)	4 (0,-)	-	-	-
Unknown spp	9 (0,-)	2 (0,-)	-	-	2 (1,0)	-	-

Table 4. The percentage of trees by tree species that were sampled, the percentage of trees of each species with suitable cavities, and the percentage of trees of each species with suitable or marginal cavities that were detected within forest plots located in Cass County, Minnesota, USA during 2016–2017.

Tree species	% of all trees sampled	% of all trees with suitable cavities	% of all trees with suitable or marginal cavities
American basswood (<i>Tilia americana</i>)	15.54	18.67	17.18
American elm (<i>Ulmus americana</i>)	0.26	–	–
Balsam fir (<i>Abies balsamea</i>)	1.71	–	–
Balsam poplar (<i>Populus balsamifera</i>)	0.42	–	–
Bigtooth aspen (<i>Populus grandidentata</i>)	5.95	4.00	3.52
Black ash (<i>Fraxinus nigra</i>)	3.90	–	0.88
Black cherry (<i>Prunus serotina</i>)	0.01	–	–
Black spruce (<i>Picea mariana</i>)	0.01	–	–
Box elder (<i>Acer negundo</i>)	0.07	–	–
Bur oak (<i>Quercus macrocarpa</i>)	4.02	1.33	1.76
Eastern cottonwood (<i>Populus deltoides</i>)	0.01	–	–
Eastern hophornbeam (<i>Ostrya virginiana</i>)	0.01	–	–
Eastern larch (<i>Larix laricina</i>)	0.03	–	–
Eastern white pine (<i>Pinus strobus</i>)	1.69	1.33	1.76
Green ash (<i>Fraxinus pennsylvanica</i>)	3.10	0.67	0.44
Hackberry (<i>Celtis occidentalis</i>)	0.05	–	–
Jack pine (<i>Pinus banksiana</i>)	0.34	–	–
Northern pin oak (<i>Quercus ellipsoidalis</i>)	0.18	–	–
Northern red oak (<i>Quercus rubra</i>)	10.77	16.00	13.22
Northern white-cedar (<i>Thuja occidentalis</i>)	0.48	–	–
Paper birch (<i>Betula papyrifera</i>)	10.62	3.33	4.41
Quaking aspen (<i>Populus tremuloides</i>)	15.32	16.00	17.18
Red maple (<i>Acer rubrum</i>)	7.45	6.67	9.25
Red pine (<i>Pinus resinosa</i>)	5.94	–	–
Sugar maple (<i>Acer saccharum</i>)	10.29	27.33	25.11
White spruce (<i>Picea glauca</i>)	0.31	–	–
Yellow birch (<i>Betula alleghaniensis</i>)	0.71	2.00	2.20
Unidentified ash spp (<i>Fraxinus spp</i>)	0.08	–	–
Unidentified pine spp (<i>Pinus spp</i>)	0.05	0.67	0.44
Unidentified aspen spp. (<i>Populus spp</i>)	0.50	1.33	2.20
Unknown spp	0.18	0.67	0.44

Table 5. The species-specific number of suitable cavities detected; percentage of cavities in live, dying, and dead trees; and percentage of trees examined in the live, dying and dead classes in Cass County, Minnesota, USA during 2016–2017. Health status classifications (described in Appendix 1) were assigned to broader classifications as follows: healthy (1), health-impacted (2), and dead trees (3–7). Tree species were included only if at least one suitable cavity was found.

Tree species	Number of suitable cavities	Cavities in healthy trees (%)	Healthy trees (%)	Cavities in health-impacted trees (%)	Health-impacted trees (%)	Cavities in dead trees (%)	Dead trees (%)
American basswood (<i>Tilia americana</i>)	29	41.38	85.48	37.93	10.24	20.69	4.29
Bigtooth aspen (<i>Populus grandidentata</i>)	6	—	60.27	16.67	23.29	83.33	16.44
Bur oak (<i>Quercus macrocarpa</i>)	3	66.67	84.80	33.33	13.51	—	1.69
Eastern white pine (<i>Pinus strobus</i>)	2	—	65.32	—	16.13	100.00	18.55
Green ash (<i>Fraxinus pennsylvanica</i>)	1	—	81.14	—	14.91	100.00	3.95
Northern red oak (<i>Quercus rubra</i>)	26	26.92	66.54	42.31	23.36	30.77	10.10
Paper birch (<i>Betula papyrifera</i>)	5	—	60.82	40.0	19.97	60.00	19.21
Quaking aspen (<i>Populus tremuloides</i>)	27	3.70	34.07	40.74	37.53	55.56	28.39
Red maple (<i>Acer rubrum</i>)	12	8.33	50.73	66.67	39.23	25.00	10.04
Sugar maple (<i>Acer saccharum</i>)	43	9.30	61.03	76.74	32.50	13.95	6.47
Yellow birch (<i>Betula alleghaniensis</i>)	3	33.33	59.62	33.33	32.69	33.33	7.69
Unidentified pine spp (<i>Pinus spp</i>)	1	—	—	—	—	100.00	100.00
Unidentified aspen spp (<i>Populus spp</i>)	2	—	5.41	—	—	100.00	94.59
Unknown spp	2	—	38.46	—	—	100.00	61.54

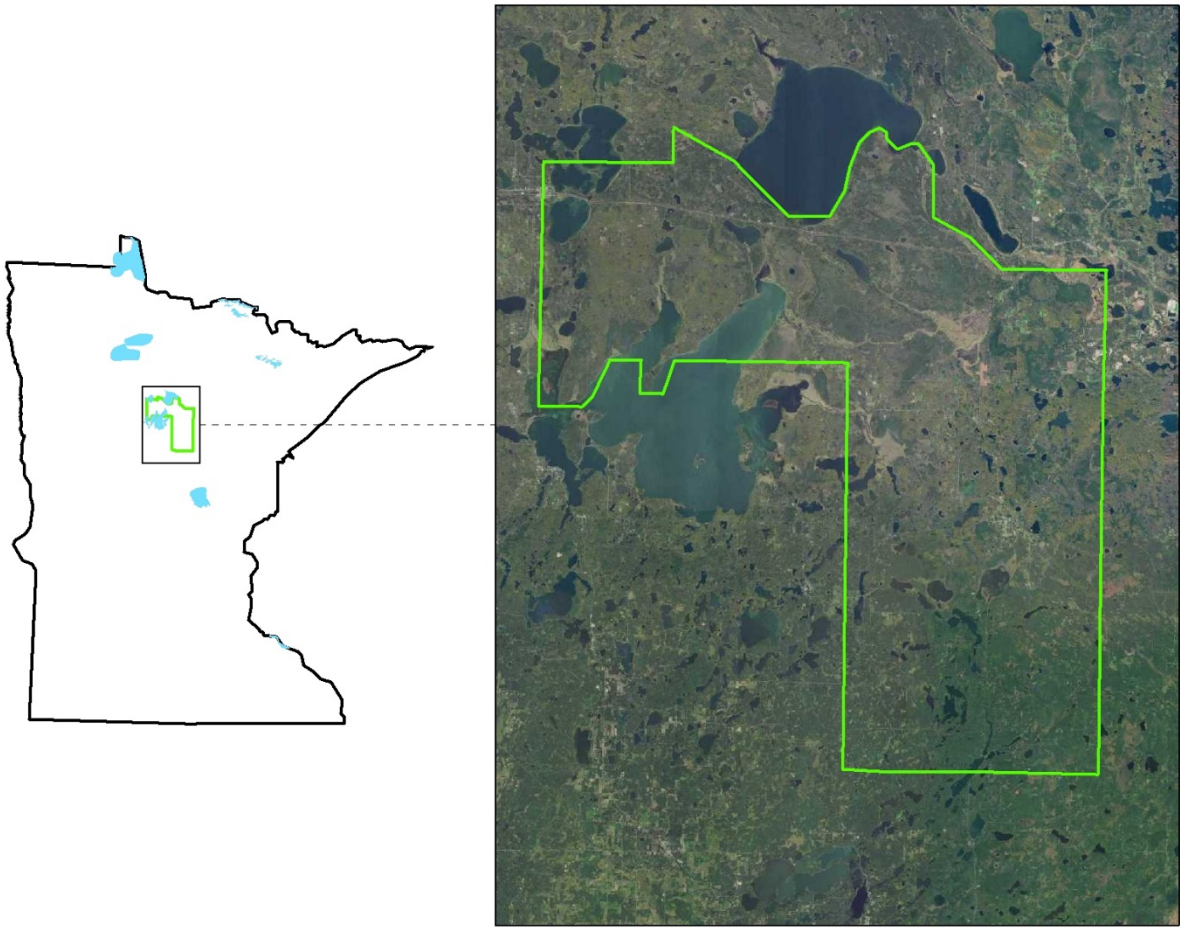


Figure 1. Location of the wood duck-LiDAR project in Cass County, Minnesota, USA.

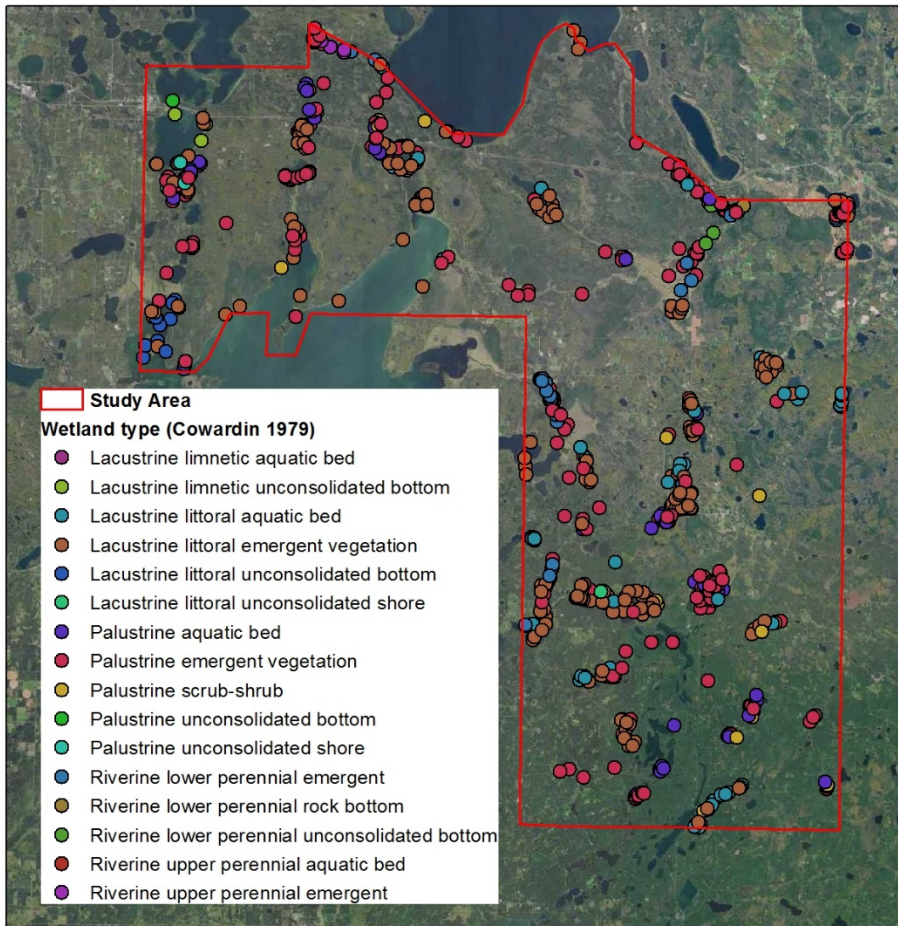


Figure 2. Location of wetland plots of different National Wetland Inventory classes (Cowardin et al. 1979) surveyed in Cass County, Minnesota, USA during Summer and Fall 2016 and 2017.

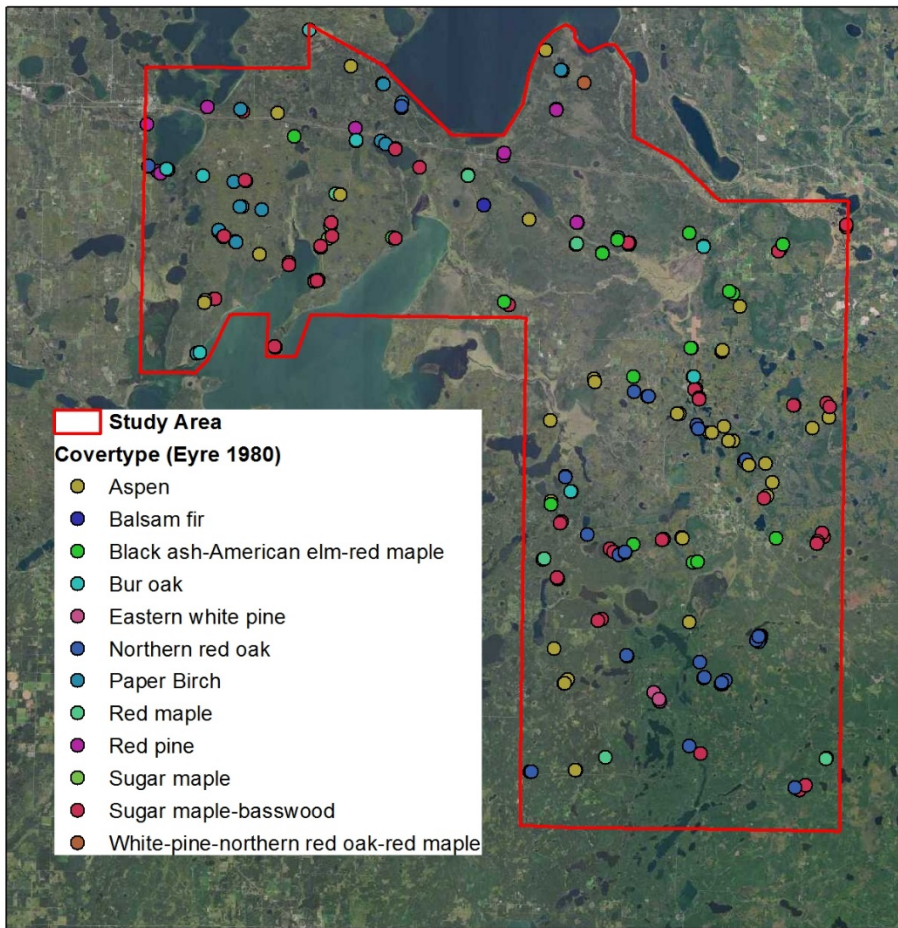


Figure 3. Location of forest plots of different cover types (Eyre 1980) that were surveyed in Cass County, Minnesota, USA during Fall 2016, Spring 2017, and Fall 2017.

Appendix 1. Numerical codes used in the classification of the health status of trees (from Thomas 1979).

Health status	Description
1	Live tree that has no defects or injuries that will threaten its long-term health.
2	Live tree with defects that contribute to a decline in health. Indicators may include decay on the bole, fungi, large dead limbs, and substantial cracks.
3	Recently dead tree with bark, limbs, and twigs substantially intact.
4	Dead tree that has lost some limbs and almost all twigs.
5	Dead tree that has lost most limbs and all twigs.
6	Dead tree with a broken top and hard bole wood.
7	Dead tree with a broken top and soft bole wood.

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EVALUATING INSECTICIDE EXPOSURE RISK FOR GRASSLAND WILDLIFE ON PUBLIC LANDS

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SUMMARY OF FINDINGS

Increasing evidence suggests that acute toxicity to pesticides may be more important than agricultural intensity in explaining declines in grassland-dependent wildlife. Although neonicotinoids (systemic insecticides routinely used on corn and soybeans) are currently under scrutiny for their effects on birds and pollinators, other insecticides are commonly used in Minnesota's farmland regions that may also have negative effects on non-target organisms. Minnesota Department of Natural Resource (MNDNR) wildlife managers and members of the public have reported concerns about foliar-application insecticides in particular. Such insecticides are used on a variety of crops but their use has been especially important for controlling soybean aphid outbreaks in Minnesota's farmland regions. Concerns have previously been raised about the impacts of chlorpyrifos, a broad-spectrum organophosphate, and other foliar-application insecticides on water quality and human health, prompting the Minnesota Department of Agriculture (MDA) to release guidelines for voluntary best management practices for their use. Although lab studies have shown chlorpyrifos and other insecticides used to target aphids are highly toxic to non-target organisms, including economically important game species and pollinators, fewer studies have investigated the environmentally-relevant exposure risk of free-ranging wildlife to these chemicals. Our research project is assessing the direct and indirect exposure risk of grassland wildlife to common soybean aphid insecticides along a gradient from soybean field edge to grassland interior. During summer 2017, we sampled 2 treatment and 2 control sites in southwestern Minnesota. We are currently processing our samples to quantify chemical residues and to assess the effects of insecticide exposure on the invertebrate community. Additionally, we will sample an additional 6 treatment and 2 control sites during summer 2018. The data we obtain on the environmentally-relevant exposure risk of wildlife to these insecticides will be used to help natural resource managers and private landowners better design habitats set aside for grassland wildlife in Minnesota's farmland region.

INTRODUCTION

Grassland habitat loss and fragmentation is a major concern for grassland-dependent wildlife throughout the Midwestern United States (US). In particular, habitat loss due to agricultural intensification has been implicated as a primary reason for the declines of many grassland nesting birds (Sampson and Knopf 1994, Vickery et al. 1999). However, concerns are increasingly being raised about the impacts of pesticides on birds and other wildlife in agriculturally-dominated landscapes (e.g., Hopwood et al. 2013, Hallmann et al. 2014, Main et al. 2014, Gibbons et al. 2015), and some evidence exists that acute toxicity to pesticides may be more important than agricultural intensity in explaining grassland bird declines in the U.S. (Mineau and Whiteside 2013).

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Soybean aphids were first discovered in southeastern Minnesota during 2000 and subsequently spread throughout the farmland zone by 2001 (Venette and Ragsdale 2004). Although these aphids pose significant risks to agriculture, their presence does not automatically translate to reduced yield or income (Venette and Ragsdale 2004). In response to concerns over yield loss, the University of Minnesota Extension Office (hereafter, UM Extension) released guidelines on how to scout for aphids and when to consider treatment for infested fields (UM Extension 2014). Foliar applications of insecticides using ground sprayers or planes are common treatment methods when chemical control of aphids is necessary. The 2 most common insecticides used are chlorpyrifos and lambda-cyhalothrin (MDA 2005, MDA 2007, MDA 2009, MDA 2012, MDA 2014a) but bifenthrin is also frequently used (N. Davros, unpublished data; E. Runquist, unpublished data). Withholding times vary by chemical (lambda-cyhalothrin: 45 d; bifenthrin: up to 14 d; chlorpyrifos: 28 d); thus, the timing of product use within the growing season should be considered. If retreatment is necessary due to a continued infestation, landowners are encouraged to use an insecticide with a different mode of action to prevent resistance (UM Extension 2014) or reduce the impact of insecticide-resistant aphids (UM Extension 2017, UM Extension 2018). Therefore, multiple chemicals may be used on the same field at different times of the year in some situations. Alternatively, landowners may choose to use a product that combines 2 or more chemicals together (e.g., chlorpyrifos + lambda-cyhalothrin), and such products are readily available on the market.

Lambda-cyhalothrin (common trade names include Charge, Demand, Excaliber, Grenade, Hallmark, Icon, Karate, Kung-fu, Matador, Samurai, and Warrior) is a broad-spectrum pyrethroid insecticide that affects the nervous systems of target- and non-target organisms through direct contact, ingestion, and inhalation [National Pesticide Information Center (NPIC) 2001]. Although lambda-cyhalothrin is considered low in toxicity to birds, it is highly toxic to pollinators such as bees (NPIC 2001). Further, field studies have shown lower insect diversity and abundance in fields exposed to lambda-cyhalothrin (Galvan et al. 2005, Langhof et al. 2005, Devotto et al. 2006). Because insects are an especially important source of protein for birds during the breeding season, fewer insects could mean reduced food availability for fast-growing chicks.

Bifenthrin (common trade names include Bifenture, Brigade, Discipline, Empower, Tundra, and Xpedient) is a broad-spectrum pyrethroid insecticide that affects the central and peripheral nervous systems of organisms by contact or ingestion (Johnson et al. 2010). Bifenthrin is low in toxicity to birds, including game species such as bobwhite quail (*Colinus virginianus*) and mallards (*Anas platyrhynchos*) (LD₅₀ values of 1800 mg/kg and <2150 mg/kg, respectively; Johnson et al. 2010). However, there are exposure risks for birds that feed on fish and aquatic insects because bifenthrin is very highly toxic to aquatic organisms (Siegfried 1993, Johnson et al. 2010). Some non-target terrestrial insects are also susceptible to bifenthrin (Siegfried 1993). For example, bifenthrin is very highly toxic to bumblebees, with one study showing 100% mortality by contact (Besard et al. 2010).

Chlorpyrifos (common trade names include Dursban, Govern, Lorsban, Pilot, Warhawk, and Yuma) is a broad-spectrum organophosphate insecticide that also disrupts the normal nervous system functioning of target- and non-target organisms through direct contact, ingestion, and inhalation (Christensen et al. 2009). Although first registered for use in the U.S. in 1965, its use as an ingredient in residential, pet, and indoor insecticides was removed in 1997 (except for containerized baits) due to human health concerns (Christensen et al. 2009, Alvarez et al. 2013 and references therein, MDA 2014b). Further, MDA recently released guidelines for best management practices for the use of chlorpyrifos due to water quality concerns (MDA 2014b). Lab studies have shown chlorpyrifos to be toxic to a variety of aquatic and terrestrial organisms (reviewed in Barron and Woodburn 1995), and some bird and beneficial insect species are especially susceptible to acute toxicity from chlorpyrifos exposure (Christensen et al. 2009,

MDA 2014a). Chlorpyrifos is very highly toxic to gallinaceous bird species such as the ring-necked pheasant (*Phasianus colchicus*) and domesticated chickens (*Gallus gallus domesticus*), with a lethal dose causing death in 50% of treated animals (LD₅₀) of 8.41 mg/kg and 32-102 mg/kg, respectively (Tucker and Haegele 1971, Christensen et al. 2009). Several other bird species are also particularly susceptible to chlorpyrifos, including American robins (*Turdus migratorius*), common grackles (*Quiscalus quiscula*), and mallards (Tucker and Haegele 1971, Christensen et al. 2009). Yet few field studies have been able to document direct mortality of birds from chlorpyrifos exposure (e.g., Buck et al. 1996, Martin et al. 1996, Booth et al. 2005), and an ecotoxicological risk assessment conducted by Solomon et al (2001) concluded that the available evidence did not support the presumption that chlorpyrifos use in agroecosystems will result in extensive mortality of wildlife. However, chlorpyrifos exposure leading to morbidity (e.g., altered brain cholinesterase activity, altered behaviors, reduced weight gain) has been documented in both lab and field studies (McEwen et al. 1986, Richards et al. 2000, Al-Badrany and Mohammad 2007, Moye 2008). Thus, sub-lethal effects leading to indirect mortality (e.g., via increased predation rates) may be a concern for wildlife exposed to chlorpyrifos.

Minnesota DNR wildlife managers and members of the public have reported concerns about the effects of soybean aphid insecticides on non-target wildlife, including economically important bird and pollinator species. The common public perception is that indiscriminate spraying without first scouting for aphid outbreaks has become the norm and fewer birds and insects are observed after spraying has occurred. Yet little is known about the actual exposure risk of birds and terrestrial invertebrates to these insecticides in Minnesota's grasslands. Distances reported for drift from application of foliar insecticides vary widely in the literature (5-75 m; Davis and Williams 1990, Holland et al. 1997, Vischetti et al. 2008, Harris and Thompson 2012), and a recent butterfly study in Minnesota found insecticide drift on plants located up to 1600 m away from potential sources (E. Runquist, personal communication). The distance of travel for spray drift is dependent on several factors including droplet size, boom height or width, and weather conditions (e.g., humidity, wind speed, dew point) at the time of application. Guidelines for pesticide application are readily available to landowners and licensed applicators (MDA 2014b, MDA 2014c) so that the likelihood of spray drift can be minimized but there is likely large variation in typical application practices.

OBJECTIVES

Our goal is to assess the environmentally-relevant exposure risk of grassland wildlife to commonly-used soybean aphid insecticides, especially chlorpyrifos, in Minnesota's farmland region. In particular, we will:

1. *Direct and Indirect Exposure:* Quantify the concentration of insecticides along a gradient from soybean field edge to grassland interior to assess the potential for grassland wildlife (particularly nesting birds and their young, and beneficial insects) to be exposed to chemicals directly via contact with spray drift and indirectly through consumption of insect prey items exposed to the insecticides.
2. *Indirect Effects:* Quantify and compare the relative abundance, richness, diversity, and biomass of invertebrate prey items along a gradient from soybean field edge to grassland interior prior to and post-application to assess the indirect impact of the insecticides on food availability for grassland nesting birds and other wildlife.

STUDY AREA

Our study is being conducted within the southwest (SW), south-central (SC), west central (WC), and central (C) regions of Minnesota's farmland zone (Figure 1). Corn and soybeans combined

account for approximately 50% of the landscape across these 4 regions and up to 75% of the landscape in the SW and SC regions in particular [U.S. Department of Agriculture (USDA) 2013a, USDA 2013b]. Acres set aside as grassland habitat on public and private land account for 5.8% and 4.6% of the landscape, respectively (Davros 2015). Since 2003, these regions have also experienced some of the highest estimated use of chlorpyrifos and lambda-cyhalothrin (MDA 2005, MDA 2007, MDA 2009, MDA 2012, MDA 2014a).

METHODS

Experimental Design

A treatment study site will consist of a MNDNR Wildlife Management Area (WMA) immediately adjacent to a soybean field that will be sprayed to control for aphids. We will work in close consultation with wildlife managers and private landowner cooperators to select treatment sites. We will use sites dominated by a diverse mesic prairie mix containing warm-season grasses and forbs because this mix is commonly used by MNDNR managers and agency partners in the farmland zone to restore habitats for the benefit of grassland birds and beneficial insect species. We will also use control study sites with similar site characteristics except that control sites will have corn as the adjacent crop and they will not be sprayed with any chemicals to control aphids.

We will sample a total of 8 treatment sites and 4 control sites across 2 field seasons (summer 2017 and summer 2018). Within each treatment site prior to spraying, we will establish sampling stations at distances of <1 m, 5 m, 25 m, 50 m, 100 m, and 200 m along each of 3 transects. If the site is large enough, we will also establish a station at a distance of 400 m along each transect. This design will give us a total of 18-21 stations per site. We will establish transects and stations the same way within control sites. At all sites, transects will run perpendicular to the edge of the soybean field and will be spaced 100 m apart to reduce the likelihood of duplicate insecticide exposure from the spraying event.

Data Collection

To assess the potential for direct exposure of birds and other wildlife to soybean aphid insecticides (hereafter, target chemicals), we will deploy passive sampling devices (PSDs) to absorb any chemical drift that occurs. The PSDs will be placed in treatment fields on the day of but prior to spraying of soybeans. The PSDs will be made of Whatman™ Qualitative Filter Paper (grade 2) that is attached to 0.5 in² hardware cloth formed to a cylinder shape to approximate the size and shape of a large songbird or a gamebird chick. We will place the PSDs at two heights (ground and mid-canopy) at each of the 18-21 sampling stations per site for a total of 36-42 PSDs/site. Ground-level sampling will help represent ground-nesting birds and other wildlife that spend the majority of their time on the ground (e.g., gamebirds, small mammals, many species of invertebrates). Mid-canopy sampling will help represent above-ground nesting birds and many species of spiders and insects. We will retrieve the PSDs from the field ≤2.5 h after spraying and properly store them for later chemical analysis. At control sites, we will place PSDs at both ground and mid-canopy levels at each of the stations. We will leave the PSDs on site for the same amount of time as PSDs at treatment sites before we collect and store them for later analysis.

We will also use water-sensitive cards (Syngenta Global, Basel, Switzerland) to collect spray droplets from chemical drift. These cards change from yellow to dark blue when they encounter liquid. We will attach 2 cards next to each PSD (1 card on the vertical plane and 1 card on the horizontal plane) at each canopy layer (ground, mid) of each sampling station. The cards will later be analyzed to determine if they can be used as a quicker and cheaper method for qualitatively detecting spray drift in grasslands.

To assess the potential for birds and other insectivorous wildlife to be exposed to the target chemicals indirectly via consumption of prey items (hereafter, indirect exposure), we will sample invertebrates ≤ 5 h post-spraying at the 0 m, 5 m, and 25 m stations along each transect (total = 9 stations/site). We will sample ground-dwelling invertebrates using a vacuum trap and canopy-dwelling invertebrates using a sweepnet. Vacuum trap and sweepnet samples will both be taken along a 30 m doubled transect ($30 \text{ m} \times 2 = 60 \text{ m}$ total length sampled) to the right side of the sampling stations and parallel to the soybean field. We will combine vacuum trap and sweepnet samples taken from the same station during the same time period into one sample and properly store them for later chemical analysis. We will sample control sites using the same methods and timing, with the timing based on when we deploy the PSDs at these sites.

To quantify and compare the effects of target chemicals on the abundance, richness, diversity, and biomass of invertebrate prey items (hereafter, indirect effects), we will collect vacuum trap and sweepnet samples from the <1 -5 m, 25 m, and 100 m distances along the 3 transects at each site (total = 9 stations/site). The <1 m and 5 m distances will be combined into one distance bin for this effort. We will collect these samples 1-3 d prior to spraying and between 3-5 d and 19-21 d post-spraying at treatment sites. Samples will be taken along a 20 m doubled transect ($20 \text{ m} \times 2 = 40 \text{ m}$ total length sampled) but on the left side of the sampling stations and parallel to the soybean field. We will combine vacuum trap and sweepnet samples into one sample per station per sampling period and store them in ethanol for later sorting, identifying, drying, and weighing. During the 3-5 d and 19-21 d sampling efforts, we will also collect invertebrate samples at the same 3 distances along 1 additional transect established >60 m away from but parallel to our 3 main transects. This additional transect will provide us with post-spraying control samples to address any concerns about whether our repeat disturbance of the main transects impacts our estimates of indirect effects. We will use the same methods and timing to collect our indirect effect samples at each of our control sites. When we begin processing our samples in the lab, we will place emphasis on 4 invertebrate orders important in the diets of grassland nesting birds: Araneae (spiders), Orthoptera (grasshoppers, crickets, and katydids), Coleoptera (beetles), and Hemiptera (true bugs). All individuals from these orders will be sorted and identified to at least the family level for analysis. Quantifying the spider community will allow us to examine potential impacts on an additional trophic level since spiders are an important predator of insects.

We will use portable weather meters (Kestrel 5500AG Agricultural Weather Meters) mounted on tripods and equipped with weather vanes to measure relevant weather data (e.g., temperature, wind speed, wind direction, humidity, dew point) along the center transect at the <1 m, 100 m, and 200 m stations during the deployment of PSDs and at the <1 m, 25 m, and 100 m stations during pre- and post-spraying insect sampling at each site.

At each site, we will also collect vegetation data 1-3 days prior to spraying at all stations and again at 3-5 d and 19-21 d post-spraying at the reduced subset of stations which coincide with invertebrate sampling efforts. Multiple vegetation plots will be sampled at each station: 3 plots parallel to the field edge at each station and 1 plot at each end of the 20 m and 30 m insect sampling transects. Data collected at each plot will include percent ground cover, percent canopy cover, maximum height of live and dead vegetation, litter depth, and vertical density. Using a modified point-intercept method, we will categorize ground cover into bare ground, litter, or other [i.e., woody debris, rock, or gopher mound; Bureau of Land Management (BLM) 1996]. To determine canopy cover, we will take a nadir digital photograph of a 30 cm x 55 cm quadrat at a height of 1.5 m above the ground and use the program SamplePoint to estimate percent canopy cover (Booth et al. 2006). Canopy cover categories will include grass, forb, standing dead vegetation, woody vegetation, and other. We will measure litter depth to the nearest 0.1 cm at 1 point within the plot that represents the average condition of the plot. We will record the

maximum height of live and dead vegetation within each plot to the nearest 0.5 dm. We will measure vertical density by placing a Robel pole in the center of each plot and estimating the visual obstruction reading (VOR) from 4 m away and 1 m above the ground in each of the 4 cardinal directions (Robel et al. 1970). Finally, we will record the dominant grass and forb species (up to 3 species in each category) in each plot along the center transect plots to obtain a qualitative assessment of the vegetation present at each site.

We will send the PSD samples and invertebrate samples to the USDA Agricultural Marketing Service's National Science Lab (USDA/AMS-NSL) in Gastonia, NC for chemical residue analysis. Samples will be analyzed using a solvent-based extraction method. Extracts will be concentrated by evaporation and then analyzed using a gas chromatography/mass spectrometry-negative chemical ionization (GC/MS-NCI) or other appropriate method. Although our experimental design will focus on soybean fields sprayed with foliar insecticides to control aphids, the chemical analyses will allow us to quantify additional pesticides (e.g., neonicotinoids, fungicides) at minimal extra cost. Obtaining information about other pesticide exposure will be valuable supplementary information in support of other Section of Wildlife research and management goals.

Data Analyses

We will use mixed regression models to examine factors related to risk of direct and indirect exposure of wildlife to target chemicals. Chemical concentration will be the dependent variable. We will specify distance from soybean field edge and canopy height (when relevant) as a fixed effect. We may also include other covariates such as site, ordinal date, vegetation, and weather condition variables where appropriate. We will use similar models to examine differences in the abundance, richness, diversity, and biomass of Aranaeans, Orthopterans, Coleopterans, and Hemipterans. We will use the sampling period (i.e., 1-3 d prior to spraying, and 3-5 d or 19-21 d post-spraying) as a repeated measure in these analyses, specifying a covariance structure [e.g., autoregressive 1 (AR1)] when appropriate.

RESULTS AND DISCUSSION

During fall 2016, we surveyed 12 farmer cooperatives in 12 counties to gather more specific information about chemical spraying (e.g., type of insecticide, application method) in southern Minnesota. Congruent with MDA's pesticide usage reports (MDA 2007, MDA 2009, MDA 2012, MDA 2014a), the coops reported that chlorpyrifos, lambda-cyhalothrin, and bifenthrin have been the most commonly-used foliar soybean insecticides in recent years. Additionally, we learned that neonicotinoids have also been used in the chemical mixes used as foliar treatment of crop pests. This information is contrary to the widespread belief that neonicotinoids are only used as a prophylactic seed treatment to treat plants systemically. Based on estimates provided by 8 of the 12 coops, an average of 63% of fields were sprayed by plane (range: 40-85%) whereas 37% of fields (range: 15-60%) were sprayed by ground booms in 2016. Fewer fields could be accessed via tractor during spraying operations due to wet field conditions which may have increased the percentage of fields sprayed by plane that year.

In late winter and early spring 2017, we also mailed surveys to landowners adjacent to potential WMA study sites to learn more about their soybean aphid spraying practices and to ask for their cooperation with our study (see Appendix 1). Several potential cooperators indicated that they do not scout for aphids but instead spray regardless of infestation levels. This approach to soybean management may be a primary reason why reports of aphid resistance to pyrethroid insecticides are increasing in Minnesota and parts of North Dakota (UM Extension 2017, UM Extension 2018).

Although our mail surveys helped us identify willing cooperators, we ultimately solicited landowner cooperation by directly calling landowners and visiting their residences. This approach was more effective than mailing surveys. We are using the same cold-calling technique to solicit landowner cooperation for the summer 2018 field season.

In 2017, we sampled 2 treatment and 2 control sites from 28 July – September 14 (Table 1). We collected a total of 166 direct exposure PSD samples, 166 water-sensitive cards, 36 indirect exposure invertebrate samples, and 132 indirect effect invertebrate samples across all the sites (Table 1). Our direct exposure PSD and indirect exposure invertebrate samples are currently being processed by USDA/AMS-NSL, and only a subset of results have been received to date.

Our objective with using the water-sensitive cards was to obtain an immediate, qualitative visual assessment of insecticide drift. However, even moderately high humidity levels produced a color change in the absence of drift (Figure 2a & 2b). The cards also picked up dew droplets from the surrounding vegetation which affected discoloration. Thus, we were unable to reliably detect insecticide drift on these cards. We will be discontinuing their use in 2018.

We have completed the sorting and identification of our indirect effect invertebrate samples collected during 2017. Additional processing of these samples in the lab will begin once the summer 2018 field season has been completed.

Further results will be forthcoming once our 2018 field sampling has been completed and samples have been processed in the lab. Our goal is to sample an additional 6 treatment sites and 2 control sites during summer 2018. Late winter and April snowstorms resulted in a very wet start to the crop planting season and excessive rainfall in June further exacerbated soil moisture conditions, particularly in the SW and SC regions. Some landowners have had to replant their row crops 3 times whereas others continue to have flooding in portions of their fields. Soybean growth and development may be behind in these regions as a result. Additionally, severe storms may have directly impacted soybean aphid populations in some areas. Aphid populations may be higher this year in the regions that have experienced lower rainfall amounts and earlier planting dates (i.e., WC and C regions).

ACKNOWLEDGMENTS

We would like to thank the many MNDNR wildlife managers who have provided discussions on the topic and/or information on potential study sites. We are especially grateful to the landowners, agronomists, and pilots who have cooperated with us by returning our mail survey, calling us to provide information, and/or coordinated with us on the timing of spraying and field sampling. Tonya Klinkner contacted the cooperatives to ask about soybean spraying practices. Tonya Klinkner, Hattie Saloka, and Katie Steffl each provided assistance with supply purchasing and/or personnel hiring. Veronique St-Louis and John Giudice provided valuable feedback on initial study design, and David Andersen, Pamela Rice, Theresa Kissane Johnston, and Jessica Petersen have provided further feedback on study design and methodologies. Sophia Crosby, Chuck Fortier, Greg Gehring, John Letlebo, Casey Reep, and Haley Witt provided assistance with field data collection. Madison Fox, Bishal Parajuli, Fadzai Teramayi, and Jacob Westfield helped sort invertebrate samples, and Lindsey Christianson identified invertebrate samples. Shalesa Johnson provided assistance with data entry. This project has been funded by the Environment and Natural Resources Trust Fund (ENRTF) as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR). The MNDNR Section of Wildlife has also contributed funding through the Federal Aid in Wildlife Restoration Act. Finally, the Cooperative Fish and Wildlife Research Unit at the University of Minnesota has provided in-kind support.

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Table 1. Site location, type, and sample size information for Wildlife Management Areas (WMAs) sampled for chemical drift from soybean aphid insecticide spraying between 28 Jul - 14 Sep 2017 in Minnesota.

Site ID ^a	Agricultural region ^b	County	Site type ^c	Direct exposure		Invertebrates	
				PSDs ^d	Water-sensitive cards	Indirect exposure	Indirect effects ^e
A/Deh	SW	Jackson	Control	42	42	9	33
B/Hel ^f	SW	Jackson	Treatment	40	40	9	33
C/Roh	SW	Lyon	Control	42	42	9	33
D/Lam	SW	Murray	Treatment	42	42	9	33

^aWMA names are not provided to protect private landowner cooperators.

^bAgricultural regions: southwest (SW), south-central (SC), southeast (SE), west central (WC), central (C), east central (EC), and northwest (NW).

^cTreatment sites had adjacent soybean fields that were sprayed for aphids; control sites had adjacent corn fields that were not sprayed for aphids.

^dPSD = passive sampling device.

^e9 samples were collected during the pre-spray period and 12 samples were collected during each of the two post-spraying periods at each site.

^fFewer PSDs and water-sensitive cards were collected at this site due to transect length constraints.

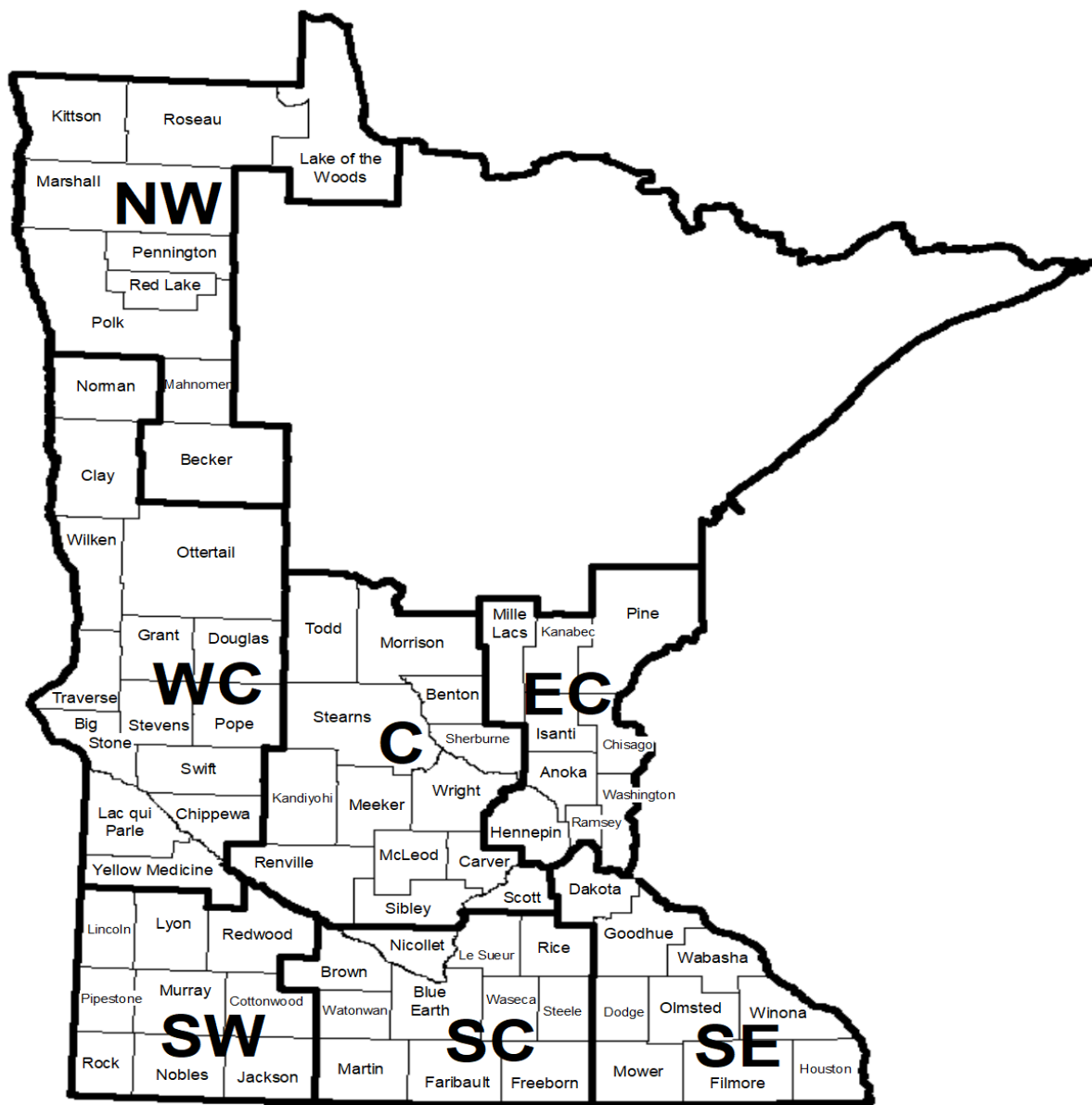


Figure 1. Minnesota's agricultural regions as outlined in MNDNR's annual August Roadside Surveys. Abbreviations: SW = southwest, SC = south central, SE = southeast, WC = west central, C = central, EC = east central, and NW = northwest.

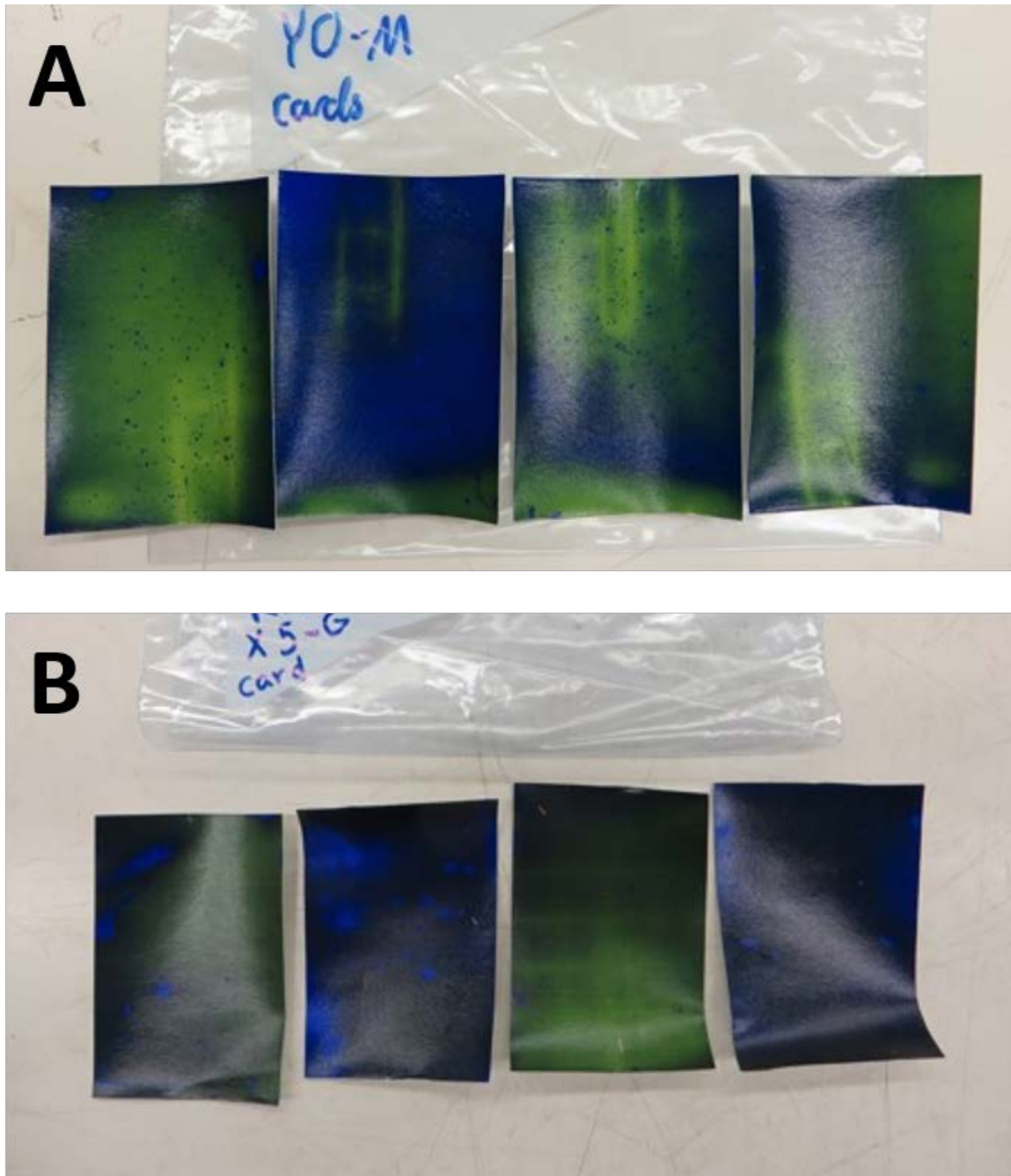


Figure 2. Water-sensitive cards were used during July-September 2017 in an attempt to qualitatively assess insecticide drift along a gradient from soybean field edges to grassland interiors of Wildlife Management Areas (WMAs) in Minnesota. Cards turned from yellow to blue when exposed to liquid but relative humidity (RH) levels above 65% also caused the cards to discolor significantly. A) Spray droplets are visible on cards placed at the mid-canopy height a distance of 0 m from the soybean edge at a treatment site; RH was 89% at the time of sampling and also caused major discoloration. B) No evidence of spray droplets is visible on cards placed on the ground at a distance of 5 m from the corn field edge at a control site; RH was 65% and caused the cards to be almost completely discolored.

APPENDIX 1. Survey sent to neighboring landowners [i.e., private landowners with property immediately adjacent to potential Wildlife Management Area (WMA) study sites in Minnesota] in March and April 2017 to assess soybean aphid spraying practices and to solicit cooperation for summer 2017 sampling efforts.

Print your name here _____

Spraying Practices Survey

PART I

1. Have you planted soybeans on your land in the past 3-5 years?
 - ☐ Yes
 - ☐ No → *please continue to Part II*
2. Were your soybeans treated with foliar insecticides in the past 3-5 years?
 - ☐ Yes
 - ☐ No → *please continue to Part II*
3. On what date(s) were foliar insecticides applied on your soybeans?
4. How was the majority of foliar insecticides sprayed on your soybeans in the past 3-5 years?
 - ☐ Ground boom
 - ☐ Aerial
 - ☐ Other (please specify):
5. Please list the foliar insecticide trade names and/or the application logistics used on your soybeans in the past 3-5 years to control aphids.
Example: "2016: Lorsban - 20 gpa through 8004 nozzles @ 50-60 psi from a 854 Rogator traveling at 6 mph to apply a 90' swath"
6. Did you hire an applicator (e.g. agricultural consultant company) to treat your soybeans with foliar insecticides in the past 3-5 years?
 - ☐ Yes (please specify company or individual):
 - ☐ No, I applied insecticides myself

PART II

1. Will you be planting soybeans on your land that borders a Wildlife Management Area (WMA) or other protected grassland in 2017?
 - ☐ Yes
 - ☐ No → *end of survey - thank you*
 - ☐ I'm not sure
2. Will you be treating these soybeans with foliar insecticides in 2017 if significant numbers of aphids occur?
 - ☐ Yes
 - ☐ No → *end of survey - thank you*
 - ☐ I'm not sure
3. How will foliar insecticides likely be sprayed on these soybeans in 2017?
 - ☐ Ground boom
 - ☐ Aerial
 - ☐ Other (please specify):
 - ☐ I'm not sure
4. Please list the foliar insecticide trade names and/or the application logistics that will likely be used on these soybeans in 2017 to control aphids.
Example: "Lorsban - 20 gpa through 8004 nozzles @ 50-60 psi from a 854 Rogator traveling at 6 mph to apply a 90' swath"
5. Will you hire an applicator (e.g. agricultural consultant company) to treat these soybeans with foliar insecticides in 2017 if chemical treatment is needed?
 - ☐ Yes (please specify company or individual):
 - ☐ No, I will apply insecticides myself
 - ☐ I'm not sure

Please return to Katelin Goebel in the envelope provided. Thank you.

Print your name here _____

Contact Information Form

1. May we contact you to identify foliar insecticide spraying date(s) in the summer of 2017?
☐ Yes
☐ No

2. What is the best way to reach you?
☐ Home phone

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☐ Cell phone

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☐ Both home & cell phones

3. In order to identify the exact date(s) of spraying, how often are you comfortable with us contacting you during the late summer of 2017?
☐ Weekly
☐ Semi-weekly
☐ As often as necessary as the spraying date approaches (no more than once daily)

4. Would you like to receive a paper copy of the LCCMR work plan for our project?
This can also be found at: http://www.lccmr.leg.nm/projects/2016/work_plans_may/_2016_03n.pdf
☐ Yes
☐ No

5. Would you like to receive a paper copy of your responses to the Spraying Practices Survey and Contact Information Form?
☐ Yes
☐ No

6. If you rent your land, please provide the name and address of your renter so we may send them a letter and survey:

Please return to Katelin Goebel in the envelope provided. Thank you.



NESTING AND BROOD-REARING HABITAT SELECTION AND SURVIVAL RATES OF RING-NECKED PHEASANTS IN PRAIRIE RECONSTRUCTIONS IN SOUTHWEST MINNESOTA

Nicole M. Davros and Lindsey N. Messinger

SUMMARY OF FINDINGS

Ring-necked pheasant (*Phasianus colchicus*) responses to the amount of grassland on 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. From early spring 2015 through summer 2018, we radiocollared 164 hens on 2 study sites in southwest Minnesota and monitored them during nesting and brood-rearing each year. We collected vegetation data on nest site selection and survival data on hens, nests, broods, and individual chicks each year. In 2016 and 2017, we also collected data on brood-rearing habitat selection. Video cameras were used to document nest predation events in 2015 and 2016. Preliminary descriptive findings are described within this report and final results are pending. Ultimately, our results will be used to better understand the factors that limit reproductive success of pheasants so that natural resource managers can prioritize their grassland management and land acquisition strategies.

INTRODUCTION

Ring-necked pheasant population dynamics are driven largely by variation in survival rates. Although adverse weather can impact survival rates in some years, 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 over the long-term and at the landscape scale (Chesness et al. 1968, Riley and Schulz 2001). Management aimed at increasing pheasant populations has instead focused primarily on providing abundant nesting cover to minimize the effects of predation and maximize reproductive success. As acres enrolled in the Conservation Reserve Program (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 and weather on pheasant population dynamics. However, the interaction between habitat and predation will no doubt remain. Thus, gaining new insights into the relationship between pheasant habitat selection and subsequent survival rates will be important for improving wildlife 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, Fogarty et al. 2017) 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). 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, Benson et al. 2013). Understanding how management at the site level (e.g., vegetation structure, composition, and diversity) 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; N. Davros, unpublished data). 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 leads 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, additional data are needed to understand the interplay between these potential limiting factors on brood habitat selection and chick survival in different grassland habitat types within Minnesota's pheasant range.

Minnesota Department of Natural Resources (MNDNR) 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. 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 (e.g., forb interseeding or other grassland reconstruction activities) and landscape (e.g., acquisition priorities) levels in the face of reduced CRP acreages. Additionally, obtaining data on individual components of pheasant population dynamics will aid in future assessment of MNDNR management activities [e.g., Prairie Plan implementation (Minnesota Prairie Plan Working Group 2011), conservation grazing] and

agricultural land use practices (e.g., cover crops, pesticide use) on Minnesota's pheasant population.

OBJECTIVES

Our overall objective is to evaluate the relative importance of within-patch diversity (e.g., sites dominated by cool-season grasses, warm-season grasses, and high diversity grass-forb mixtures) within Wildlife Management Area (WMA) project areas on pheasant productivity. Specifically, we will:

1. Evaluate pheasant nest site selection and nest, brood, chick, and adult hen (hereafter, hen) survival in relation to vegetation cover and composition.
2. Evaluate pheasant brood-rearing habitat selection in relation to vegetation cover and composition.
3. Evaluate the relative importance of different factors (e.g., vegetation, predation, weather) on nest, brood, chick, and hen survival.

Results from a pilot study during the 2015 breeding season allowed us to refine methods and protocols for the study's expansion during 2016-2018. Data collection, analysis, and interpretation are still underway. Therefore, we present preliminary results here.

STUDY AREA

Our study is being conducted in the southwest region of Minnesota, a core region of the state's pheasant range. 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 [CRP, Reinvest in Minnesota (RIM), Conservation Reserve Enhancement Program (CREP), and Wetlands Reserve Program (WRP)] and public land [MNDNR Wildlife Management Areas (WMA) and U.S. Fish & Wildlife Service (USFWS) Waterfowl Production Areas (WPA)] account for 6.3% of the landscape in this region (Davros 2016).

For our study sites, we selected 2 WMA project areas representing the grassland/wetland habitat complexes that are a goal of the Prairie Plan (Minnesota Prairie Plan Working Group 2011) and the Pheasant Summit Action Plan (MNDNR 2015). Each study site is about 9 mi² in size and contains extensive amounts of permanently protected grassland habitat. The Lamberton WMA study site (Redwood County) is a large, nearly contiguous WMA complex with >1,100 acres of permanently protected upland and wetland habitats. The Worthington Wells study site (Nobles County) has >1,500 acres of permanently protected habitat that spans multiple WMAs, the Okabena-Ocheda Watershed District, and USFWS lands.

METHODS

Data Collection

We captured hen pheasants in each study site during 6 time periods: 2 February – 15 April 2015, 7 October – 11 November 2015, 11 January – 29 April 2016, 26 September – 15 November 2016, 18 March – 14 April 2017, and 18 September – 11 October 2017 (hereafter referred to as spring 2015, fall 2015, spring 2016, fall 2016, spring 2017, and fall 2017, respectively). We used 2 capture techniques: baited walk-in traps and netting via nighttime spotlighting from a 6-wheel utility-task vehicle (UTV). Each hen was weighed to the nearest 5.0 g, measured on the right tarsus to the nearest 0.5 mm, banded with a uniquely numbered aluminum leg band, fitted with a 16.0-g necklace-style VHF radiotransmitter with integrated

mortality switch (Advanced Telemetry Systems (ATS), Isanti, MN), and released at the site of capture.

We began radiotracking hens 3-5 times per week in late April each year to determine the onset of incubation. We assumed incubation had begun when a hen's radio signal was projected from the same location for several consecutive days. We flushed each hen from her nest once between incubation day 5-20 to determine clutch size and floated 3-5 eggs to estimate hatch date (Westerskov 1950, Carroll 1988). We marked the location of nests using a global positioning system (GPS) receiver. We also placed flagging ≤ 5 m from nests to aid relocation efforts. If a hen began making large daily movements prior to being flushed, we assumed nest failure and waited for the hen to resettle and begin incubating again before attempting another flush. We used the homing technique on radiocollars emitting a mortality signal to retrieve the collars and determine a fate. We used the presence and condition of any bodily remains and the condition of the radiocollar (e.g., teeth marks, feathers plucked, body intact but frozen, frayed collar, missing crimp) and nearby evidence (e.g., predator scat, den site) to determine survival status (e.g., mortality vs. unknown) and assign a potential cause of death (e.g., predation, human/machinery, weather), if applicable.

During 2015 and 2016 only, we placed miniature color video cameras (GE 45231 MicroCam Wired Color Camera, Louisville, KY) at a random subset of nests in an attempt to document nest predation events (Cox et al. 2012). Cameras were placed at nests at the same time that hens were flushed to float eggs, and our total time near the nest was ≤ 20 min. We placed cameras 1-5 m away from the nest bowl at a height of approximately 0.3 m. Cameras had infrared light-emitting diodes (LEDs) to allow recording at night and were connected via a >20 m cable to digital video recorders (DVRs; Model MDVR14H, Super Circuits, Austin, TX) with SD memory cards for video storage and deep-cycle marine batteries for remote power. The DVRs and battery equipment were housed in waterproof containers located >20 m from nests. Video footage was reviewed and relevant video clips were archived.

Near the estimated hatch date of known nests, we monitored hen activity 2-3 times daily to pinpoint a hatching event. We assumed hatching was occurring when a hen's signal fluctuated in intensity (Riley et al. 1998). We captured 1-3 chicks by hand between day 0-2 (day 0 = hatch day) once the hen and her brood had moved away from the nest. We used 2 techniques to capture chicks. The first technique involved flushing the hen from her brood and using a decoy and playback to call chicks to us while we hid in the grass. The second technique involved flushing the hen from her brood just before sunrise during brooding and capturing chicks by hand as they scattered. We never captured more than 50% of the brood at one time. We also never kept the hen away from her remaining brood for >30 minutes to minimize risk of hypothermia for the uncaptured chicks. We discontinued chick capture attempts for a particular brood if we were unsuccessful at capturing any chicks by the end of day 2.

We transported captured chicks in a small cooler or waist belt heated with hand-warmers to a nearby field truck for processing. We determined the mass of each chick to the nearest 0.1 g and we measured tarsus length to the nearest 0.5 mm before suturing a 0.65-g backpack-style VHF radiotransmitter without mortality switch (ATS, Isanti, MN) to the chick's back (Burkepile et al. 2002, Dahlgren et al. 2010). Handling time lasted <5 min per chick and all captured chicks were returned to the hen within 30-60 min of capture. We followed the methods of Riley et al (1998) to return chicks to the hen.

We triangulated hens and their broods 2-3 times daily ≥ 3 times per week. Each bearing-coordinate pair was taken ≥ 100 m from target hens and their broods to reduce disturbance. We used specialized computer software (LOAS, Ecological Software Solutions LLC) to generate estimated locations from bearing-coordinate pairings. We monitored hens and their broods via

triangulation through the first 4 weeks post-hatching. On day 14 and day 30, we flushed the hen just before sunrise to determine brood status and size.

To estimate individual chick survival, we listened for the signal of each radiomarked chick every 1-3 days in conjunction with monitoring the hen. We relied primarily on fluctuation in the chick's signal to determine survival status, as backpack transmitters were too small to accommodate a mortality sensor. If the signal indicated that the chick was not moving, we used the homing technique to locate the transmitter and we searched the area for a carcass and any evidence for a cause of death.

We collected vegetation data at the nest site within 7 d of hatching for successful nests. For nests that failed, we also collected vegetation data at the nest site ≤ 7 d after the estimated hatch date. At each nest site, we visually estimated percent cover (Daubenmire 1959) of the upper canopy [i.e., grasses, forbs, woody vegetation, and other (e.g., logs, rocks)] using a 0.5 m² sampling quadrat. We estimated percent cover using 8 classes: 0%, 1-10%, 11-25%, 26-50%, 51-75%, 76-90%, 91-99%, and 100%. We separately estimated the cover of the ground layer (i.e., litter and bare ground) using the same 8 classes. We estimated litter depth to the nearest cm and we counted the number of grass and forb species to determine species richness within the quadrat. We also recorded visual obstruction readings from 4 m away at a height of 1 m (VOR; Robel et al. 1970) in each of the 4 cardinal directions to determine the vertical density of vegetation to the nearest 0.5 dm around the nest. Finally, we recorded the maximum height of live and standing dead vegetation within 0.5 m of the Robel pole. We repeated these sampling efforts at 2 random locations within 15 m of the nest site.

To evaluate brood habitat selection, we collected vegetation data at 5 brood locations estimated via triangulation and 10 random points outside but within 100 m of each brood's biweekly home range. Biweekly home ranges were generated twice for each brood: one home-range for the first 2 weeks of age and a second home-range for age 3-4 wk. For broods not surviving a 2-wk observation window, home ranges were generated and vegetation sampled if the brood survived for the first 7 d of the observation window. We used a Geographic Information System (GIS; ArcMap 10.2, ESRI, Redlands, CA) to estimate each biweekly home range using the minimum convex polygon tool. We generated a 100-m buffer around each home range polygon and generated random points within that buffer to identify the 10 random points for sampling. We restricted the selection of random points so that they were within the same habitat type (e.g., grassland). Any estimated brood locations or generated random points falling in habitat types other than grassland (e.g., row crop, wetlands, woodlots) were not sampled. Road right-of-ways were considered as available grassland habitat and included in sampling efforts. We collected vegetation data within 7 d of the end of each biweekly interval. At each estimated brood point, we sampled 1 center point and 3 equidistant points 10 m to the north, southwest, and southeast to capture the spatial variation associated with a brood location (i.e., hens and their broods are mobile and thus distributed around a point). We estimated percent canopy cover, litter depth, species richness, VOR, and maximum height of live and dead vegetation using the same methods described above for nest site selection. We repeated this sampling scheme at each of the 10 random points associated with each brood's biweekly home range. If a hen and her brood spent more than 50% of their time in habitat other than grassland (as defined above), we did not sample vegetation for that home range and associated random points. If a hen with a similar-aged brood had a home range that overlapped with another hen, we only sampled 5 additional random points associated with that second brood's home range.

Data Analyses

To date, we have conducted preliminary analyses on hen and nest survival. We also calculated basic descriptive statistics for nest site selection. Preliminary and final data analyses were

ongoing at the time of this report; thus, not all analyses have been included here and not all research objectives are addressed below.

We conducted a preliminary survival analysis to evaluate hen survival during the nesting and brood-rearing phases (15 April – 15 October; hereafter, breeding season) only. For hens captured between spring 2015 and spring 2017, we estimated cumulative survival using a Kaplan-Meier analysis approach in R v3.3.2 (R Core Team, 2016). The Kaplan-Meier approach assumes a known fate for each individual. As such, 36 individuals were censored at various intervals during the analysis period because they were reported missing and not relocated or their fate was otherwise unknown (e.g., slipped or failed radiocollar). We also excluded 35 individuals with capture and mortality or censor events occurring outside of the analysis period from this particular analysis.

Using 2015-2017 data, we conducted a preliminary nest survival analysis using the logistic-exposure method (Shaffer 2004) to estimate daily survival rate (DSR) of nests. We used a constant survival model (PROC GENMOD; SAS v9.3; SAS Institute, Cary, NC) which assumes that survival is constant across time and does not include any nest-specific explanatory variables.

RESULTS and DISCUSSION

We radiocollared 164 hen pheasants during the 6 trapping periods across our 2 study sites from spring 2015 through fall 2017. The baited walk-in traps were not a productive capture technique. We speculate that pheasants were not motivated to use bait due to mild winter conditions with above-average food availability each year. Only 3 hens were captured using the walk-in traps (2% of total hen captures) whereas 161 hens (98%) were captured by spotlighting. We ended spotlighting capture efforts at the onset of the nesting season, which limited our ability to increase sample sizes. In the future, we would consider using baited walk-in traps in late winter if weather conditions were severe enough to warrant this method. Winter conditions are considered severe for pheasants when snow is ≥ 6 inches deep and temperatures reach $\leq 0^\circ$ F.

From 2015-2017, cumulative survival for hens during the breeding season (183-day period pooled across years) across study areas was 0.72 ($n = 94$; CI: 0.64-0.82; Figure 1). During the 3 breeding seasons, 55% of marked individuals ($n = 51$) suffered a known mortality event. Of these mortality events, 94% were attributed to predation events, 6% to human causes (specifically, vehicle collision and agricultural equipment), and $<1\%$ to research-related marking. Although the Kaplan-Meier survival method provides a quick estimate of hen survival, the strict assumptions of this model may be inappropriate given our dataset. Because nearly 28% of individuals were censored during this analysis due to unknown fates (in particular, slipped radiocollars), our subsequent survival analyses will use methods that include expert knowledge to incorporate uncertainty in fate to refine survival estimates (Walsh et al. 2018).

We monitored 99 potential nesting attempts during the 2015-2017 seasons. We excluded 12 nests from analysis due to various reasons (e.g., hen abandoned after initial flushing event, nest area flooded prior to researcher's visit and actual nest bowl was never found). The remaining 87 nests provided 657 observation intervals and 1,244 exposure days for analysis (Shaffer 2004). Overall, DSR was 0.9777 ± 0.20 (range: 0.9674-0.9848) which results in a 59.5% overall nest success rate when extrapolated to a 23-day incubation cycle. Our nest success rate is comparable to the rate found by Clark et al. (2008) in their study of a large, contiguous grassland landscape of northern Iowa. Although the relationship between landscape fragmentation and nest success cannot be automatically inferred across study areas or regions (Benson et al. 2013), our results lend further support to the idea that landscapes with large amounts of grassland cover can benefit pheasant nest productivity in the upper Midwest. We used a constant survival model for our initial analyses; however, future analyses will examine

the role of vegetation, spatial (e.g., distance to edge), and temporal (e.g. nest age, ordinal date, year) covariates on nest DSRs. In particular, time-specific patterns of nest survival have been documented in several duck and passerine species (Grant et al. 2005, Grant and Shaffer 2012) and such analyses are likely more appropriate for pheasants given their long nesting cycle and extended breeding seasons.

During 2015 and 2016 only, we placed video cameras on approximately 40% of nests each year. Most hens were tolerant of cameras but a few hens did abandon their nests. However, these hens may have abandoned due to our other research activities (specifically, being flushed to float eggs too early in incubation) rather than the presence of the camera itself. We potentially captured 2 predation events on camera in 2016 but the video qualities were low due to obstruction from vegetation and windy conditions, which greatly reduced our ability to clearly view activity at the nests. One notable observation included a rooster visiting a hen at her nest almost daily during late incubation. Although all nests were visible when cameras were first placed, the rapid growth of vegetation during the nesting cycle quickly impacted our ability to view nest contents or activities in the immediate area. Windy conditions often compounded our inability to review camera footage by causing vegetation to blow in front of the camera. In the future, we would consider using cameras to document nest predation events but we would alter our camera set-up (e.g., distance to nest, height of camera) to reduce the impact of vegetation and wind on the quality of the footage.

We captured and radiomarked 84 chicks between day 0-2 during the 2015-2017 breeding seasons. During the 2016 and 2017 field seasons only, we recaptured 3 chicks between day 12-15 and replaced their 0.65 g transmitters with sutured 1.1-g backpack-style transmitters (ATS, Isanti, MN). Recapturing radiomarked chicks at this age was relatively easy and may be a viable option to replace lighter transmitters with heavier ones that have a longer battery life, thereby allowing monitoring of chicks beyond 4 wk of age in future work. We attributed known fates ($n = 38$) as follows: 32% died due to predation, 26% died from exposure, 24% of chicks died due to unknown causes, 11% died due to human-induced causes (specifically, agricultural operations, vehicle collisions, and researcher activities), and 8% survived beyond 30 days. Similar to our hen survival analyses, our future chick survival analyses will use methods that incorporate expert knowledge about uncertain fates to refine estimates (Walsh et al. 2018).

During 2015-2017, we collected vegetation data from 90 nest sites. We calculated means and standard errors (SE) for 2 groups of comparisons: nest sites versus random points (Table 1), and successful versus depredated nests (Table 2). We included all nests regardless of nest fate (e.g., successful, depredated, abandoned, other failure) for the comparison of nest sites versus random points. Percent cover of forbs was similar between nest sites and random points ($\bar{X} \pm \text{SE} = 16.4 \pm 2.10\%$ vs. $20.8 \pm 2.40\%$, respectively; Table 1, Figure 2a); however, successful nests had more than twice as much forb cover compared to depredated nests ($18.8 \pm 3.01\%$ vs. $8.3 \pm 2.97\%$, respectively; Table 2, Figure 2b). The percent cover of standing dead vegetation was greater at nest sites compared to random points nearby ($15.0 \pm 1.36\%$ vs. $9.9 \pm 1.06\%$, respectively) but hatched nests and depredated nests had similar amounts of standing dead cover ($15.1 \pm 1.54\%$ and $13.2 \pm 4.20\%$, respectively). Vertical density of the vegetation, as measured by VOR, was similar between nest sites and random points (5.2 ± 0.21 dm vs. 5.3 ± 0.22 dm, respectively; Figure 3a) yet successful nests had greater vertical density compared to depredated nests (5.4 ± 0.26 dm vs. 4.5 ± 0.60 dm, respectively; Figure 3b). Although preliminary, these findings have practical implications for wildlife management activities. For example, previous MNDNR research found that neither burning nor mowing were successful management techniques for increasing forb diversity in warm-season grass-dominated stands (Davros et al. 2014), yet our results show that forb cover is positively associated with pheasant nest success. Therefore, managers should consider other options for increasing forb cover in

prairie reconstructions to benefit pheasants. Additionally, we found that hens selected nest sites with more standing dead canopy cover, which is especially important for visual concealment early in the nesting season, and they were more successful when nests had greater vertical density, which is also assumed to provide visual concealment. However, recent studies suggest that increased structural heterogeneity of the vegetation may better conceal nests from olfactory-based nest predators (Fogarty et al. 2017, Fogarty et al. 2018). Management actions that create vegetation that provides both visual and olfactory concealment may be key to creating more productive nesting habitat.

We monitored 55 broods for survival during the 3 years of our study. During 2016-2017 only, we collected vegetation related to brood habitat selection for 40 broods. We continued to monitor each brood until 4 wk of age or until complete brood loss, whichever came first. We documented at least one hen who re-nested after losing her brood between 1-2 wk of age. Although rare, other researchers have also documented second brood attempts after early losses of first broods (Dumke and Pils 1979; A. Annis and T.J. Fontaine, personal communication; T. Bogenschutz, personal communication; T. Lyons, personal communication).

The final results from this study will relate pheasant survival rates to nesting and brood-rearing habitat selection. Ultimately, the information gained will help managers better understand factors that may limit pheasant productivity so that they can prioritize their management activities in an era of reduced grassland habitat on the landscape.

ACKNOWLEDGMENTS

We thank area wildlife staff, especially K. Kotts, W. Krueger, J. Markl, C. Netland, B. Schuna, D. Trauba, C. Vacek, and J. Zajac for their valuable discussions on issues and management efforts related to pheasant brood habitat. J. Giudice, V. St-Louis, M. Grund, M. Larson, J. Lawrence, L. Cornicelli, and G. Hoch reviewed earlier drafts of the research proposal and provided valuable input on the design and methodology of this study. T. Fontaine, D. Hoffman, T. Lyons, and S. Chiavacci provided great discussions and feedback on field methods and equipment. We also thank M. Adamek, B. Bermel, J. Bushman, S. Crosby, C. Fortier, K. Goebel, G. Gehring, M. Howell, Q. Huber-Heidorn, J. Johnson, J. Letlebo, N. Smetana, C. Reep, M. Rice, N. Schmidt, M. Rice, H. Witt, and J. Youngmann for their dedication to field work and data collection, and E. Andstedt, S. Buck, K. Deweese, S. Endres, B. Nosbush, R. Tebo, and M. Tuma for their volunteer efforts during hen trapping. Staff at the Nicollet wildlife office, the Windom wildlife office, the Talcot Lake wildlife office, and Blue Mounds State Park loaned fleet equipment during our hen capture efforts. This study would not be possible without the permission of the many landowners who have graciously allowed us onto their land as part of our monitoring efforts. This project was funded by the Federal Aid in Wildlife Restoration Act (Pittman-Robertson) and the MN DNR Section of Wildlife.

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Table 1. Descriptive statistics for vegetation surveys at sites used for nesting by ring-necked pheasant hens and nearby random points (≤ 15 m away) as a comparison in southwest Minnesota during the 2015-2017 breeding seasons.

	Nest sites ($n = 90$)		Random points ($n = 90$)	
	Mean	SE	Mean	SE
% Canopy cover				
Grasses	40.3	2.80	42.9	2.49
Forbs	16.4	2.10	20.8	2.40
Standing dead	15.0	1.36	9.9	1.06
Species richness				
Total	4.0	0.27	4.5	0.28
Grasses	1.8	0.10	1.9	0.09
Forbs	2.2	0.23	2.5	0.23
Maximum height (cm)				
Live vegetation	53.1	4.74	57.2	5.21
Dead vegetation	56.9	6.64	44.8	4.60
Litter depth (cm)	2.9	0.31	4.2	0.29
Vertical density (dm) ^a	5.2	0.21	5.3	0.22

^aVertical density is the average visual obstruction reading (VOR) as determined by using a Robel pole.

Table 2. Descriptive statistics for vegetation surveys at successful versus depredated nest sites of ring-necked pheasants in southwest Minnesota during the 2015-2017 breeding seasons.

	Successful nests (<i>n</i> = 52)		Depredated nests (<i>n</i> = 17)	
	Mean	SE	Mean	SE
% Canopy cover				
Grasses	41.1	3.42	43.0	8.35
Forbs	18.8	3.01	8.3	2.97
Standing dead	15.1	1.54	13.2	4.20
Species richness				
Total	4.3	0.37	3.8	0.63
Grasses	1.9	0.13	1.8	0.22
Forbs	2.4	0.30	1.9	0.62
Maximum height (cm)				
Live vegetation	53.1	6.56	63.7	7.73
Dead vegetation	55.2	8.42	50.2	12.80
Litter depth (cm)	2.5	0.28	3.0	0.47
Vertical density (dm) ^a	5.4	0.26	4.5	0.60

^aVertical density is the average visual obstruction reading (VOR) as determined by using a Robel pole.

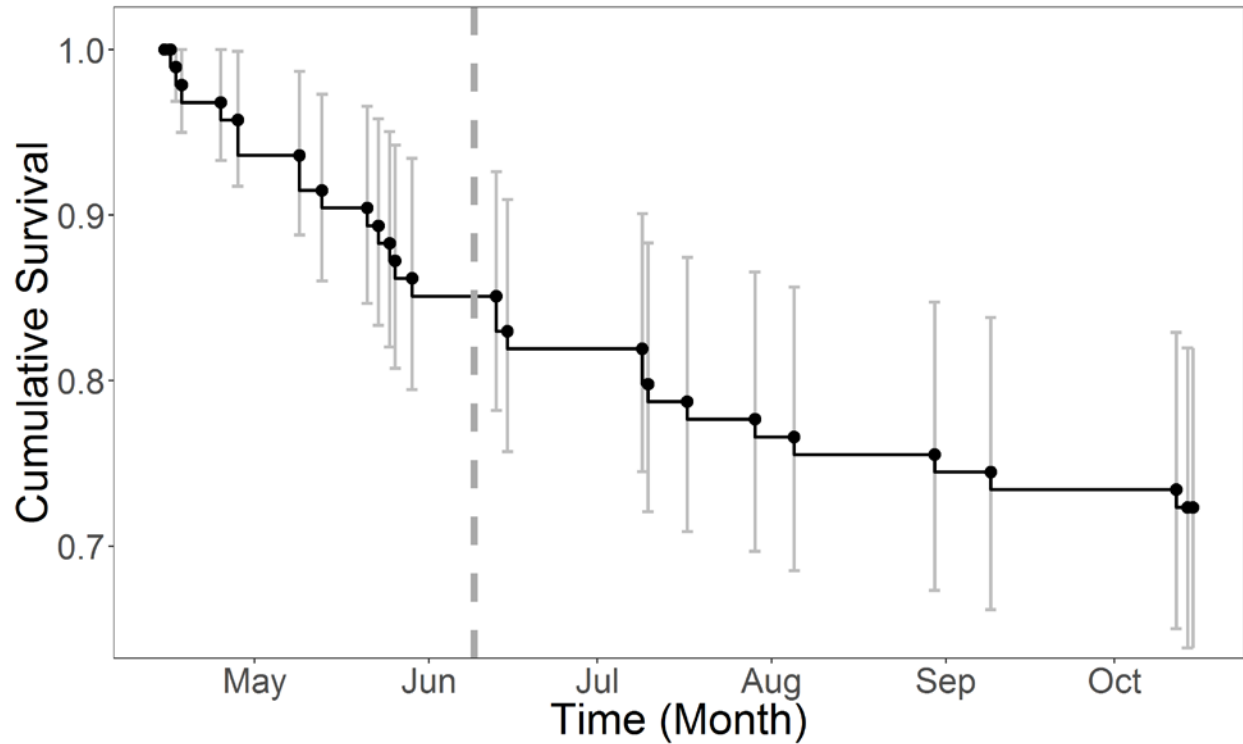


Figure 1. Cumulative survival of radiocollared ring-necked pheasant hens during the 2015-2017 breeding seasons (15 April – 15 October) in southwest Minnesota. Points represent survival estimates at intervals where mortality events took place. Error bars (vertical gray lines extending from each point) represent the upper and lower 95% confidence interval for each survival estimate. The 10-year average (2007-2016) for peak hatch of pheasant nests in Minnesota, as estimated by MNDNR's annual August roadside count surveys, is 12 June and is shown with the vertical gray dashed line.

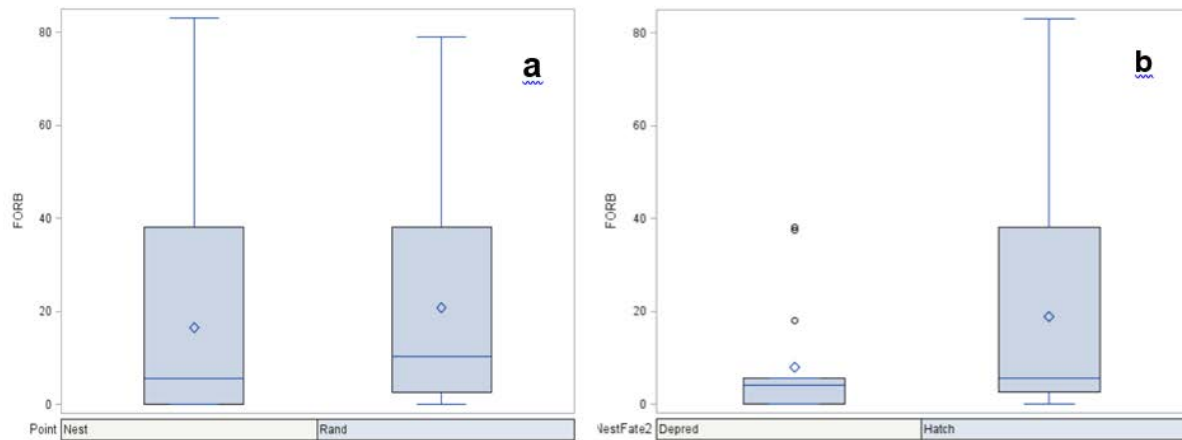


Figure 2. Box plot comparisons of the estimated percent cover of forbs at (a) nest sites versus nearby random points (<15 m away) and (b) depredated versus successfully hatched nests of ring-necked pheasants in southwest Minnesota during 2015-2017.

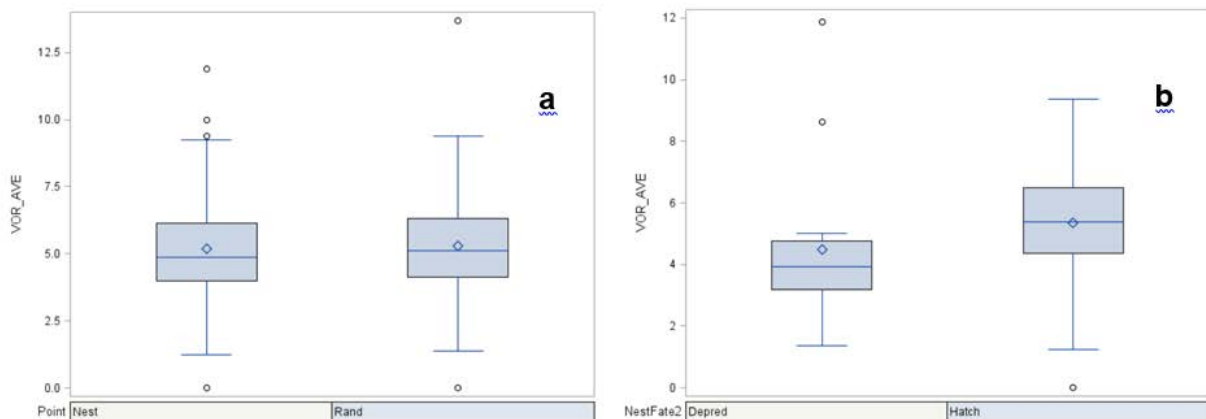


Figure 3. Box plot comparisons of vegetation vertical density, as measured by a visual obstruction reading (VOR) using a Robel pole, at (a) nest sites versus nearby random points (<15 m away) and (b) depredated versus hatched nests of ring-necked pheasants in southwest Minnesota during 2015-2017.

ASSESSING THE CONTRIBUTION OF HARVEST MORTALITY TO OVERALL MORTALITY RATES OF GRAY AND FOX SQUIRRELS ON PUBLIC LANDS IN MINNESOTA

Ryan G. Tebo and Andrew S. Norton

SUMMARY OF FINDINGS

Squirrel hunting is a popular activity in Minnesota, but hunter perceptions of squirrel declines on publicly-owned land near the Twin Cities Metropolitan Area of Minnesota (hereafter, Metro) has led interested user groups to express concerns over squirrel populations in recent years. To explore these concerns, we conducted a study to compare squirrel mortality rates on a site with heavy hunting pressure (Whitewater Wildlife Management Area [WWMA]) to a paired non-hunted site (Whitewater State Park [WSP]) in southeastern Minnesota. From July 2015 – September 2016, we trapped and radiocollared 107 gray and fox squirrels (*Sciurus carolinensis* and *S. niger*, respectively) on WWMA and 101 gray squirrels on WSP. Using survival models that accounted for uncertainty in fates, we compared differences in annual survival, survival during the hunting season, and survival outside of the hunting season between sites. Annual survival on WWMA and WSP was 0.251 (0.146 – 0.365) and 0.493 (0.368 – 0.624), respectively. During the hunting season (mid-September – February), there was a 0.993 probability survival was higher on WSP. Survival of squirrels on WWMA was 0.492 (0.389 – 0.596), whereas survival on WSP was 0.630 (0.529 – 0.723). Outside of the hunting season, there was little difference in survival rates between the 2 sites. WWMA squirrels had a 0.709 (0.448 – 0.900) survival rate while WSP squirrels had a 0.704 (0.554 – 0.839) rate. On WWMA, we were interested in the relationship between survival and sex, age, or distance to hunter access points (e.g., parking lots, walking trails, roads) during time periods within the hunting season. We found no evidence of differences in survival between sex and age class of squirrels during this time, but distance to hunter access points did affect survival rates. Specifically, squirrels found closer to hunter access points on WWMA had lower survival rates, especially during the first 6-weeks of the hunting season ($p(\beta_{\text{Dist}}) < 0 = 0.935$). Survival of squirrels closest to hunter access points was 0.484 (0.345 - 0.624), whereas squirrels 1-km and 2-km from access points had survival rates of 0.687 (0.490 - 0.847) and 0.798 (0.437 - 0.969), respectively. Reduced survival rates due to hunting mortality were clear in this study, but exploited populations are known to respond favorably to high levels of harvest with increased recruitment following removal of a large segment of the population. Those looking to manage properties for adequate squirrel hunting opportunity from year to year need to begin by identifying pathways of recruitment by assessing the site itself as well as properties in the surrounding landscape. In the case of large tracts of land with good squirrel habitat, the site itself may be able to harbor sub-populations of squirrels that act as source populations for more heavily-harvested areas of the property. On smaller properties, the ability to restock populations from neighboring private lands or refuges could be a consideration.

INTRODUCTION

Many factors cause squirrel populations to fluctuate naturally (e.g., mast abundance, population density, disease outbreaks; see Barkalow et al. 1970, Nixon et al. 1974, Nixon et al. 1975,

Healy and Welsh 1992, Descamps et al. 2009, Vander Haegen et al. 2013) by affecting reproduction, immigration, emigration, or mortality. As a game species, hunting mortality is often assumed to be compensatory to natural mortality in determining overall squirrel mortality rates. However, previous research suggests hunting mortality can be additive to non-hunting mortality in other upland game species (Bruggink et al. 2013).

The number of gray and fox squirrels harvested during the fall hunting season is correlated with pre-hunt densities and the amount of effort expended by hunters early in the hunting season (Nixon et al. 1974, Nixon et al. 1975). A study in Virginia found the annual mortality rate and the rate of juvenile recruitment was higher in hunted squirrel populations (Mosby 1969). In an Ohio study, approximately 50% of the harvest was comprised of squirrels born the previous spring and summer and there was no difference observed in the harvest sex ratio for young-of-the-year or adult squirrels (Nixon et al. 1975). Annual mortality rates were 70-80% for young-of-the-year squirrels and nearly 80% for adult squirrels. Hunting accounted for nearly 60% of the annual mortality. The researchers concluded that the population could not sustain itself with this level of mortality and that squirrels were likely immigrating from surrounding habitats (Nixon et al. 1975). In Illinois, fox squirrel annual mortality rates were 79% on a hunted site and 44% and 45% on non-hunted sites whereas mortality rates outside of the hunting season were not significantly different (Herkert et al. 1992). Reproductive intensities did not differ between the sites and the researchers also concluded that the hunted population was being sustained by immigration. In a small Minnesota study, researchers found an annual survival rate of 27% for gray squirrels on a hunted site, with 74% of the mortalities due to hunting (Longley 1963). The annual survival rate was 48% the following year when the site was not hunted. Reproductive rates were 2.4 young per litter regardless of the prior year's squirrel hunt status (Longley 1963).

Small game hunting is a popular recreational activity in Minnesota with approximately 292,000 hunters buying licenses each year since the late 1990s (Dexter 2009, Dexter 2014). Nearly 15% of small game hunters pursue gray and fox squirrels with an estimated take of 5.1 gray and 3.9 fox squirrels per hunter (Dexter 2009, Dexter 2014). The combined gray and fox squirrel harvest and the number of squirrel hunters have each declined by 13.8% since the late 1990s; however, the combined take per hunter has declined by only 3.3% during this same time (Dexter 2009, Dexter 2014). In an effort to better understand barriers to hunter participation, the Minnesota Department of Natural Resources (MNDNR) conducted a survey of squirrel hunters (Dunbar 2009). Survey results indicated more hunters in the Metro believed squirrel populations were declining (51%) than their statewide counterparts (19%). Metro hunters also indicated that they had limited access to private land, and heavy hunting pressure existed on publicly-owned land (Dunbar 2009). The MNDNR Section of Wildlife has considered changes to the squirrel season structure in the Metro based on these survey results. However, because a paucity of information exists with respect to the impacts that the current hunting regulations have on squirrel population growth rates, no changes have been made to date.

OBJECTIVE

1. Assess mortality rates of radiocollared squirrels across multiple seasons (fall/early hunting season, winter/late hunting season, spring, and summer) on heavily-hunted public land and nearby non-hunted land.

METHODS

Capture and Handling

We trapped gray and fox squirrels during July-September 2015 and May-September 2016. We used wire box traps (48 x 15 x 15 cm; 2.5 x 1 cm mesh) baited with sunflower seeds, dried corn, peanut butter, and/or hand-picked black walnuts. To identify initial trapping locations, we used

ArcGIS 10.2 (ESRI, Redlands, California, USA) to select patches of oak habitat that looked to be suitable squirrel habitat. In these areas and where terrain allowed, we created a grid of points 25-m apart and placed traps at these points. In areas with challenging terrain or insufficient contiguous oak habitat, traps were selectively placed at locations that researchers deemed to be likely squirrel-use locations. Traps were checked at least twice per day (i.e., late morning and late evening) to reduce the amount of time squirrels remained in the traps. Trapping ceased during inclement weather.

Upon capture, we identified the sex of each squirrel and determined the reproductive status of females. We weighed squirrels in the trap to the nearest 10 g using a digital hanging scale. We used a modified handling cone to restrain squirrels which allowed us to handle and radiocollar without sedation (Koprowski 2002). Handling cones were constructed of denim with hook and loop straps to help secure the squirrel and a zipper opening to allow access to the head and neck during collar attachment. Once in the handling cone, a removable plastic funnel was attached around the squirrel's neck to protect handlers from bites during collaring (McCleery et al. 2007). We only collared squirrels weighing ≥ 300 g in an effort to keep the transmitter weight below 3% of the animal's body mass. Squirrels received a 7-g, 10-g, or 13-g VHF necklace-style radiocollar (models M1525 and M1535, Advanced Telemetry Systems, Isanti, MN) depending on the weight of each individual squirrel. Each transmitter was equipped with an integrated mortality sensor that changed the pulse rate of the signal if an animal did not move for 8-12 h. Optimal battery life was 362 d for the 13-g collar and 302 d for the 7- and 10-g collars. Upon completion of the handling procedure, all squirrels were immediately released. All non-target captures were released immediately.

Tracking

Following capture, radiocollared squirrels were monitored for mortality biweekly using a vehicle-mounted non-directional radiotelemetry antenna system. Beginning on the first day of the squirrel hunting season (19 September 2015 or 17 September 2016), squirrels were monitored twice weekly until the end of October. Squirrels were then monitored once weekly until the end of December, and biweekly until 1 March. When a radiocollar transmitted a mortality signal, researchers used homing techniques in an attempt to recover the collar and determine the cause of mortality. Squirrels remaining on-air beyond 1 March continued to be monitored biweekly until death or battery failure.

Data Analysis

To investigate squirrel survival, we developed survival models based on traditional biostatistical survival analysis approaches (Kalbfleisch & Prentice 1980), and we accounted for uncertainty in fates using data augmentation methods in a Bayesian framework (Walsh et al. 2017). Our data were interval censored because we did not know the exact time squirrels went off-air. We partitioned monitoring intervals to fit biologically-relevant time frames, resulting in approximately 24 2-week intervals. Sources of mortality were placed into 4 categories: harvest, other (natural), dropped collar, and battery failure. Because we included dropped collar and battery failure as mortality sources, this allowed us to forgo assumptions of censoring being independent of fate. To account for uncertainty in fate, we incorporated a data augmentation technique that assigned fates for each Markov chain Monte Carlo iteration based on prior predictive probabilities based on the belief the fate was associated with a specific cause (Walsh et al. 2017). For example, if a squirrel on WWMA was heard during each monitoring event but went missing following the opening weekend of the hunting season, we may assign a 0.9 probability the squirrel was harvested, and a 0.1 probability the squirrel dispersed beyond our search radius or there was a mechanical failure in the collar transmission (i.e., right-censored cause). Using this approach, we modeled yearly survival, survival during the hunting season, survival during the first 6 weeks

of hunting season, and survival outside of the hunting season. We ran 3 individual chains for 100,000 iterations each, and discarded the first 50,000 iterations as burn-in. We separately evaluated the relationship between survival and site, sex, age, or distance from hunter access points during relevant time periods. For modeling survival in relation to hunter access points, we chose distances of 0-, 1-, and 2-km from access points to obtain estimates. We did not use DIC values to compare models; instead, we used beta values and credible intervals to evaluate strength of evidence of an effect and effect sizes (Heisey et al. 2010).

RESULTS

Trapping and Monitoring

We successfully captured 132 squirrels (119 gray, 13 fox) during the 2015 and 2016 trapping seasons on WWMA, 107 of which received radiocollars. In an effort to distribute captures throughout WWMA, trapping locations were spread out across the unit (Figure 1). Fifty-one males and 56 females received radiocollars. Based on their lactation status, 21 female squirrels that received collars (38%) showed evidence of prior reproductive activity. Weights of collared squirrels on WWMA ranged from 410-920 g (\bar{x} = 655 g)

We captured 124 squirrels (123 gray, 1 fox) on WSP during the 2015 and 2016 trapping seasons, 101 of which received radiocollars. Trapping locations were spread out across the interior of WSP (Figure 2). Fifty males and 51 females were collared. Of 49 radiocollared females checked for reproductive status, 22 (45%) showed signs of prior reproductive activity. Weights of collared squirrels on WSP ranged from 370-840 g (\bar{x} = 649 g).

From 1 July - late August 2015, our trapping success was very low on both sites and we captured only 10 squirrels on each site (3564 trap checks; 0.56% capture rate). However, following this period of low capture success, we switched to fresh-picked walnuts as the main bait type. In the remaining 3 weeks of the 2015 trapping season following the change, we captured 46 and 39 squirrels on WWMA and WSP, respectively (1181 trap checks; 7.2% capture rate). When available, we used walnuts as the predominant bait-type for the remainder of the study. The preference for walnuts may have been due in part to a seasonal change in behavior where caching food became the ultimate goal of squirrels once trees began producing the year's walnut crop. Overall, our capture rates for WWMA and WSP for the duration of the study were 1.99% (6,629 trap checks) and 5.02% (2,470 trap checks), respectively. Two squirrels on WWMA and 4 squirrels on WSP originally captured in 2015 were recaptured during the 2016 trapping season and fitted with new radiocollars.

Twenty-nine (27.88%) squirrels were definitively harvested by hunters on WWMA (Figure 3). In addition, 9 (8.65%) animals were lost to natural mortality events, 9 (8.65%) dropped their collars, 13 (12.50%) collars were unrecoverable (e.g., in a tree or inaccessible), 1 (0.96%) collar was recovered but fate was uncertain, 19 (18.27%) went missing due to unknown reasons, and 24 (23.07%) went missing due to presumed collar battery failure (Figure 3). On WSP, 21 (20.79%) squirrels were lost to natural mortality events, 15 (14.85%) dropped their collars, 20 (19.80%) collars were unrecoverable, 8 (7.92%) went missing due to unknown reasons, and 37 (36.63%) went missing due to presumed collar battery failure (Figure 3). We removed 3 of the 107 radiocollared squirrels on WWMA from the survival analysis due to capture complications.

Of squirrels captured in 2015, all known harvest mortalities (13) occurred within the first 6 weeks of the hunting season. The last observed harvest mortality was 27 October 2015. In 2016, 10 of 15 (67%) known harvests occurred during the first 6 weeks of the hunting season. The latest harvest mortality recorded was 21 February 2017. In addition, 8 of 12 (67%) squirrels that went missing for reasons not deemed to be collar battery failure went missing during the first 6 weeks of the hunting season in 2015. Four of 7 (57%) missing squirrels did so during the first 6 weeks

of the 2016 hunting season. Although it cannot be confirmed, we believe it is very likely some, if not most of these squirrels were harvested.

We placed signs throughout WWMA and at popular parking areas to inform hunters about the study and encourage them to report the harvest of any radiocollared squirrels. Additionally, we placed an ear tag that included a callback telephone number on squirrels captured in 2016. Despite these efforts, only 4 of the 29 (14%) recorded harvests were reported. We found most radiocollars from harvested squirrels cut off in the presumed location of harvest or in parking lots. Other squirrels were found deceased with noticeable gunshot wounds but were left in the woods or unrecovered by hunters.

We found the majority (85.29%) of unrecoverable collars in trees and particularly tree cavities. Whether these squirrels died or dropped their collars is unknown; however, we categorized a majority of these losses as dropped collars. Examples that led us to this presumption include numerous cases where collars fluctuated between mortality and normal signal, an indication that the collars were still subject to movement in the tree cavities. On another occasion, a collar was emitting a mortality signal until researchers reached the tree, at which point the collar switched out of mortality mode and an uncollared squirrel exited a cavity in the tree. As a final example, one collar that had been unrecoverable in a tree for months was found on the ground by researchers in late March, with clear signs that the zip-tie attachment had been chewed through.

Squirrel Survival

Annual survival of squirrels on WWMA and WSP was 0.251 (0.146 – 0.365) and 0.493 (0.368 – 0.624), respectively (Figure 4). During the hunting season (mid-September – February), there was a 0.993 probability survival was higher on WSP. Survival of squirrels on WWMA was 0.492 (0.389 – 0.596) whereas survival on WSP was 0.63 (0.529 – 0.723) during the season. Outside of the hunting season, survival rates were similar between the sites. WWMA squirrels had a 0.709 (0.448 – 0.900) survival rate and WSP squirrels had a 0.704 (0.554 – 0.839) survival rate.

On WWMA, we were interested in the relationship between survival and sex, age, or distance to hunter access points during time periods within the hunting season. During the first 6 weeks of the hunting season and during the entire hunting season, we found little evidence that male and female survival differed ($p(\beta_{\text{Sex}}) > 0 = 0.542$ and 0.777 , respectively). Male survival during the first 6-weeks of the hunting season was 0.553 (0.419 – 0.682) and female survival was 0.563 (0.428 – 0.693). Male and female survival during the entire hunting season was 0.372 (0.249 – 0.504) and 0.435 (0.304 – 0.568), respectively.

We found very little evidence that differences in age class affected survival rates on WWMA during the first 6 weeks of the hunting season and during the entire hunting season ($p(\beta_{\text{Age}}) > 0 = 0.518$ and 0.608 , respectively). Adult survival during the first 6 weeks of the hunting season was 0.590 (0.452 – 0.722) and juvenile survival was 0.587 (0.442 – 0.723). Adult and juvenile survival during the entire hunting season was 0.430 (0.296 – 0.569) and 0.407 (0.273 – 0.549), respectively.

Our results indicated that squirrels found closer to hunter access points on WWMA had lower survival rates, especially during the first 6 weeks of the hunting season ($p(\beta_{\text{Dist}}) < 0 = 0.935$). Survival of squirrels closest to hunter access points was 0.484 (0.345 - 0.624), whereas squirrels 1-km and 2-km from access points had survival rates of 0.687 (0.490 - 0.847) and 0.798 (0.437 - 0.969), respectively.

DISCUSSION

We found annual survival of radiocollared squirrels on WWMA was nearly half that found on WSP. Hunting mortality appeared to account for the differences found between the 2 sites, especially when examining squirrel survival during the first 6 weeks of the hunting season. Prior studies in Ohio, Illinois, and Minnesota (Longley 1963, Nixon et al. 1975, Herkert 1992, respectively) all found marked differences in annual survival rates between hunted and non-hunted sites. In Minnesota, Longley (1963) found squirrel survival was 27% when hunting was open to the public, but increased to 48% when the site remained closed to hunting the following year. Results from Longley (1963) are nearly identical to annual survival rates found on our 2 study sites. Researchers in Illinois concluded that additive mortality was occurring in heavily exploited populations, resulting in reduced squirrel numbers in successive years of high harvest (Herkert 1992).

Nixon et al. (1975) stated that squirrel numbers will decline after several years of hunting pressure when survival rates are low. When hunting mortality reduces resident squirrel numbers to such low levels, compensatory reproduction alone may not be enough to maintain fall-winter squirrel numbers (Nixon et al. 1974, Herkert 1992). With annual survival rates near 25% on our hunted site, we believe that this is unsustainable if relying on the resident squirrel population to replenish numbers from year to year. Instead, it is likely necessary for squirrels to immigrate from other areas to stabilize populations that are subject to heavy hunting mortality similar to WWMA (Nixon et al. 1974, Nixon et al. 1975, Herkert 1992). This colonization is highly dependent on nearby areas that serve as refugia or receive light hunting pressure (Herkert 1992).

The squirrel hunting season in Minnesota opens mid-September and runs through the end of February. We predicted the most detrimental time period to the squirrel population on the hunted site would be during the early months of the season. Our results support our prediction as we did not record a single known harvest beyond October during the first year of the study. The same trend continued in 2016, when the majority of confirmed hunting mortalities occurred during this same 6-week period. Similarly, significant differences in success of hunters in Ohio were found when comparing the months of September (40.1%), October (35.9%), and November (24.9%; Nixon et al. 1975). We believe once the firearm deer season began in Minnesota in early November, much of the attention shifted away from squirrels resulting in limited harvests throughout the remainder of the season. One advantage of the season beginning in mid-September is that summer-born juvenile squirrels should be weaned prior to being subject to harvest (Nixon et al. 1974). Having juvenile squirrels available to hunters for harvest would possibly provide a buffer to adult mortality once the hunting season begins (Nixon et al. 1975).

We also predicted males and juveniles would have higher mortality rates compared to females and adults due to the expanded ranges of males and likelihood of juveniles to travel longer distances to establish a home range (Barkalow 1970). However, we found no evidence for either prediction in our study. Even when hunting pressure was at its peak, we found that males only had a slightly lower survival rate than females. Similarly, juveniles had only slightly lower survival rates than adults. Nixon et al. (1975) also reported no difference in the sex ratios of squirrels shot on their huntable site across their 10-year study. Additionally, Nixon et al. (1975) found little evidence for a difference in survival between juvenile and adult squirrels during the hunting season.

The size of WWMA allowed us to model the effects of distance to hunter access points on survival rates as we were able to capture squirrels that were much less accessible to hunters (i.e., >2 km from the nearest access point) compared to squirrels captured closer to roadways

and parking areas. We hypothesized that squirrel survival during the hunting season would be positively associated with distance from access points. Indeed, we found that squirrel survival steadily increased as distance from nearest access points increased. As other studies have noted, a key component for recruitment of squirrels into a population is having a source population that helps restock local populations annually (Nixon et al. 1974, Nixon et al. 1975, Herkert 1992). WWMA is large enough that many areas within the boundaries of the site may receive comparatively light squirrel hunting pressure which in turn may help with recruitment of squirrels into more heavily-hunted areas of the site. Because these areas likely exist on WWMA, we believe a huntable annual population is retained. However, very few public lands in the Metro have acreages as large as WWMA which may lead to potential problems if similar squirrel survival rates occur on these smaller tracts. Public lands that are isolated pockets of habitat with no nearby population sources are especially at risk (Nixon et al. 1974), and years of consistent harvest may be detrimental to squirrel populations on these properties.

MANAGEMENT IMPLICATIONS

Many factors cause squirrel populations to fluctuate annually. Mast abundance is one of the most important and well-documented factors in annual squirrel survival, with favorable seed crops leading to increases in survival, reductions in emigration of young squirrels, and increases in fecundity of females the following year (Nixon et al. 1975). As a result, managing squirrel populations on public lands via harvest can become challenging because these sometimes drastic fluctuations are hard to predict. However, our results show that one potential way to offset high hunting mortality is to have areas that act as refuges for squirrels. In the case of large tracts of land with good squirrel habitat, the site itself may be able to harbor sub-populations of squirrels that act as source populations for more heavily harvested areas of the property. On smaller properties, the ability to restock populations from neighboring private lands or refuges would be a necessity (Nixon et al. 1974).

On the surface, a 25% annual survival rate of squirrels on our hunted site seems lower than what would be considered sustainable. However, exploited populations are known to respond favorably to high levels of harvest with increased recruitment following removals of a large segment of the population (Allen 1954, Jordan 1971, Herkert 1992), and rapid recolonization can occur if a source population of squirrels is available nearby (Herkert 1992). Using this information, managers can identify pathways of recruitment by assessing surrounding private lands or refuges. Doing so will allow them to make management-unit decisions regarding season length and harvest strategies so that squirrel hunting opportunities are retained across time.

ACKNOWLEDGMENTS

We would like to thank the employees at Whitewater State Park and Whitewater WMA, especially Brent Anderson, Colin Wright, Don Nelson, Christine Johnson, Dave Dahl, and Steve Elringer for their support. We appreciate the tolerance and curiosity of the Park and WMA visitors. We would like to thank Tonya Klinkner for handling all of our travel arrangements and project equipment purchases. Finally, we thank our technicians Natalie Schmidt, Sean Gibbs, Eric Anstedt, Alicia Freeman, Kelly Deweese, Brandon Berndt, Quinlan Eatwell, Andy Nelson, Calypso Gagorik, and Gwen Jutz for their assistance during the 2015 and 2016 field seasons. This study was funded by MNDNR and through the Federal Aid in Wildlife Restoration Act.

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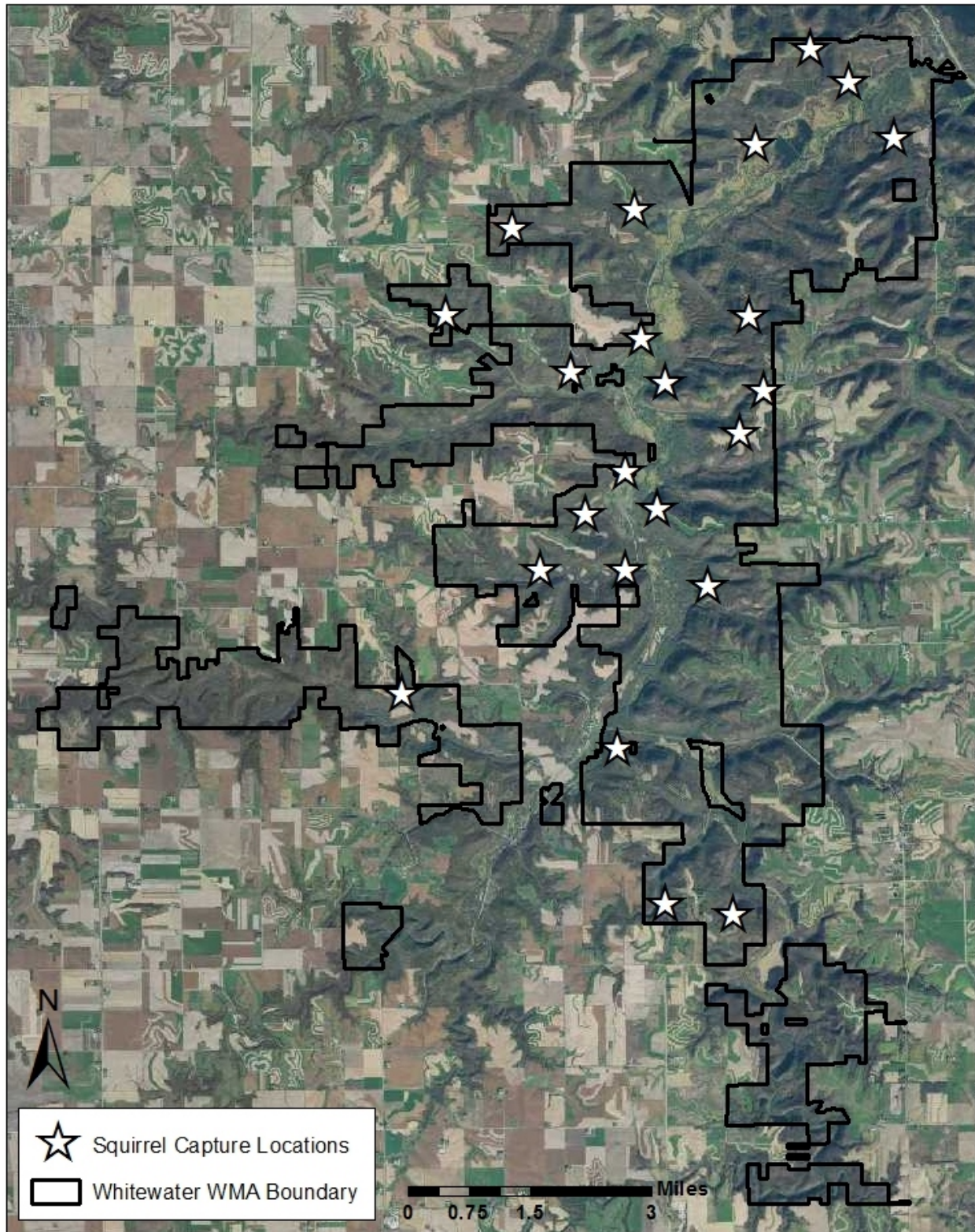


Figure 1. Capture locations for gray and fox squirrels (as indicated by white stars) during 6 July 2015 – 14 September 2016 in Whitewater Wildlife Management Area, Minnesota (outlined in black).

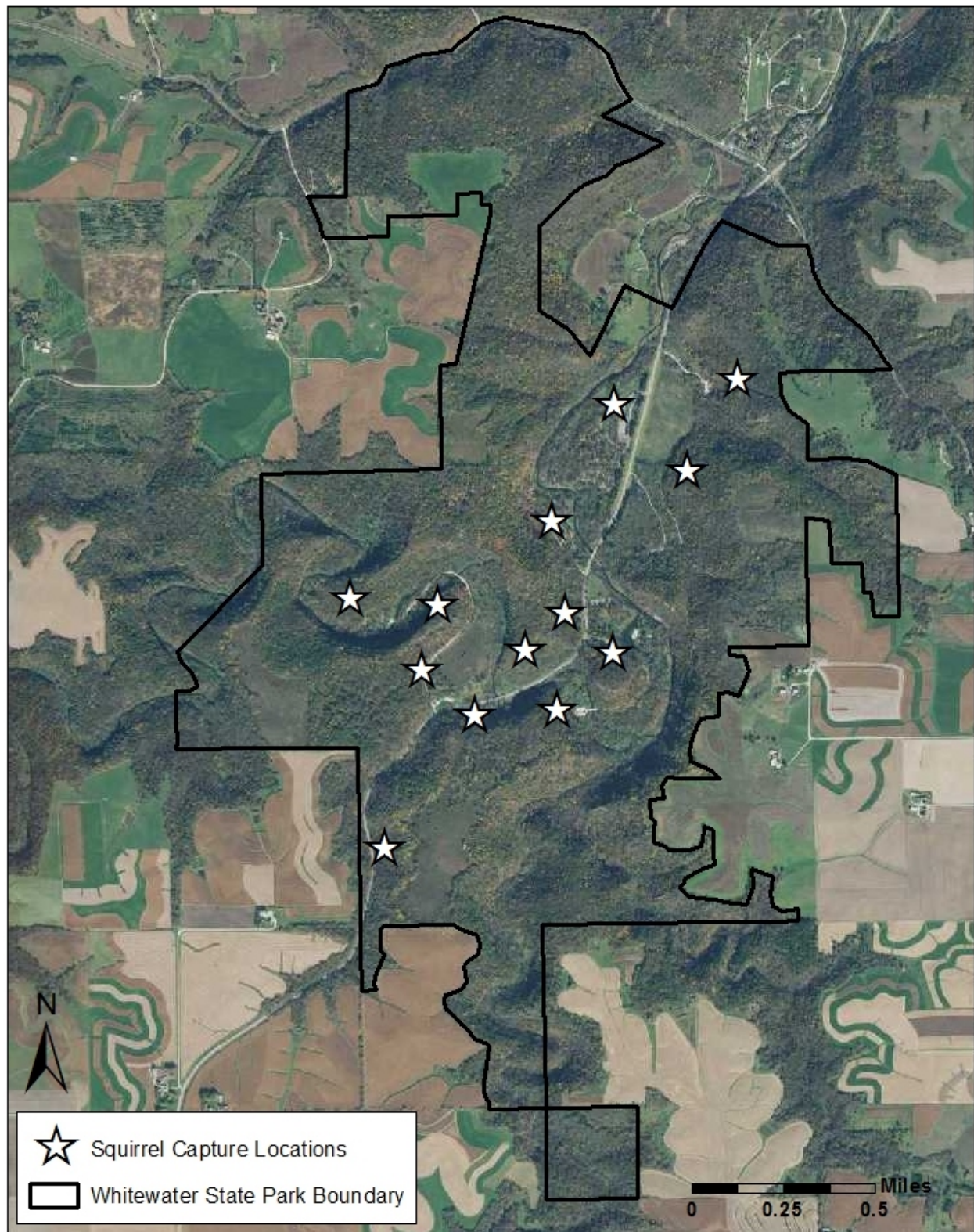


Figure 2. Capture locations for gray and fox squirrels (as indicated by white stars) during 4 August 2015 – 15 September 2016 in Whitewater State Park, Minnesota (outlined in black).

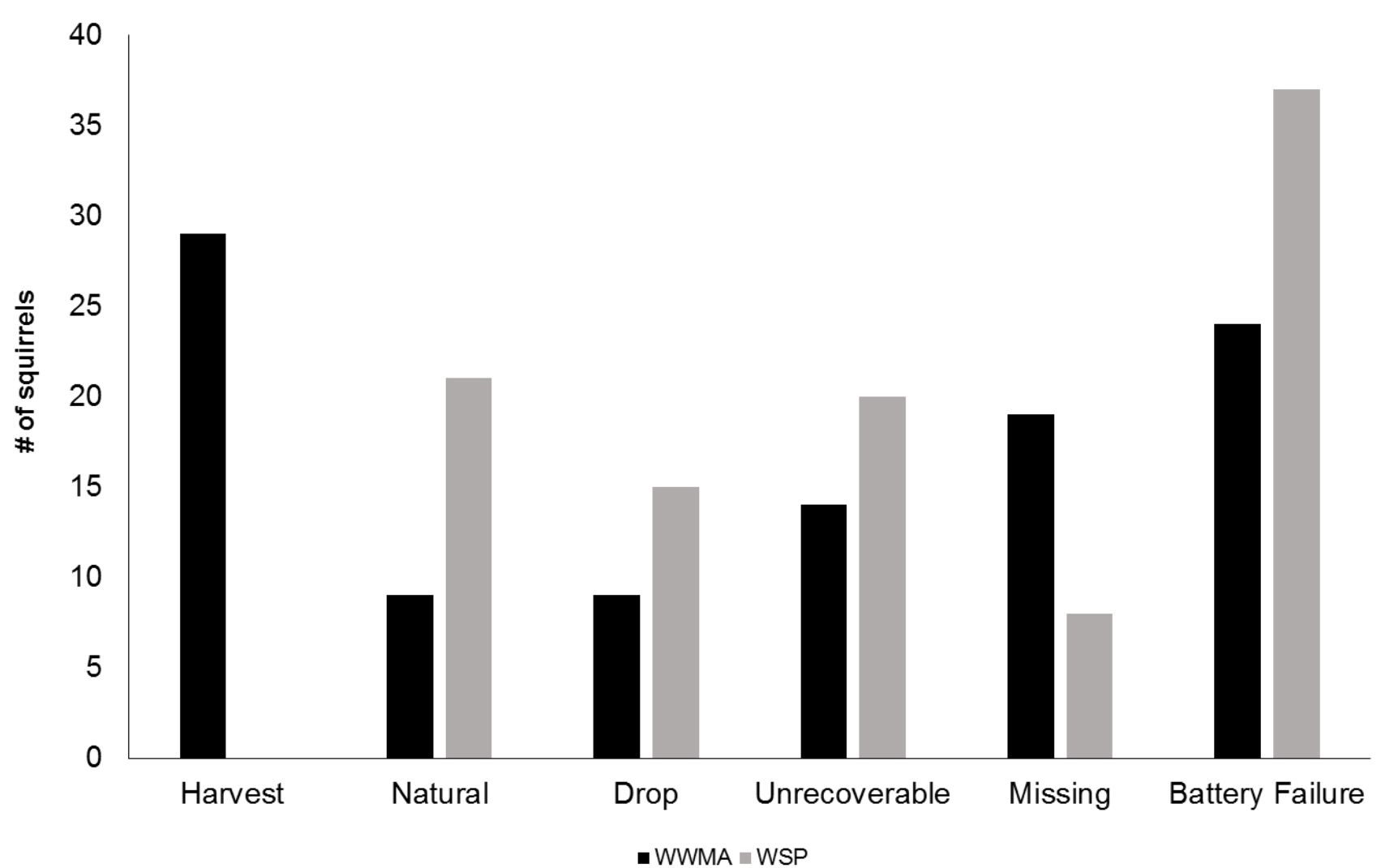


Figure 3. Fates of gray and fox squirrels in Whitewater Wildlife Management Area ($n = 104$; black bars) and Whitewater State Park ($n = 101$; gray bars), Minnesota during 2015 – 2017.

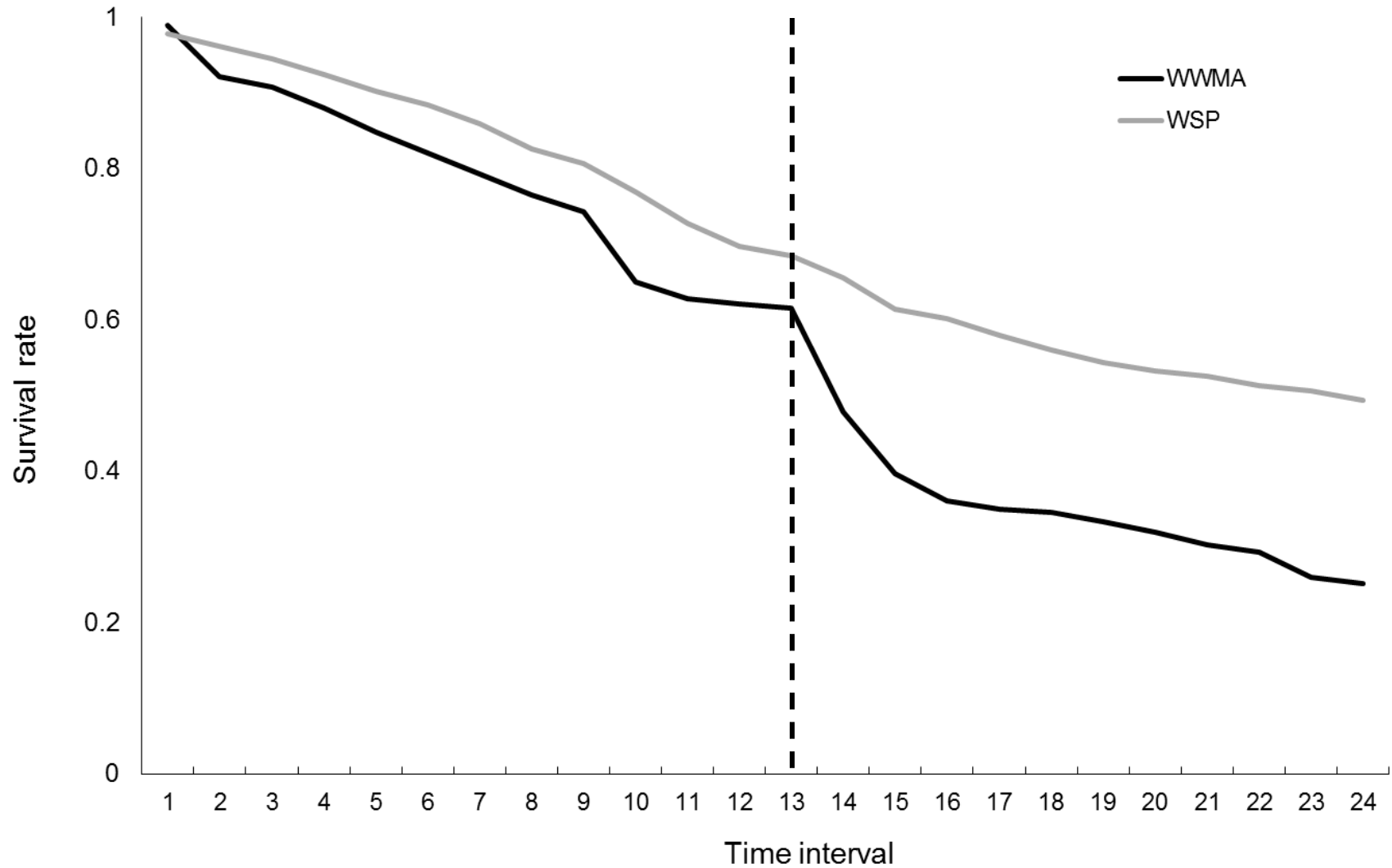


Figure 4. Annual survival curve of gray and fox squirrels on Whitewater Wildlife Management Area ($n = 104$; black line) and Whitewater State Park ($n = 101$; gray line), Minnesota during 2015 – 2017. Intervals are each approximately 2 weeks in length. The vertical dashed line indicates the beginning of the squirrel hunting season.



BEHAVIORAL RESPONSES OF FEMALE ELK TO HUNTING IN NORTHWESTERN MINNESOTA

Ryan G. Tebo, Gino J. D'Angelo¹, and Louis Cornicelli

INTRODUCTION

Currently, Minnesota Department of Natural Resources (MNDNR) is undertaking research to study home range sizes, movements, and seasonal habitat use of adult female elk (*Cervus elaphus*) in an effort to improve habitats and management strategies for the species in Minnesota. Although we continue to grow our knowledge-base on elk in Minnesota, little is known about how elk respond to direct human disturbance in the agriculture-dominated landscape. The Minnesota elk hunting season presents an opportunity to study behavior of elk relative to their period of greatest vulnerability to harvest. Most licenses available during each year's elk hunt are for antlered bull elk only. However, disturbances during the hunting season would not be restricted to bulls due to the harem behavior exhibited during the rut. We compared GPS locations of collared adult cow elk with GPS locations of hunters to quantify elk behavior in response to hunter pressure. Information gleaned from this study will allow managers to identify habitat needs of elk during times of increased human disturbance while also balancing opportunities for elk harvest through management of publicly accessible property. Hunters will also benefit from this information by learning how their encounters with elk can influence elk behavior and movements during the hunting season.

OBJECTIVES

1. Quantify changes in elk movements during the hunting season compared to pre- and post-hunt time periods.

STUDY AREA

Minnesota's elk herd is found in 3 distinct herds (Kittson-Central, Caribou-Vita, & Grygla) which reside in Kittson, Roseau, and Marshall Counties, respectively. The landscape consists of >50% agricultural land but is interspersed with publicly accessible Wildlife Management Areas (WMAs), lands owned and managed by The Nature Conservancy, Conservation Reserve Program (CRP) grasslands, small woodlots, and wetlands. In recent years, the Caribou-Vita and Kittson-Central herds have had numbers sufficient to allow limited hunting whereas the Grygla herd has declined and has not been hunted since 2012.

METHODS

During 2016 and 2017, elk hunting was open for the Kittson-Central and Caribou-Vita herds. Hunting was closed for the Grygla herd during both years. The 2016 elk season was open from 10-18 September, and licensed hunters could legally harvest antlered bull elk only. Five licenses were available during the 2016 season in the Kittson County Central Elk Zone (hereafter, Zone 20; Figure 1) where 14 previously GPS-collared adult cow elk

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resided, and 2 licenses were available in the Kittson County Northeast Elk Zone (hereafter, Zone 30, Figure 1) where 3 previously GPS-collared adult cow elk resided. In 2017, the season was open from 9-17 September and 7-15 October. Six antlered bull elk-only licenses and 2 antlerless-only licenses were available in Zone 20, and 5 antlered bull-only licenses were available in Zone 30 during 2017. In 2016, all 20 elk (17 in hunted areas, 3 in non-hunted) collars were programmed to collect hourly locations beginning 9 d prior to the hunting season through 9 d following the close of the season. Once the 9-day hunting season began, locations were increased to 15 min from 2 hr prior to sunrise until 2 hr after sunset, while remaining at 1-hr locations during the night. The same pattern was followed for the 2017 hunting season; however, due to collar battery restrictions only 10 of the 20 collars collected increased locations as described above. In addition, hunters were asked to participate in data collection by carrying a small GPS-logger programmed to take locations every 15 min, simultaneous with locations recorded by the elk collars. Hunter-carried GPS-loggers were activated prior to the hunter's first day afield and hunters attached the unit to the outside of a backpack or related item so that it could record their movements during hunting hours. Upon completion of the hunter's time afield, units were returned to researchers so that movement data could be downloaded from the units. Hunters were also provided a daily hunt log datasheet to record any encounters with elk, with an emphasis on identifying any encounters with ear-tagged and/or GPS-collared animals (Figure 2).

As a preliminary analysis, we calculated daily kernel density utilization distributions using hourly locations for GPS-collared elk from data collected in 2016 to evaluate whether elk movements differed between pre-hunt, hunting season, and post-hunt time periods. Calculations were processed using the *adehabitatHR* package in Program R (ver. 3.4.0; R Development Core Team 2017).

RESULTS AND DISCUSSION

In 2016, all 7 hunters carried a GPS-logger during their time afield. Five of 7 completed and returned the daily hunt log sheet. In 2017, 10 of 13 carried a GPS-logger during their time afield and 7 hunters returned a daily hunt log sheet.

Elk in the Kittson-Central and Caribou-Vita herds, which were subjected to hunting pressure, had smaller utilization distributions during the hunting period in 2016 compared to pre-and post-hunt time periods (Figure 3). The average daily area used decreased from 749.3 ha to 375.6 ha once the hunting season was in effect. Following the hunting season, utilization distributions of elk in hunted areas increased to 448.2 ha. Elk in the non-hunted Grygla herd had utilization distributions that increased during the hunting season, showing a pattern opposite to elk subjected to hunting pressure (Figure 3).

Results from our preliminary analysis show evidence that adult cow elk decreased their movements in response to hunting pressure. Additionally, we anecdotally noted instances where elk split into smaller groups away from other herd members in response to interactions with hunters. Following these disturbances, collared elk took at least 2 wk to regroup with their previous herd members.

We continue to analyze the data and plan to utilize the Movement Ecology Tools extension (ArcMET; Wall 2014) in ArcGIS (ver 10.4.1, Environmental Systems Research Institute 2017, Redlands, CA) to quantify movements of collared elk during 2016 and 2017 in relation to hunter pressure during the hunting season. In addition to quantifying overall movement metrics, we anticipate calculating movement metrics during day and night time periods, investigating path tortuosity, and linking hunter and elk movement locations to identify potential disturbance events.

ACKNOWLEDGMENTS

We would like to thank the Environment and Natural Resources Trust Fund, MNDNR, the Wildlife Restoration (Pittman-Robertson) Program, and the Rocky Mountain Elk Foundation for funding this study. We thank Advanced Telemetry Systems for their willingness to design a GPS-logger for specific use by hunters during this study. Additionally, we would like to thank the staff in Region 1 for their support and assistance coordinating this study, and the willingness of the hunters to carry the GPS-units and record their daily sightings.

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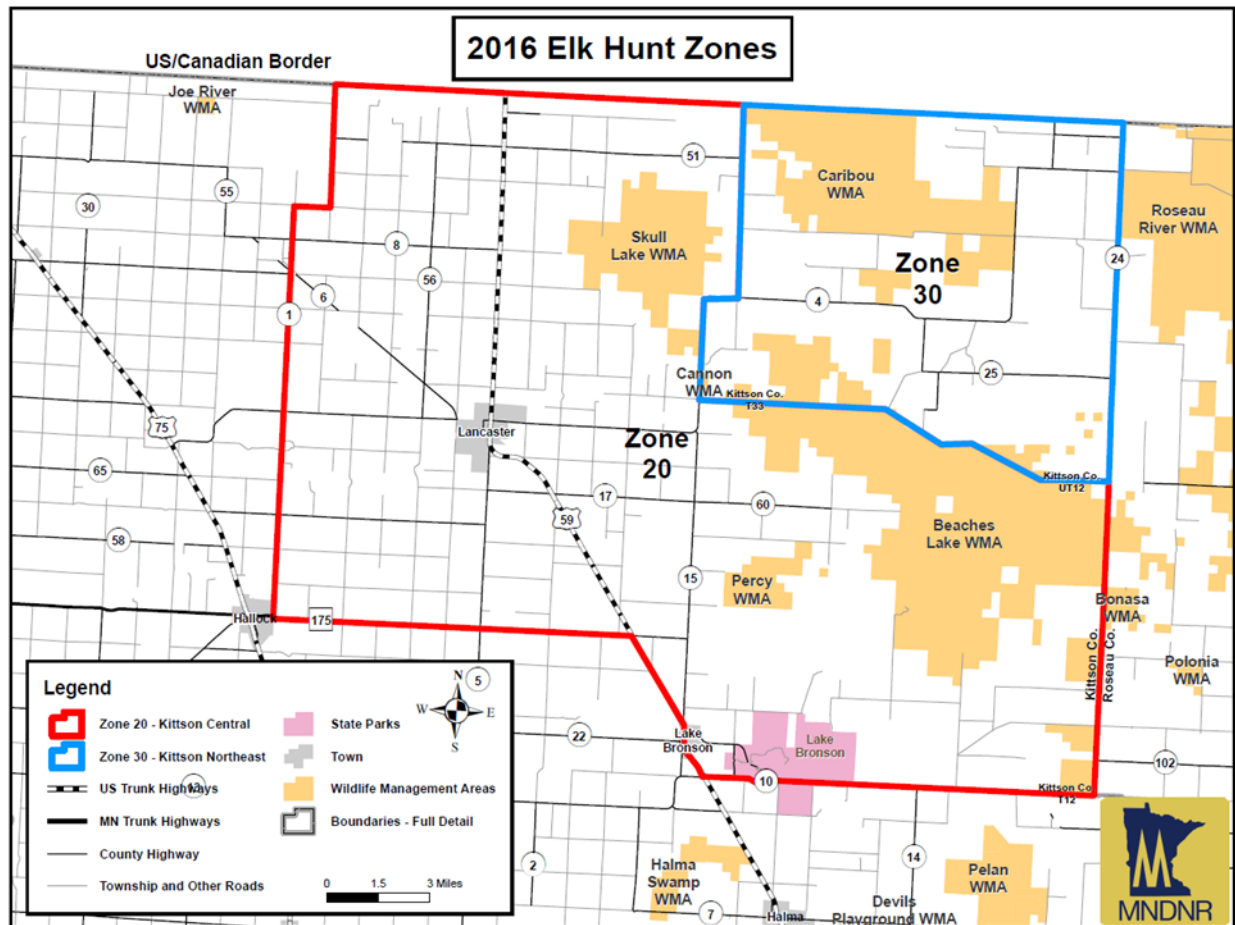


Figure 1. 2016 and 2017 Minnesota elk hunting zones found in Kittson and Roseau counties, Minnesota. Zone 20 included the Kittson-Central herd and Zone 30 included the Caribou-Vita herd.

2016 Minnesota Elk Hunt – Daily Hunter Log

Hunter Name: _____

GPS Unit ID: 037607

Please complete a brief summary of your daily hunting activities during the elk season to help us evaluate elk behaviors in response to hunting.

Saturday, September 10, 2016	Sunday, September 11, 2016	Monday, September 12, 2016
I hunted today: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	I hunted today: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	I hunted today: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Time I started hunting for the day (entered the field): 6 AM / PM	Time I started hunting for the day (entered the field): 6 AM / PM	Time I started hunting for the day (entered the field): 6 AM / PM
Time I stopped hunting for the day (left the field): 8:15 AM / PM	Time I stopped hunting for the day (left the field): 8:15 AM / PM	Time I stopped hunting for the day (left the field): 8 AM / PM
Total hours hunted: 14:15	Total hours hunted:	Total hours hunted:
Number of elk observed: 0	Number of elk observed: 0	Number of elk observed: 2 Cow + calf
Number of collared elk observed:	Number of collared elk observed:	Number of collared elk observed:
I shot at an elk today: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	I shot at an elk today: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	I shot at an elk today: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Time shots fired: AM / PM	Time shots fired: AM / PM	Time shots fired: AM / PM
Was elk harvested? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Was elk harvested? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Was elk harvested? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Comments:	Comments:	Comments:
Tuesday, September 13, 2016	Wednesday, September 14, 2016	Thursday, September 15, 2016
I hunted today: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	I hunted today: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	I hunted today: 6 <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Time I started hunting for the day (entered the field): 6 AM / PM	Time I started hunting for the day (entered the field): 6 AM / PM	Time I started hunting for the day (entered the field): AM / PM
Time I stopped hunting for the day (left the field): 7:30 AM / PM	Time I stopped hunting for the day (left the field): 8 AM / PM	Time I stopped hunting for the day (left the field): 8 AM / PM
Total hours hunted:	Total hours hunted:	Total hours hunted:
Number of elk observed: 1 Bull	Number of elk observed: 0	Number of elk observed: 0
Number of collared elk observed:	Number of collared elk observed:	Number of collared elk observed:
I shot at an elk today: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	I shot at an elk today: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	I shot at an elk today: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Time shots fired: 2 AM / PM	Time shots fired: AM / PM	Time shots fired: AM / PM
Was elk harvested? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Was elk harvested? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Was elk harvested? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Comments: I walked a woods and	Comments:	Comments:

Chased elk out to 2 hunters.

Figure 2. Example hunter log sheet filled out by a hunter during the 2016 Minnesota elk hunting season.

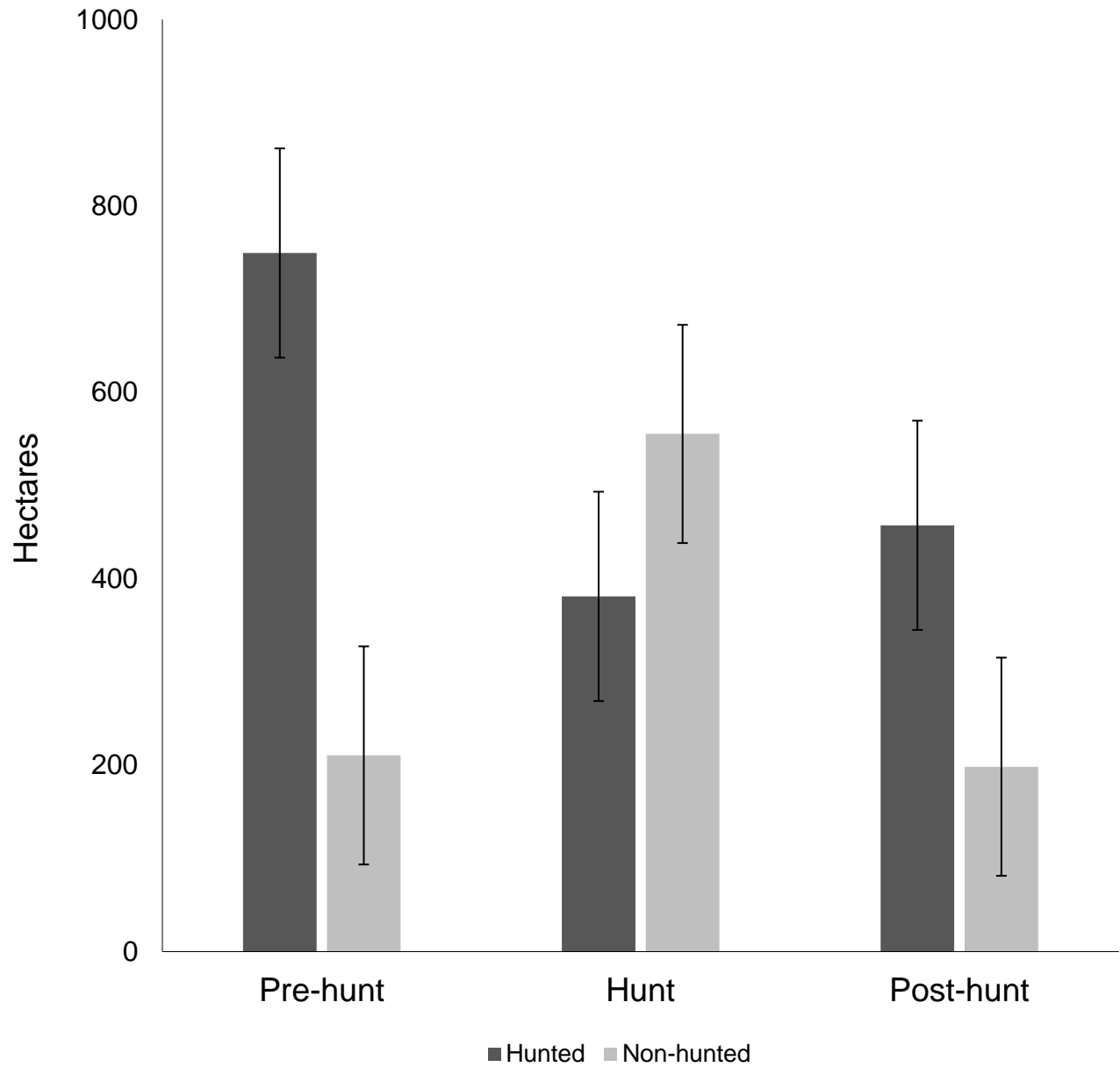


Figure 3. Daily utilization distributions (in hectares) of GPS-collared cow elk in hunted (dark gray) and non-hunted (light gray) zones during pre-hunt, hunting season, and post-hunt time periods in northwest Minnesota during fall 2016.

Human Dimensions Summaries



THE 2017 WATERFOWL HUNTING SEASON IN MINNESOTA: A STUDY OF HUNTERS' OPINIONS AND ACTIVITIES

Susan A. Schroeder and Steven D. Cordts

SUMMARY OF FINDINGS

The Section of Wildlife in the Minnesota Department of Natural Resources in collaboration with the Cooperative Fish and Wildlife Research Unit at the University of Minnesota conducted a survey of waterfowl hunters' participation and harvest, motivations, involvement, satisfaction, and opinions about bag limits, season dates and youth waterfowl opportunities. This study was conducted in an effort to obtain information on hunters' preferences relative to management alternatives associated with season dates and bag limits for waterfowl in the state. Variation in satisfaction with waterfowl hunting opportunity by geographic region and species was also of substantive interest in the study; as were preferences for potential changes to sandhill crane hunting regulations.

Ducks were revealed to be the most commonly sought waterfowl among Minnesota hunters. More hunters hunted the Central Zone than the North or South Zones during the 2017 season. The majority of duck hunters (71%) and goose hunters (63%) were satisfied with their hunting experience. Hunters were supportive of the Youth Waterfowl Hunting Day. Roughly two-thirds of hunters indicated that the 6-duck overall, 2-hen mallard, and 3-wood duck bag limits were "about right". A minority in each instance believed that the limits were either too high, too low, or did not have an opinion. Around 10% of respondents reported that waterfowl hunting was their "most important recreational activity." Social aspects of waterfowling were found to be most important attributes of involvement in the activity. Hunters were supportive of allowing open water hunting on large water bodies, but opposed restricting the use of motorized decoys. North Zone duck hunters preferred a straight 60 day season, compared to Central and South. While South Zone hunters preferred a split season with a later end date, compared to Central and North. Hunters, on average, supported amendments to sandhill crane hunting in Minnesota, including an expanded hunt boundary.

INTRODUCTION

Obtaining information on hunters' preferences for aspects of hunting activities can inform season setting, regulations, and resource management decisions that optimize opportunities for hunters' to have preferred experiences (Schroeder et al., 2019; Schroeder et al., 2006; Vaske, Fedler, and Graefe, 1986). The Minnesota Department of Natural Resources (DNR) regularly conducts assessments of the attitudes, opinions, and behaviors of hunters and other stakeholders to achieve these goals. The number of registered waterfowl hunters in the state has fallen by one-third since 2000. Waterfowl hunting generates substantive economic (Grado et al., 2011) and social benefits (Arnett and Southwick, 2015), in addition to contributing to wildlife conservation (Vrtiska et al., 2013). Therefore, declining waterfowl hunter numbers are a cause for concern for DNR as the trustee of waterfowl in the state, and the agent responsible for facilitating citizens' connection to wildlife resources.

Objectives

This study was undertaken with the following objectives:

1. Describe hunter effort in Minnesota in 2017 including: species and seasons hunted; number of days hunted; effort during weekdays, weekends, and opening weekend; and management regions hunted.
2. Describe hunting satisfaction with waterfowl (duck and goose) hunting in Minnesota in 2017.
3. Examine the importance of various experiences preferences (related to bagging waterfowl) and actualization of those experience in waterfowl hunting during 2017.
4. Examine importance of and involvement in waterfowl hunting to Minnesota and intentions to participate in the future.
5. Determine Minnesota waterfowl hunters' opinions concerning bag limits and other management strategies for maintaining waterfowl numbers;
6. Determine Minnesota waterfowl hunters' opinions on season dates and split seasons.
7. Determine Minnesota waterfowl hunters' opinions changes to sandhill crane hunting.
8. Determine Minnesota waterfowl hunters' support for and participation in Youth Waterfowl Hunting Day.
9. Determine demographics of waterfowl hunters in Minnesota.
10. Examine trends in waterfowl hunters' characteristics and opinions over time.

METHODS

Sampling

The primary sample frame consisted of Minnesota residents aged 18 and older that purchased a state waterfowl stamp for the 2017 season. 3,600 individuals were randomly selected to participate, stratified by four geographic regions corresponding to waterfowl hunt zones (North, Central, South) and the Minneapolis-Saint Paul Metropolitan region, by zip code of residence (900 hunters per strata). Additional samples of 900 pictorial waterfowl stamp purchasers, and 900 sandhill crane permit holders were randomly selected for participation.

Data Collection

Data were collected using a repeat-mail design. Participants were sent a survey packet with a cover letter, questionnaire and self-addressed, postage-paid return envelope. Mailing lists were adjusted between rounds. Non-respondents were sent three additional survey packets. In the fourth mailing, participants were also provided a \$1 incentive in an effort to increase response rate. A one page survey was sent to non-respondents after the fourth round in order to quantify non-response bias. Data collection occurred between January and May, 2018.

Data Entry and Analysis

Data were keypunched, and analysis was conducted using the Statistical Program for the Social Sciences (SPSS for Windows 21). Basic descriptive statistics and frequencies were calculated for the statewide, pictorial, and crane permit holder samples. The three research strata and regional results were compared using one-way analysis of variance (ANOVA) and cross-tabulations. Frequency weights were applied to calculate statewide estimates based on the proportion of hunters that reside in each of the geographic strata in the sample.

RESULTS

Response Rate and Respondent Demographic Characteristics

Collection efforts yielded an overall response rate of 48.6% 134 of the original 3,600 addresses were undeliverable or otherwise invalid resulting in an adjusted sample of 3,466 individuals.

Response rates ranged from 50.3% to 45.8% by strata. A non-response bias was detected when respondents were compared to the population of hunters; where respondents tended to be older. However, when analytical weights were applied to the data no meaningful differences in estimates were detected when compared to the unweighted results. Therefore, the data were not weighted by age.

Respondents were on average 44.5 years old, and had lived in Minnesota for an average of 41 years. On average, respondents possessed an annual household income of approximately \$113,000. Roughly 39% of respondents had completed a 4-year college degree or greater.

Participation and Waterfowl Hunting Activities

The vast majority (87%) of waterfowl permit holders reported that they went waterfowl hunting during the 2017 season. Of those that reported hunting, 94% hunted ducks and a majority hunted Canada geese during the regular season. Early season goose hunting, other geese, and sandhill crane were less commonly sought targets. Average season bags for ducks, Canada geese and other geese were 11.0, 6.4, and 1.6 respectively.

Satisfaction

Overall, (71%) respondents were satisfied with their duck hunting experience. Forty-nine percent of duck hunters reported that they were satisfied with harvest, and more than 50% were satisfied with duck hunting regulations. A slightly lower plurality of goose hunters (63%) reported that they were satisfied overall, while a similar pattern to duck hunters was observed for satisfaction with harvest and regulations.

Importance, Motivation and Involvement in Waterfowl Hunting

Around half of respondents said that waterfowl hunting was one of their most important recreational activities, while 28% reported that waterfowl hunting was no more important than others. Ten-percent indicated that waterfowl hunting was their most important recreational activity, and 11% indicated that it was less important than other activities. Respondents agreed that waterfowl hunting provided an opportunity to be with family and friends, and that it is one the most enjoyable things that they do. Fewer respondents indicated that waterfowl hunting was a central part of their life. Four dimensions were identified among factors that influence hunters' satisfaction with waterfowl hunting; seeing ducks and geese, attracting waterfowl with decoys and calls, bagging a lot of waterfowl, and other specialized aspects of bagging waterfowl. On average, respondents rated seeing ducks and geese as the most important determinant of their satisfaction, and bagging a lot of waterfowl as the least.

Opinions about Youth Waterfowl Hunting Day

A majority (73%) of respondents supported the Youth Waterfowl Hunting Day, and 46% strongly supported it. Twelve-percent of respondents reported participating in the Youth Waterfowl Hunting Day, escorting an estimated 18,027 youth hunters to the field in 2017.

Opinions about Waterfowl Regulations

Respondents' opinions about current duck bag limits were queried. The majority (~two-thirds) indicated that the 6-duck, 2-hen mallard, and 3-wood duck bag limits were "about right." Minorities in each instance reported that they felt the bag limits were either too high, too low, or did not have an opinion on the matter. Respondents indicated preference for the current three zone (North, Central, and South) configuration. Support was also expressed for the potential to allow open water hunting on a select number of large water bodies. Respondents, on average, expressed the least support for restricting the use of motorized decoys.

Opinions about Season Dates and Splits

Respondents were asked to indicate their preference for alternative configurations of season dates and splits. An option for a continuous 60 day season, and two different split seasons were presented, as was a “no preference” alternative. Fifty-eight percent of respondents in the North Zone preferred a continuous season, while 43% of South Zone hunters preferred the split season with the latest end date. A similar pattern was observed with respect to season opening dates, with North Zone hunters preferring an early start date and Central and South Zone hunters preferring a later one.

Opinions about Sandhill Crane Hunting in Minnesota

Five potential changes to sandhill crane hunting regulations were presented to participants for evaluation. Respondents, on average, supported a) expanding the crane hunting area, b) moving the opening day to an earlier date, c) expanding the size of the current zone, d) increasing the season length from 37 to 58 days, and e) increasing the daily bag limit from one to two birds. Mean values ranged from 3.8 (a) to 3.3 (e), where 1 = strongly oppose, 3 = neutral, and 5 = strongly support.

Comparisons to Previous Assessments

Satisfaction with waterfowl hunting in 2017 was higher than in several previously studied years including 2000, 2005, 2007, 2010, 2011, and 2014 (but not 2002). Support for the Youth Waterfowl Hunting Day was also higher than in years where it was examined.

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THE 2016 RUFFED GROUSE HUNTING SEASON IN MINNESOTA

Susan A. Schroeder

SUMMARY OF FINDINGS

The Minnesota Cooperative Fish and Wildlife Research Unit in collaboration with the Minnesota Department of Natural Resources (MNDNR) conducted a survey of ruffed grouse (*Bonasa umbellus*) hunters following the 2016 season. A statewide survey of small game hunters pre-screened for grouse hunting participation was conducted to understand: grouse hunting behavior and intensity of participation, motivations for grouse hunting, and preferences for regulations and hunting experiences.

INTRODUCTION

Ruffed grouse populations undergo a periodic cycle (Zimmerman et al., 2008). During the 20th century, grouse hunter participation in Minnesota has fluctuated with the population cycle. This variation has implications for managing grouse hunting experience, especially given the potential for wide differences in expectations and real experiences, and ultimately satisfaction (Schroeder et al., 2019).

Objectives

1. Evaluate grouse hunters' patterns and rates of participation and harvest
2. Understand grouse hunters' satisfaction with the activity
3. Determine factors that motivate participation and influence satisfaction
4. Understand differences in metro-county, and non-metro county hunters' beliefs, attitudes and behaviors about grouse hunting

METHODS

Sampling

The sample frame for this study was Minnesota residents aged 18 and over that possessed a small game license for the 2016 hunting seasons. A random sample of 8,000 hunters was selected from this population for participation; stratified by place of residence (4,000 residents of the 7-county area surrounding Minneapolis/Saint Paul, and 4,000 non-metropolitan county residents). Hunters were mailed a screening postcard to assess participation in grouse hunting. 1,312 postcards (668 metropolitan and 644 non-metropolitan) were returned from grouse hunters willing to participate.

Data Collection

Data were collected following the recommendations of Dillman (2000). Participants were sent a survey packet containing a personalized cover letter, questionnaire and a self-addressed business-reply envelope. Three full survey packets were distributed to non-respondents at roughly 3-week intervals. Following distribution of the third survey packet, a shortened survey was sent to non-respondents to assess non-response bias.

Analysis

Descriptive statistics were generated for all variables, and mean and proportion differences in outcomes were estimated between metro and non-metro hunters on variables of interest using paired sample *t*-tests and chi-squared tests respectively.

RESULTS

Response Rates

Of the 668 surveys sent to pre-screened metropolitan county hunters, 461 were returned. This represents a response rate of 69%. Of the 644 surveys sent to pre-screened non-metropolitan county hunters, 426 were returned for a response rate of 74%. Thirty-eight and 52 short surveys used to gauge non-response bias were returned from metro and non-metro hunters respectively.

Grouse Hunting Participation and Harvest

Grouse hunters spent on average 9.3 days afield during the 2016 season. Over the course of the season, hunters harvested roughly 4 grouse, or 0.43 grouse per person/per day. Grouse hunters, on average, had been hunting grouse for 36 years. Respondents took an average of 6.4 trips during the 2016 season to hunt grouse. Differences were observed between metro and non-metro hunters in trip length, with metro hunters traveling an average of 151 miles per trip and non-metro hunters traveling 88 miles per trip.

Satisfaction, Crowding, and Change in Quality of Hunting over Time

Overall, hunters were satisfied with grouse hunting in Minnesota with a majority indicating that they were either “slightly”, “moderately” or “very” satisfied. However, differences were observed when examining various aspects of satisfaction with grouse hunting access, the number of grouse flushed, and quality of grouse habitat. For instance, a smaller proportion of respondents indicated that they were either “very” or “moderately” satisfied with the number of grouse they flushed during the 2016 season.

The majority of respondents did not experience crowding while hunting grouse in 2016, with 80% indicating either “not at all” or “somewhat” when asked to evaluate their perceptions of the number of other hunters or people they encountered while hunting. Among those hunters that did experience interference, ATVs and other off-highway vehicles were the most commonly reported source.

When asked to compare the quality of grouse hunting in Minnesota in 2016 to five and ten years ago, most hunters felt that grouse hunting in 2016 was about the same. However, on average, hunters felt that the number of grouse they flushed and the amount of crowding they experienced was slightly worse today when compared to the past.

Grouse Management and Trust in MNDNR

Hunters were asked about their perceptions of bag limits for grouse management. Items measured hunters’ beliefs that bag limits should be set based on grouse biology (biology), what is fair (fairness), what is desirable (social desirability), what is right (injunctive), and what other hunters commonly think bag limits should be (descriptive). On average, hunters agreed with items measuring these beliefs about grouse bag limits, with the exception of social desirability. Differences were also found between the ratings of metro and non-metro hunters, where metro hunters more strongly agreed that grouse bag limits should be set based on the species biology and what is right.

On average, hunters agreed with statements expressing aspects of trust in MNDNR including a belief that the MNDNR “does a good job managing ruffed grouse”, “can be trusted to make decisions about ruffed grouse”, and that the MNDNR “listens to grouse hunters’ concerns,” among others. However, agreement on these items was only slightly positive. (~3 on a 5 point scale).

Importance of Grouse Hunting, Motivations, and Preferences

Very few hunters reported that grouse hunting was either their most important recreational activities, or their least important recreational activity. The vast majority indicated (>80%) that grouse hunting was either less important than their other recreational activities or no more important than their other recreational activities. Slightly greater than 10% of respondents reported that grouse hunting was one of their most important recreational activities.

Respondents were asked how important a variety of experiences was to them as determinants of their satisfaction with grouse hunting. On average, the most important experiences for grouse hunters were: enjoying nature and the outdoors, reducing tension and stress, and seeing grouse in the field. Hunters placed less importance on harvest-related experiences including: bagging enough grouse to share, bagging enough grouse to freeze for the future, and bagging a daily limit. Hunters reported actually experiencing activity-general motives, especially those related to enjoying nature and reducing stress. Harvest orientation was further examined, with around 85% of respondents agreeing strongly that “a grouse hunting trip can be enjoyable even if no grouse are bagged” and one-third agreeing that “the more grouse I bag the happier I am.”

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NEONICOTINOIDS ON THE LANDSCAPE: EVALUATING AVIAN EXPOSURE TO TREATED SEEDS IN AGRICULTURAL LANDSCAPES

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SUMMARY OF FINDINGS

Neonicotinoid pesticides [e.g., imidacloprid (IMI), thiamethoxam (TMX), thiacloprid (THIA), clothianidin (CLO)] are commonly applied to agricultural seeds (e.g., corn, soybean, wheat, sunflower) and are known to cause lethal and sub-lethal effects in birds. Neonicotinoid-treated seeds could be available to wildlife through spillage or exposure to treated seeds near or at the soil surface after planting (de Leeuw et al. 1995, Pascual et al. 1999, Lopez-Antia et al. 2016). Using several lines of evidence, we examined sub-lethal exposure and the potential for exposure of wildlife to these pesticides in agricultural landscapes of Minnesota in 2016 and 2017. We documented exposed seeds at the soil surface in plots at 35% of 71 fields sampled after planting. We also quantified the rate of seed spills during planting season and documented 329 seed spills in the 76 townships surveyed in the spring. We documented birds and mammals eating treated seeds through field studies with trail cameras. We quantified consumption of treated seeds for 11 species of birds and 9 species of mammals, and in many cases we estimated that more than 25% of the LD₅₀—the amount of ingested substance to kill 50% of a test sample—was ingested. Seed exposure experiments conducted under environmental conditions indicated that neonicotinoids are persistent on the seed surface for as long as 30 days in the environment, so wildlife can ingest neonicotinoids on treated seeds for at least 30 days after planting.

We also conducted laboratory experiments using domestic chickens (*Gallus gallus domesticus*) to identify non-lethal and lethal sampling methods that could lead to measurement of individual- and population-level exposure, including residues in the excreta and blood of birds. Mean residue concentrations in chickens dosed in the lab were highest in the brain. In decreasing order of concentration, residues were also detected in liver, spleen, muscle, blood, kidney, then feces. Residues in chicken fecal samples collected in the lab had the highest frequency of detection in all tissues tested.

Finally, we collected field samples from prairie grouse leks and from hunter-harvested birds to evaluate whether wild birds were exposed to sub-lethal doses. Seventy-three of 82 (89%) liver samples collected from sharp-tailed grouse (*Tympanuchus phasianellus*) and 32 of 45 (71%) greater prairie-chickens (*Tympanuchus cupido*) contained concentrations above the Method Limit of Quantification (MLOQ) for at least 1 neonicotinoid. Similarly, 95 of 109 (87%) sharp-tailed grouse fecal pellets and 51 of 59 (86%) fresh greater prairie-chicken fecal pellets collected from leks have been analyzed and had concentrations above the MLOQ for ≥1 neonicotinoid. Most of the detected concentrations were <10 ng/g, which explains why earlier studies with higher detection thresholds than the current study concluded a more rapid

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clearance of neonicotinoids from vertebrates than we found. Only 3 greater prairie-chicken livers and 9 sharp-tailed grouse livers had CLO concentrations >10 ng/g, and 3 greater prairie-chicken and 7 sharp-tailed grouse livers had IMI >10 ng/g. Similarly, only 2 greater prairie-chicken pellets and 5 sharp-tailed grouse pellets had CLO >10 ng/g, and 9 greater prairie-chicken and 14 sharp-tailed grouse pellets had IMI >10 ng/g. These results show that wildlife were exposed to neonicotinoids through treated seeds, a large proportion of prairie grouse in Minnesota had quantifiable residues of neonicotinoids, and wildlife may have experienced both sub-lethal and lethal effects. Further research is necessary to evaluate individual- and population-level effects of these rates of ingestion of neonicotinoid-treated seeds.

INTRODUCTION

Neonicotinoids are the most widely used pesticides worldwide (Mineau and Palmer 2013), comprising 25% of the global agricultural chemical market. Their action is highly specific to invertebrates, with relatively low toxicities for vertebrates compared to pesticide options predating the early 1990s (Tomizawa and Casida 2005, Jeschke et al. 2011). This high specificity contributed to their widespread and rapid adoption beginning in 1994 with the registration of imidacloprid in the United States.

Recently, neonicotinoids have received a lot of attention because of their potential toxicity to bees and other pollinators and their possible role in colony collapse disorder. Several neonicotinoid treatments were banned or placed under a moratorium in Europe in 2013, and neonicotinoids are currently under registration review by the Environmental Protection Agency (EPA) in the United States. The Minnesota Department of Agriculture (MDA) recently conducted a special registration review of neonicotinoid pesticides with an emphasis on pollinators (MDA 2016). However, recent concern has not been limited to pollinators; the American Bird Conservancy called for research on the effects of neonicotinoids on birds and a ban on neonicotinoid seed treatments (Mineau and Palmer 2013). Evidence is accumulating that vertebrates are also adversely affected by these pesticides (see reviews in Mineau and Palmer 2013, Gibbons et al. 2014). MDA (2014) acknowledged that, “Although neonicotinoids are less toxic to vertebrates than to arthropods, direct consumption of neonicotinoid treated seeds may expose birds and other taxa to acute or chronic doses.”

The most likely route of exposure to large doses of neonicotinoids for birds is ingestion of treated seeds (Goulson 2013, Gibbons et al. 2014), although numerous other mechanisms exist (e.g., soil, trophic transfer; SERA 2005, Douglas et al. 2015). Ingestion of a small number of neonicotinoid-treated seeds is lethal to birds; for example, a single treated corn kernel can kill a blue-jay sized bird (see reviews in Mineau and Palmer 2013, Gibbons et al. 2014). However, toxicity generally varies by chemical and species, given differences in genetic and physiological factors including size, metabolic, and digestive processes. Lethal impacts are rapid and difficult to detect in the wild although a few pesticide poisoning incidents have been detected (Greig-Smith 1987, Fletcher et al. 1995, Berny et al. 1999, de Snoo et al. 1999). Sub-lethal exposure might be easier to detect in the wild than lethal exposure if mortality events are relatively small and carcasses rapidly removed by scavengers. Sub-lethal effects in birds in the lab include hyporeactivity, lack of coordination, wing drop, immobility, disruption of migratory coordination, eggshell thinning, reduced egg hatching rate, impaired testicular function, and low weight in chicks (Cox 2001, Lopez-Antia et al. 2013 and 2015, Tokumoto et al. 2013, Mineau and Palmer 2013, Eng et al. 2017). Avian reproduction can be affected by consumption of just 1/10th of a treated corn seed per day during egg-laying (Mineau and Palmer 2013).

Thirty bird species were observed picking up treated seeds from cereal fields in Spain, and 3.1% of red-legged partridge (*Alectoris rufa*) gut contents collected by hunters tested positive for imidacloprid after planting of winter cereal crops (Lopez-Antia et al. 2016). Dead and poisoned

partridges have been found in agricultural fields in France following use of imidacloprid-treated seed (Berny et al. 1999). The EPA estimated that ~1% of seeds remain accessible to granivores after planting (as reported by Goulson 2013, Lopez-Antia et al. 2015). Use of neonicotinoid “treated articles,” such as seed, is not currently tracked by the U.S. government due to the exemption in 40CFR §152.25(a). Yet, almost all corn planted in the Midwestern U.S. has been treated with these pesticides (Stokstad 2013); most soybean, wheat, and sunflower seeds are treated also; and neonicotinoids are widely used with other application methods for other crop types.

Studies of neonicotinoid effects on vertebrates are overwhelmingly laboratory-based (91% of studies), which limits our ability to interpret the significance of findings in more natural settings (Gibbons et al. 2014). Higher densities of exposed seeds generally result in greater attraction of birds to fields (Murton et al. 1963, Feare et al. 1974). Bednarska et al. (2013) identified a need for feeding rate information in the field to allow extrapolation of lab data to the field. Lopez-Antia et al. (2013) pointed to a “need for evaluation of real exposure to coated seed ingestion by wild birds, including feeding behavior analyses and estimation of food intake rates.” Despite these calls for field studies and the time elapsed, the information deficits identified have still not been sufficiently addressed. Importantly, the U.S. still lags behind Europe (Berny et al. 1999, Lopez-Antia et al. 2013, 2016) in field-based studies focused on neonicotinoids and wildlife. We are therefore conducting a study to determine whether wild birds are exposed to neonicotinoid-treated seeds in agricultural landscapes in Minnesota. Preliminary data from our ongoing studies are reviewed below.

OBJECTIVES

The overarching objective of our research was to ascertain whether birds are exposed to neonicotinoid-treated seeds in agricultural landscapes. Specifically, we aimed to:

1. Quantify the rate of seed spillage and surface seed exposure after planting within fields.
2. Identify birds consuming neonicotinoid-treated seeds and quantify consumption per foraging bout.
3. Quantitatively link exposure and chemical residues in tissue, blood, and excreta to neonicotinoid concentrations in chickens (lab study).
4. Determine whether neonicotinoid exposure in wild prairie grouse can be detected from non-lethal sampling methods or from hunter harvested birds.

STUDY AREA

We conducted the field portions of our study in agricultural regions of Minnesota. Most field components were conducted in the agriculturally-dominated western portion of the state including the quantification of actual seed spills (Figure 1a), seeds on the soil surface and seed consumption at simulated seed spills (Figure 1b) in the spring. Field samples of prairie grouse came from the northwestern part of this region and also the east-central part of the state where agriculture was present but comprised a smaller proportion of the landscape (Figure 1c).

METHODS

Quantifying Seed Spills

All chemically treated seeds (e.g., neonicotinoids, fungicides, other pesticides) are unnaturally colored, as mandated by the Federal Seed Act. These seeds are highly visible and easily identified by their unusual color (e.g., pink, blue, green, purple), which is used to prevent accidental feeding to livestock. We quantified the frequency of actual seed spills on the landscape by inspecting fields with visual access from roads, field access points, and roadsides in agricultural areas. We hoped to avoid bias in spill rates that might result from obtaining

permission to access privately-owned fields on foot, but this method makes the implicit assumption that spill rates associated with refilling and overfilling hoppers is similar for fields that are adjacent to roads and fields that are not adjacent to roads.

We identified 211 townships in the western third and southeastern part of the state with ≥ 50 miles of roads and $\geq 50\%$ of the area in corn, soybeans, and/or wheat production using the Department of Transportation (DOT) Roads Layer (DOT 2008) and 2014 Cropland Data Layer (USDA-NASS 2015), respectively, in ArcGIS. These criteria were used to select townships with visual access to fields from roads while also not being so restrictive that the spatial distribution of the sample was constrained. We drew a spatially balanced sample of 50 townships and surveyed the 38 most western townships selected due to a later start to planting during the spring of 2016. In 2017 we selected 50 different townships and again surveyed the 38 westernmost townships due to a late start to planting. We surveyed a total of 76 townships during the 2 years of the study. We began in the southern counties and worked north beginning in late April as crops were planted.

We recorded locations and approximate number of seeds in spills near *recently planted* fields with the DNRSurvey mobile computer application. Documenting only *recently planted* fields allowed for control in temporal variation in the timing of planting. For example, a field that has not been planted yet will not have a spill at the time of sampling, which is different from a spill not occurring during planting. Thus, by only including recently planted fields in our estimates, we measured spills during planting. We defined a “field” as a quarter of a quarter-section (i.e., 40 acres). We recorded each quarter of a quarter-section in agricultural production, whether any part of it was recently planted (i.e., before early seedling stage), documented the amount (number of seeds) of spilled seed on the road, field edge, or visible in the field, and crop type (when possible). To determine the proportion of seed spills that contained neonicotinoid-treated seed, we collected seeds from accessible spills (e.g., along public roads and rights-of-way) and quantified 7 neonicotinoids (Chen et al. 2014).

Quantifying Seeds on the Soil Surface

To estimate the amount of seed at the soil surface after planting, we used a 1-m² frame to define plots in recently planted fields and counted all treated seeds visible within the frame after planting (Lopez-Antia et al. 2016). We sampled 5 plots in a field corner and 5 plots in the field center as estimated visually from field boundaries while standing in the field. For corner locations, we randomly selected 1 field corner per field by flipping a coin twice and paced 15 m and 30 m along each edge in an L shape that had the field corner for a vertex for a total of 5 measurements (i.e., 1 plot at vertex, 2 plots at 15 m, and 2 plots at 30 m). This approach incorporated sampling parallel and perpendicular to planting rows, and we suspected that seed exposure would be greater at the end of rows where planters turn sharply than within rows. For field centers, we paced 15 m in each cardinal direction to sample for a total of 5 measurements, including the center.

In 2016, we sampled 36 fields on DNR-managed Wildlife Management Areas (WMAs) that were farmed by private individuals under contract through Cooperative Farming Agreements (CFAs), 2 privately farmed fields on private land where we had permission, and 10 fields farmed by DNR staff on WMAs. In 2017, we sampled 6 privately farmed fields in CFAs and 17 privately owned and farmed fields with landowner consent. During 2017, neonicotinoid-treated seed was not permitted on WMAs. When seeds were exposed, we could determine whether they were treated; however, we did not dig up seeds for confirmation. In 4 cases, 2 fields were known to be planted by the same farmer, but in 3 cases, the fields were planted to different crop types, with different planting equipment used for each crop type in 2 of 3 cases where equipment type used was known.

Quantifying Decay of Neonicotinoids on Treated Seeds on the Soil Surface

To determine how long neonicotinoids persist on the seeds left on the soil surface we distributed hundreds of seeds on the soil surface of a tilled field near Bemidji to experience UV, microbial, rainfall, and other ambient conditions. After environmental exposure for 0, 1, 2, 4, 8, 16, and 30 days, we collected 5-7 seeds of each type to quantify decay of neonicotinoids under environmental conditions. We recorded daily precipitation and cloud cover during the experiment. We conducted the experiment in 2016 with 2 types of commercially available corn seed treatments (CLO and TMX) and commercially treated soybeans (IMI). In 2017, we repeated the experiment, but also put out wheat seeds (CLO, but the seed treatment was applied locally rather than through an industrial application). After field collection, seeds were stored frozen until shipping to a laboratory at Southern Illinois University Carbondale (SIUC) for neonicotinoid analysis.

Documenting Consumption of Treated Seeds

In 2016, we selected 12 WMAs to place trail cameras to observe wildlife consuming seeds at simulated spills in planted fields. The available data on CFAs on DNR-managed land indicated 7,420 acres (3,003 ha) of row crops in 341 CFAs in Region 4 (southern region) and 2,431 acres (984 ha) of row crops in 66 CFAs in Region 1 (northwest region; M. Benage and J. Williams, respectively, pers. comm.). We selected WMAs with a land cover composition similar to that of the surrounding landscape using the 2014 National Cropland Data Layer (USDA-NASS 2015) in ArcGIS 10.2 (ESRI 2015). Working on WMAs minimized bias in farming activities that might result from prior knowledge of the study. Furthermore, neonicotinoid-treated seed has been commonly used by private farmers on WMAs and many DNR managers reported difficulty finding seeds that had not been treated. We prioritized this portion of the study in 2016 because farmers and managers were prohibited from planting neonicotinoid-treated seeds on WMAs beginning in 2017.

Camera locations were selected to minimize risk of theft and to view a recently planted field to document foraging at a simulated seed spill and on exposed or submerged seeds or seedlings. In 2016, spills were simulated with 1,000 corn ($n = 15$ spills) or soybean seeds ($n = 2$ spills) to allow determination of the time it takes for birds to discover spills and the number of seeds consumed in each foraging bout by individual animals. Additionally, we placed cameras at 2 fields on privately-owned land where we had obtained permission. Cameras were deployed in each location for 3–6 weeks after planting. At each field, 2 motion-activated cameras were deployed—one that captured 1 image/sec in still photos and 1 that captured 1 min of video when triggered by motion. The camera set for still photos also took photos at 5-min intervals between 0600–0800 hr and 1830–2030 hr to document birds foraging in fields during sunrise and sunset periods during the planting season. Images were examined to identify species of wildlife consuming seeds and the number of seeds consumed per foraging bout.

In 2017, we included more privately-owned fields, which were generally larger than fields planted on WMAs. We placed 1 camera at each of 24 privately-owned fields in addition to placing cameras at 16 WMAs. We simulated 20 more corn spills, 23 soybean spills, and 9 wheat spills of 1,000 seeds each. Instead of capturing still images at simulated spills, which often produced ambiguous information about whether seeds were ingested, we instead set the cameras to record video only. Cameras were programmed to capture a 1 min video whenever the motion sensor was triggered. We checked cameras once weekly to replace batteries and data cards and deployed cameras in each location for 2–3 weeks. When we checked simulated spills, we restocked with an additional 1,000 seeds of the same seed type if 25-50% of the seeds remained but switched to a different seed type (after removing any remaining seeds) if <25% remained.

Linking Field and Laboratory Exposure Concentrations in Birds

We quantitatively linked field sample concentrations to laboratory exposure concentrations through work with University of Minnesota-College of Veterinary Medicine (UMN-CVM) and SIUC. We determined how many days post-exposure that imidacloprid (i.e., the most common seed treatment in Minnesota, J. Zachmann, MDA, pers. comm.) was detectable in both non-lethally and lethally collected samples from dosed birds. A non-lethal method to determine sub-lethal exposure would facilitate data collection during spring planting when spills would be expected to be most numerous.

At UMN-CVM, domestic chickens (*Gallus gallus domesticus*) were orally exposed to imidacloprid (IMI) for 7 days and serially sampled during and after the course of exposure to simulate repeated sub-lethal exposures. Chickens served as our model species given their suitability to captivity and close taxonomic relationship with wild grouse (Family Phasianidae). Small sample sizes are commonly used in dosing studies because the differences among treatment groups are expected to be very large and variability within groups low (e.g., Berny et al. 1999, Bednarska et al. 2013). We exposed chickens ($n = 5$) to 1%, 5%, and 20% of the LD_{50} (104.1 mg/kg IMI, Kammon et al. 2010) daily for 7 days by giving ~1.5 kg birds a daily IMI bolus of 1.04 mg/kg/day, 5.20 mg/kg/day, and 20.80 mg/kg/day (i.e., low, medium, and high dosage, respectively). The LD_{50} is the single dose that is expected to be lethal to 50% of test subjects. The LD_{50} would be reached if chickens ingested ~260–946 corn seeds (depending on application rate to seeds, which varies among seed companies). Stated differently, 3–10 seeds is comparable to the low, or 1%, LD_{50} dose. Thus, these were realistic doses. Prairie grouse (0.6–1.2 kg) are smaller than chickens and thus smaller doses (e.g., 104–780 seeds for the low-dose treatment, depending on bird weight) would be expected to produce similar results. Other neonicotinoids have a higher LD_{50} than IMI, so lethality would be expected at much higher seed ingestion levels for those pesticides.

The full laboratory experiment was completed only for chickens in the low- and medium-dosage groups because chickens in the high-dosage group were humanely euthanized on day 1 due to severe neurological and respiratory depression. Prior to exposure, baseline blood and excreta samples were collected. Sequential blood and excreta samples were collected on experiment days 1–21. Blood samples were collected at 0, 8, and 24 hours post-exposure and then on days 8, 14, and 21 post-exposure. Chickens that were considered at endpoint and euthanized had blood samples taken immediately before euthanasia. The low-dosage group was sampled for feces 1 day earlier than the medium group due to logistical challenges. Samples of internal organs (i.e., brain, kidney, liver, spleen) and muscle were taken from chickens that died during the treatment period or on day 21, whichever came first. Chickens were weighed on all days of sampling. Samples were sent to SIUC for residue analysis (Chen et al. 2014).

Descriptive statistics and graphing of the available data from these lab studies were performed to gain a preliminary sense of how IMI concentrations changed over time and in response to dose on a tissue-specific basis. According to best practices, we used geometric rather than arithmetic mean for chemical concentration data, which are typically lognormally distributed. Arithmetic mean is often biased high. Further statistical analyses will be conducted once we obtain the full dataset, including metabolites (i.e., neonicotinoids modified through metabolic processes) and feed concentrations.

Detecting Neonicotinoids in Free-Ranging Birds

We also collected samples from wild birds using both invasive and non-invasive methods to identify ways to assess exposure to neonicotinoids in the field. Fresh fecal pellets and blood samples from trapped prairie grouse were collected during lek visits for a genetic study in spring 2015 and again in 2017 for this study. Samples were stored frozen until shipped to the lab at

SIUC. Hunters also voluntarily submitted harvested prairie grouse in fall 2015, 2016, and 2017. Tissues and fecal pellets are being tested for thiacloprid (THIA), acetamiprid (ACE), thiamethoxam (TMX), IMI, clothianidin (CLO), dinotefuran (DIN), and nitenpyram (NTP).

DNR staff also assisted with lethal collections of granivorous birds observed foraging on treated seeds in the spring of 2016 under federal permit MB682323-0 issued to DNR. We are examining exposure to neonicotinoids using ingesta and tissue residue levels according to Chen et al. (2014) at SIUC.

RESULTS

Quantifying Seed Spills

We observed 212 large seed spills that were visible from the road during surveys in 2016 and 117 spills during surveys in 2017. However, we missed the peak of planting in many of the townships surveyed because both the springs of 2016 and 2017 were very wet and crops were planted later than usual. Planting in 2017 was later than in 2016, and we observed standing water in many fields during the spring planting season. At the time of our road-based surveys in 2016, 79,386 acres of corn, 82,341 acres of soybeans, 76,895 acres of wheat, and 21,427 acres of other crops were planted in the areas surveyed, amounting to 60.5% of the acres surveyed having been planted at the time of our survey. Spill rates in the areas surveyed were calculated as 4 spills/10,000 ac corn, 15 spills/10,000 ac soybeans, 6 spills/10,000 ac wheat, and 15 spills/10,000 ac other crop types. In 2017, 40,110 acres of corn, 23,556 acres of soybeans, and 33,749 acres of wheat, and 14,957 acres of other crops were planted during our surveys, or 23% of acres surveyed were planted at the time of our survey. Spill rates of 2 spills/10,000 ac corn, 27 spills/10,000 ac soybean, 7 spills/10,000 ac wheat planted were calculated. Extrapolating statewide requires the assumption that spill rates visible in fields adjacent to roads are representative of spill rates in fields located elsewhere. If spills near roads are more likely to be cleaned up than those less visible to passersby, then this assumption may not be tenable. Yet, we did not observe spills being cleaned up during our surveys. Furthermore, most spills occur during hopper refilling, and this often occurs near field access points along roads. Thus, we think our assumptions are reasonable. Applying our spill rates across the acres farmed statewide (8,450,000 acres of corn, 7,550,000 acres of soybeans, and 1,321,000 acres of wheat were planted in Minnesota during 2016 [National Agricultural Statistics Service (NASS); last accessed 5 June 2017 [National Agricultural Statistics Service](#)], we estimate nearly 15,000 large seed spills statewide in 2016 and expect that if there is a bias, our estimates are biased low. In 2017, 8,050,000 ac of corn, 8,150,000 acres of soybeans, and 1,160,000 acres of spring wheat were planted (NASS; last accessed 5 March 2018 [National Agriculture Statistics Service](#)), which extrapolates to ~25,000 spills during the planting season. Spills increased as we moved from south to north, and the proportion of fields planted during our surveys also increased as we moved south to north.

Quantifying Seeds on the Soil Surface

We documented exposed seeds at the soil surface in plots in 25 of the 71 fields where we sampled 10 1-m² plots in 2016 and 2017, and when areas outside plots were included, 40 fields had exposed seeds at the soil surface (Table 3). Seeds were exposed in ≥ 1 centrally located plot in 20% of fields measured. Exposed seeds were detected in ≥ 1 corner plot of 30% of fields measured. The quantity of exposed seeds on the surface of fields was 0.47 seeds/m² (range: 0-69) in the center of fields and 0.77 seeds/m² (range: 0-51) in the edges of fields, which is an order of magnitude lower than that reported by Lopez-Antia et al (2016). Most (72%) of the fields we measured were planted to corn, 24% were planted to soybeans, and 4.2% were planted to wheat (Table 4). Most (73%) sampled fields were on public land but 81% of the sampled fields on public land were planted by private cooperating farmers with their own

equipment. We suspect that spill rates are influenced by the type of equipment used for sowing (Lopez-Antia et al. 2016) and the seed type.

Quantifying Decay of Neonicotinoids on Treated Seeds on the Soil Surface

Neonicotinoids decayed on the surface of seeds relatively quickly, but concentrations exceeding 10 ng/g were present on all seeds after 16 days, and on IMI treated seeds after 30 days (Figure 2). We did not have a 30 day sample for CLO treated seeds because no seeds remained on the soil surface after 30 days, presumably due to wildlife consumption because the seeds were not removed from the tilled field by people.

Documenting Consumption of Treated Seeds

We reviewed images collected by trail cameras at simulated spills during spring 2016 ($n = 188,399$ photos and 12,602 videos) and 2017 ($n = 39,653$ videos). We documented ring-necked pheasants (*Phasianus colchicus*), Canada geese (*Branta canadensis*), American crows (*Corvus brachyrhynchos*), mourning doves (*Zenaida macroura*), wild turkeys (*Meleagris gallapavo*), blue jays (*Cyanocitta cristata*), brown thrasher (*Toxostoma rufum*), rose-breasted grosbeak (*Pheucticus ludovicianus*), various species of sparrows (Emberizidae) and blackbirds (Icteridae), as well as white-tailed deer (*Odocoileus virginianus*), black bears (*Ursus americanus*), raccoons (*Procyon lotor*), rodents, Eastern cottontails (*Sylvilagus floridanus*) and white-tailed jackrabbits (*Lepus townsendii*) consuming treated seeds. Consumption rates (seeds/min), the number of seeds eaten per 1 min video, and the total seeds eaten by an individual in consecutive videos are indicated in Table 1.

To estimate the toxicity of consuming neonicotinoid treated seeds, we estimated species-specific LD₅₀ concentrations using standard metabolic scaling procedures (EPA T-REX³) with estimated toxicity values for surrogate species, the mass of surrogate species, and product-labeled concentrations of chemical on a treated seed (in mg/seed; Bayer Crop Science and Syngenta). Toxicity values (LD₅₀ in mg/kg-bw) for surrogate species were acquired from EPA draft risk assessments or other documents (DeCant and Barrett 2010, Anon 2012, EPA_HQ-OPP-2011-0865-0242, EPA-HQ-OPP-2008-0844-1256, EPA-HQ-OPP-2011-0581-0093) to create the potential toxicity assessment (Table 2) for species observed consuming treated seeds in images. These metrics are useful for the assessment of risk in birds and mammals. In summary, potential exposure concentrations were much closer to estimated LD₅₀ concentrations for birds than mammals.

Linking Field and Laboratory Exposure Concentrations in Birds

We collected 72 blood samples; 100 fecal samples; 15 samples of muscle, brain, liver, and kidney; and 103 eggs during laboratory IMI exposures of chickens. Based on a detection limit of 0.10 ng/g, IMI was detected more frequently and for a longer duration post-exposure in fecal samples (90.9%, ≤ 21 days post exposure) than blood (32.9%, ≤ 7 days post exposure; Table 5). Blood concentrations increased from the first samples taken at the start of the experiment (hr 0) to hr 8 and declined again at hr 24 (Figure 3); after this time, samples did not contain detectable IMI except for 1 sample taken on day 8. Fecal IMI concentrations followed a 3rd order polynomial pattern, increasing from the start of the experiment (day 0) until approximately day 6, decreasing until day 18 and holding steady or slightly increasing by day 21 (Figure 4). As expected, the low dose group tended to exhibit lower IMI fecal concentrations than birds in the medium dose group. IMI was rapidly removed from blood, but the change in concentrations varied 17,234-fold (c.f., 279-fold in feces; fold change is maximum detected concentration/minimum detected concentration across all groups and times), and thus blood

³ [EPA T-REX guide](#)

may provide a more sensitive indicator of an acute exposure than feces. By contrast, fecal samples provided a more integrated, longer, and more consistent detection in exposed birds (Figure 3) and thus may be more applicable to field applications where time from chemical exposure will be more variable.

IMI was measured in internal organs, which were collected on the final day of the experiment, depending on when birds were euthanized (Figure 5). Low- and medium-dosed birds were euthanized on day 21, whereas high-dosed birds were euthanized after showing clinical signs of distress on day 1. Detection frequency of IMI was highest in kidney, liver, and spleen (73.3%), although muscle and brain also exhibited similar detection frequencies (66.7%). Geometric mean tissue concentrations were highest in brain and lowest in the kidney (Table 6).

For analytical method quality assurance and control, we used matrix spiked recovery tests, procedural blanks, and recoveries of surrogate standards. IMI (25 ng) was spiked into muscle ($n = 5$) or blood ($n = 5$) and analyzed. Mean (\pm SD) recoveries were $86.7 \pm 5.8\%$ and $90.9 \pm 4.9\%$ in tissue or blood, respectively. One procedural blank was processed for every 10 samples, and no target compound was detected in any blanks. Good analytical performance was indicated by surrogate standards with recoveries ranging from 75% to 98%. Similar methods were used for THIA, ACE, TMX, and CLO and the method limit of quantification was calculated by multiplying the standard deviation from replicates with a Student's t -value appropriate for a 99% confidence level. Thus, the method limit of quantification (MLOQ) for IMI was 0.3 ng/g in tissue and 0.4 ng/mL in blood, for THIA was 0.7 ng/g and 0.6 ng/mL, for ACE was 0.7 ng/g and 0.8 ng/mL, for TMX 0.8 ng/g and 0.8 ng/mL, and for CLO was 0.7 ng/g and 0.7 ng/mL in tissue and blood respectively. Minimum detectable concentrations were lower and ranged 0.1–0.3 ng/g for the 5 neonicotinoids, but we took a more conservative approach for reporting and interpretation.

Detecting Neonicotinoids in Free-ranging Birds

Field-collected prairie grouse samples sent for neonicotinoid analysis included 61 sharp-tailed grouse fecal pellet groups and 34 greater prairie-chicken fecal pellet groups collected in 2015, and 46 and 27 pellet groups, respectively, in 2017 (no sample collection occurred in 2016). We also collected 5 blood samples from trapped sharp-tailed grouse, as well as 2 brains and 3 breast muscles from sharp-tailed grouse for which we had whole carcasses and sent them for neonicotinoid analysis. Hunters submitted livers from 11 prairie-chickens, 22 sharp-tailed grouse, and 3 prairie-chicken/sharptail hybrids during fall 2015, 17 prairie-chickens, 33 sharp-tailed grouse, and 2 pheasants during fall 2016, and 17 prairie-chickens and 27 sharp-tailed grouse during fall 2017.

Seventy-three of 82 (89%) livers collected from hunter-harvested sharp-tailed grouse, 32 of 45 (71%) greater prairie-chicken livers, and 3 of 3 sharptail/prairie-chicken hybrids from hunter-submitted samples had concentrations above the MLOQ for at least 1 neonicotinoid. Three of 3 blood samples analyzed tested negative for neonicotinoids. Dinotefuran and NTP were not detected in any samples. Neonicotinoids above the MLOQ in prairie-chicken livers included IMI (64%), CLO (27%), and THIA (2%) and in sharp-tailed grouse livers included IMI (79%), CLO (37%), THIA (5%), and ACE (1%). Maximum concentrations of neonicotinoids in prairie-chicken livers were 22.0 ng/g IMI, 15.0 ng/g CLO, and 1.1 ng/g THIA. (Note that ACE and TMX were reported in a previous report, but detected concentrations were below the MLOQ; 0.21 ng/g, ACE, and 0.43 ng/g TMX). Maximum concentrations detected in livers of harvested sharp-tailed grouse were 84.5 ng/g IMI, 21.0 ng/g CLO, 1.18 ng/g THIA, 0.71 ng/g ACE, and 0.5 ng/g TMX, again with TMX below the more conservative MLOQ. Similarly, 51 of 59 (86%) fresh prairie-chicken fecal pellets and 95 of 109 (87%) sharp-tailed grouse pellets collected from leks during springs of 2015 and 2017 contained concentrations above the MLOQ for at least one

neonicotinoid. The most commonly detected neonicotinoid in the greater prairie-chicken fecal pellets was IMI (51%), followed by CLO (37%), and THIA (3%). Acetamiprid and TMX were not detected in feces, perhaps due to differences in the way they are metabolized or excreted. Maximum concentrations of IMI, CLO, and THIA in feces were 14.0 ng/g, 44.8 ng/g, 1.05 ng/g, respectively. In sharp-tailed grouse pellets, neonicotinoids above the MLOQ were IMI (62%), CLO (40%), and THIA (4%). Maximum concentrations were 39.7 ng/g IMI, 32.3 ng/g CLO, 0.9 ng/g THIA, with ACE and TMX below the MLOQ (0.2 ng/g and 0.5 ng/g, respectively). However, most of the detected concentrations were <10ng/g, which is below the detection limit in tissues in some other laboratories. Only 3 greater prairie-chicken livers and 9 sharp-tailed grouse livers had CLO concentrations >10ng/g, and 3 greater prairie-chicken and 7 sharp-tailed grouse livers had IMI>10ng/g. Similarly, only 2 greater prairie-chicken pellets and 5 sharp-tailed grouse pellets had CLO >10ng/g, and 9 greater prairie-chicken and 14 sharp-tailed grouse pellets had IMI >10ng/g.

Birds collected while foraging on treated seeds included 1 ring-necked pheasant, 5 red-winged blackbirds (*Agelaius phoeniceus*), 2 yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), 4 brown-headed cowbirds (*Molothrus ater*), and 5 common grackles (*Quiscalus quiscula*). Two brown-headed cowbird livers tested positive for exposure to IMI and CLO. One yellow-headed blackbird liver tested positive for IMI. Livers of all other birds collected while foraging on treated seeds tested negative for recent neonicotinoid exposure, indicating that this was either their first exposure or that previous exposures were not recent enough to detect.

DISCUSSION

We found that neonicotinoid-treated seed is common on the landscape during the spring planting season, both on seeds available on the soil surface and in seed spills. We also documented numerous avian and mammalian species consuming treated seeds at simulated spills, some of which ingested amounts that would be expected to produce lethal and sub-lethal effects. Samples obtained from wild birds during the fall hunting season also indicated recent exposure in a large proportion of harvested birds, which is consistent with consumption of treated seeds during planting of winter wheat in September and October in Minnesota. Indeed, several of the hunter-submitted sharp-tailed grouse carcasses contained wheat. These findings indicate a need for much more study into the exposure rates of wildlife to neonicotinoids. Population-level effects are possible based on the consumption rates, availability of treated seed, and persistence of neonicotinoids on seeds under environmental conditions that we observed. Thus, lethal and sub-lethal effects should receive more attention in wild populations, especially in granivorous species that consume seeds as part of their diet.

Field studies on neonicotinoids in vertebrates have been infrequent to date, in part due to methodological obstacles for field detection and in part due to the difficulty of isolating variables in field settings where variables cannot be easily controlled. We identified several methodological options that can be applied in field situations, including detection of residues in feces and tissues. Notably, fecal samples provide a non-invasive means to detect exposure in birds, which can be especially important for species of concern. Fecal samples also could be collected from the GI tract of live birds or from hunter-killed birds. For game species and more common species, internal organs like livers can also serve as an indicator of neonicotinoid exposure in lethal collections and livers are fairly easy for non-specialists to locate. Berny et al. (1999) reported that liver and kidney had the most consistent imidacloprid concentrations in fatally exposed wild birds, whereas crop and gizzard provided inconsistent concentrations. However, Lopez-Antia et al. (2015) reported that imidacloprid could be consistently detected in crops and livers of dosed partridges (*Alectoris rufa*). We had few ingesta samples, but our

results also indicated that liver and kidney provide more consistent imidacloprid concentrations than other tissues.

Previous studies have demonstrated that neonicotinoids (e.g., thiamethoxam) are excreted primarily through the kidneys in mammals (Bednarska et al. 2013, Tomizawa and Casida 2005). Ongoing analytical work to measure metabolites of imidacloprid in feces and the uric acid wash in birds is expected to provide a more sensitive (i.e., higher fold concentration change) assay than current parent compound (i.e., imidacloprid unmodified by metabolic processes) data. Further work will be required to quantify how the potential environmental imidacloprid exposure scenarios (concentration, duration, and frequency) influence the detection of parent compound and metabolites in feces and the uric acid wash in birds. Refining non-invasive collection is necessary because UV light can and microbial degradation may degrade neonicotinoids (Lu et al. 2015; Lu et al. 2016; Ma et al. 2014). Thus, pellet freshness is an important consideration. Most studies have suggested a rapid metabolism and elimination (~48 hours) of parent (i.e., unchanged) compound in the urine after *single* oral doses (Bednarska et al. 2013; Tomlin 2004). Other studies have had 10-fold lower detection thresholds in tissues, which explains the discrepancy between our study and others.

The highest concentration of IMI detected in livers of harvested prairie grouse (84.5 ng/g) was higher than that of chickens in the low and medium dose group at the end of the experiment. However, it was lower than the high LD₅₀ group after early euthanization. Similarly, the highest concentration of IMI detected in field-collected feces (39.7 ng/g) was consistent with the 1% dose group, lower than the 5% dose groups within 3 days of exposure, and was generally higher than both dose groups 2 weeks post-exposure, although samples varied substantially. We cannot know if this indicates a higher initial exposure or how much the passage of time since exposure might have reduced these levels, but given that 1% LD₅₀ (1.04 mg/kg) is comparable to the dose received after consuming 3–10 corn seeds and that IMI can be detected in tissues for as long as 21 days post-exposure, we consider it likely that this finding reflects a high initial exposure to IMI.

This research provides evidence contrary to several popularly held beliefs that wildlife do not eat treated seeds because they are unpalatable, that seeds are always drilled below the soil surface and are thus not available for wildlife, and that packaging labels are sufficient to protect wildlife from harmful effects. We encourage other researchers to replicate our study, and to pursue additional field studies of wildlife, to ensure that objective data are available to evaluate the risks of neonicotinoids to wildlife.

ACKNOWLEDGEMENTS

We would like to thank Curt Vacek, Beau Liddell, Steve Piepgras, Eric Nelson, Eric Thorson, and Nate Thom for their assistance with bird collections during spring. We thank Judy Markl, Bill Schuna, Randy Markl, Nick Trauba, Joe Stangel, Stein Innvaer, Curt Vacek, Brad Olson, Rob Baden, Mark Palm, Randy Prachar, and Jessica Parson for assisting with field planting information. We would like to thank Glacial Ridge National Wildlife Refuge, Talcot Lake Wildlife Management Area, and Roseau River Wildlife Management Area for accommodating technicians during field work. We would like to thank Judy Markl, Mark Palm, and Al Killian for acquiring seed. Traver Fields, Alisha Mosloff, Rachel Krebs, and Megan Zagorski surveyed for seed spills. Clarinda Wilson, Sophia Crosby, Rachel Haindfield, and Nicole Benson collected fecal pellets from leks. Robert Wright assisted with DNR survey. Ernesto Dominguez managed captive dosing experiments at University of Minnesota. Hongli Tan and Timothy DeKoster assisted with laboratory analysis of neonicotinoid residues at Southern Illinois University in

Carbondale. Laura Gilbert assisted with contracts, purchases, expense reporting, and generally anything asked of her with a smile. Mike Larson provided comments that improved this report.

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Table 1. Birds and mammals documented eating seeds at simulated spills in Minnesota during 2016 and 2017 by seed type (corn, soybean, & wheat in separate sections of the table). Consumption rates (seeds consumed/min), the range of seeds consumed in 1 min videos, and the maximum amount of seeds consumed by an individual in consecutive videos.

Species	Scientific name	Corn consumption rate (seeds/min)	Sample size	Range (seeds eaten per 60 s video)	Max seeds eaten per feeding bout
Common grackle	<i>Quiscalus quiscula</i>	3.2	27	1-5	5
Blue jay	<i>Cyanocitta cristata</i>	27.7	4	2-4	6
Ring-necked pheasant	<i>Phasianus colchicus</i>	15.3	9	1-21	21
Red-winged blackbird	<i>Agelaius phoeniceus</i>	1.9	28	1-6	6
Brown thrasher	<i>Toxostoma rufum</i>	2.46	5	1-3	3
American crow	<i>Corvus brachyrhynchos</i>	28.1	16	1-24	24
Black-billed magpie	<i>Pica hudsonia</i>	12	1	2	2
Wild turkey	<i>Melagris gallapavo</i>	174.2	2	1-150	150
White-tailed deer	<i>Odocoileus virginianus</i>	54.2	8	5-111	650
13-lined ground squirrel	<i>Ictidomys tridecemlineatus</i>	7.7	24	1-13	22
Raccoon	<i>Procyon lotor</i>	11.9	32	4-21	268
Eastern cottontail	<i>Sylvilagus floridanus</i>	3.1	14	1-6	35
White-tailed jackrabbit	<i>Lepus townsendii</i>	3.8	5	3-5	43
Eastern gray squirrel	<i>Sciurus carolinensis</i>	3.1	4	1-4	23
Fox squirrel	<i>Sciurus niger</i>	3.3	9	2-6	48
Striped skunk	<i>Mephitis mephitis</i>	13	1	13	13
Red fox kit	<i>Vulpes vulpes</i>	2.1	5	1-3	3
Red fox adult	<i>Vulpes vulpes</i>	n/a	2	1-2	2

Species	Scientific name	Soybean consumption rate (seeds/min)	Sample size	Range (seeds eaten per 60 s video)	Max seeds eaten per feeding bout
Ring-necked pheasant	<i>Phasianus colchicus</i>	18.9	21	1-36	68
Canada goose gosling	<i>Branta canadensis</i>	33.6	2	3-7	9
White-tailed deer	<i>Odocoileus virginianus</i>	107.6	36	3-317	800
13 lined ground squirrel	<i>Ictidomys tridecemlineatus</i>	6.9	15	1-14	14
Raccoon	<i>Procyon lotor</i>	9.7	4	5-8	61
Eastern cottontail	<i>Sylvilagus floridanus</i>	9.4	12	1-14	14
Fox squirrel	<i>Sciurus niger</i>	1.0	1	1	1

Species	Scientific name	Wheat consumption rate (seeds/min)	Sample size	Range (seeds eaten per 60 s video)	Max seeds eaten per feeding bout
Red-winged blackbird	<i>Agelaius phoeniceus</i>	10.5	2	2-5	5
American crow	<i>Corvus brachyrhynchos</i>	29.8	4	4-30	61
Mourning dove	<i>Zenaidura macroura</i>	16.2	32	1-31	73
Song sparrow	<i>Melospiza melodia</i>	1.6	6	1-2	2
Wild turkey	<i>Meleagris gallapavo</i>	199.7	5	153-215	700

Table 2. Estimation of potential avian and mammalian acute toxicity from different levels of treated seed consumption for focal species using surrogate species and metabolic scaling approaches as described in EPA's T-REX model. Mammalian scaling factor was 0.75 and avian scaling factor was 1.15. Neonicotinoid chemicals (CHEM) evaluated were clothianidin (CLO), imidacloprid (IMI), and thiamethoxam (TMX).

CHEM	Focal species	Seed	Surrogate	Surrogate LD ₅₀ (mg/kg)	Estimated LD ₅₀ (mg/kg)	Max % of LD ₅₀	Seeds (#) for LD ₅₀	Time to LD ₅₀ (mins)
CLO	American crow	Corn	Bobwhite quail <i>Colinus virginianus</i>	200	174	38.2	63	2
CLO	Black-billed magpie	Corn	Bobwhite quail	200	200	7.0	29	2
CLO	Blue jay	Corn	Bobwhite quail	200	224	39.4	15	0.5
CLO	Brown thrasher	Corn	Bobwhite quail	200	228	21.9	14	6
CLO	Common grackle	Corn	Bobwhite quail	200	216	26.8	19	6
CLO	Red-winged blackbird	Corn	Bobwhite quail	200	239	56.6	11	6
CLO	Ring-necked pheasant	Corn	Japanese quail <i>Coturnix japonica</i>	423	271	5.5	379	25
CLO	Wild turkey	Corn	Japanese quail	423	221	12.5	1195	7
CLO	American crow	Wheat	Bobwhite quail	200	174	1.8	3384	114
CLO	Mourning dove	Wheat	Bobwhite quail	200	206	5.6	1300	80
CLO	Red-winged blackbird	Wheat	Bobwhite quail	200	239	0.9	571	54
CLO	Song sparrow	Wheat	Bobwhite quail	200	259	0.6	363	227
CLO	Wild turkey	Wheat	Japanese quail	423	221	1.1	64457	323
IMI	Blue jay	Corn	House sparrow <i>Passer domesticus</i>	41	34	280	2	0.1

CHEM	Focal species	Seed	Surrogate	Surrogate LD ₅₀ (mg/kg)	Estimated LD ₅₀ (mg/kg)	Max % of LD ₅₀	Seeds (#) for LD ₅₀	Time to LD ₅₀ (mins)
IMI	Common grackle	Corn	House sparrow	41	34	183	3	0.9
IMI	Red-winged blackbird	Corn	House sparrow	41	37	387	2	0.8
IMI	Ring-necked pheasant	Soy	Japanese quail	17	11	83.5	81	4
TMX	Blue jay	Corn	Mallard <i>Anas platyrhynchos</i>	576	804	11.0	55	2
TMX	Common grackle	Corn	Mallard	576	804	7.2	70	22
TMX	Red-winged blackbird	Corn	Mallard	576	889	15.2	40	21
IMI	White-tailed deer	Corn	Mouse <i>Mus musculus</i>	131	1063	1.0	65471	1208
IMI	13-lined ground squirrel	Corn	Mouse	131	233	66.6	33	4
IMI	Raccoon	Corn	Mouse	131	700	3.3	8098	681
IMI	Eastern cottontail	Corn	Mouse	131	384	8.7	401	129
IMI	White-tailed jackrabbit	Corn	Mouse	131	479	3.5	1216	320
IMI	Eastern gray squirrel	Corn	Mouse	131	297	20.8	111	36
IMI	Fox squirrel	Corn	Mouse	131	328	26.1	184	56
IMI	Striped skunk	Corn	Mouse	131	470	1.2	1105	85
IMI	Red fox adult	Corn	Mouse	131	595	0.1	3598	1799
IMI	White-tailed deer	Soy	Mouse	131	1063	0.2	374916	3484
IMI	13-lined ground squirrel	Soy	Mouse	131	233	7.4	189	27
IMI	Raccoon	Soy	Mouse	131	700	0.1	46375	4781

CHEM	Focal species	Seed	Surrogate	Surrogate LD ₅₀ (mg/kg)	Estimated LD ₅₀ (mg/kg)	Max % of LD ₅₀	Seeds (#) for LD ₅₀	Time to LD ₅₀ (mins)
IMI	Eastern cottontail	Soy	Mouse	131	384	0.6	2296	244
IMI	Fox squirrel	Soy	Mouse	131	328	0.1	1052	1052
CLO	White-tailed deer	Corn	Mouse	427	3466	0.3	228769	4221
CLO	13-lined ground squirrel	Corn	Mouse	427	759	19.1	115	15
CLO	Raccoon	Corn	Mouse	427	2282	0.9	28297	2378
CLO	Eastern cottontail	Corn	Mouse	427	1251	2.5	1401	452
CLO	White-tailed jackrabbit	Corn	Mouse	427	1562	1.0	4248	1118
CLO	Eastern gray squirrel	Corn	Mouse	427	967	5.9	387	125
CLO	Fox squirrel	Corn	Mouse	427	1070	7.5	642	195
CLO	Striped skunk	Corn	Mouse	427	1532	0.3	3861	297
CLO	Red fox	Corn	Mouse	427	1940	0.0	12573	6287
TMX	White-tailed deer	Corn	Rat <i>Rattus norvegicus</i>	1563	7135	0.1	470899	8688
TMX	13-lined ground squirrel	Corn	Rat	1563	1563	9.3	238	31
TMX	Raccoon	Corn	Rat	1563	4697	0.5	58247	4895
TMX	Eastern cottontail	Corn	Rat	1563	2575	1.2	2884	930
TMX	White-tailed jackrabbit	Corn	Rat	1563	3215	0.5	8744	2301
TMX	Eastern gray squirrel	Corn	Rat	1563	1991	2.9	796	257
TMX	Fox squirrel	Corn	Rat	1563	2203	3.6	1322	401
TMX	Striped skunk	Corn	Rat	1563	3154	0.2	7948	611
TMX	Red fox adult	Corn	Rat	1563	3994	0.0	25880	12940

CHEM	Focal species	Seed	Surrogate	Surrogate LD ₅₀ (mg/kg)	Estimated LD ₅₀ (mg/kg)	Max % of LD ₅₀	Seeds (#) for LD ₅₀	Time to LD ₅₀ (mins)
TMX	White-tailed deer	Soy	Rat	1563	7135	0.0	3893016	36180
TMX	13 lined ground squirrel	Soy	Rat	1563	1563	0.7	1964	285
TMX	Raccoon	Soy	Rat	1563	4697	0.0	481541	49643
TMX	Eastern cottontail	Soy	Rat	1563	2575	0.1	23844	2537
TMX	Fox squirrel	Soy	Rat	1563	2203	0.0	10928	10928

Table 3. Exposed seeds on the soil surface after planting in 3 categories of field types in Minnesota during 2016 and 2017. Cooperative Farming Agreements (CFAs) are privately farmed areas on public land. Public fields were farmed by DNR staff with older planting equipment. Private lands were fields where we obtained landowner permission to survey fields after planting. We did not dig up seeds to determine whether they were treated, so if no seeds were on the surface, we did not know whether the seeds were treated.

Field type	# fields	Treated (T) or not treated (N)	Exposed seeds in center plots	Exposed seeds in corner plots	Exposed seeds outside plots	Spills
CFA (private equipment, public land)	42	18T, 2N	4 (10%)	4 (10%)	17 (40%)	4 (10%)
Public (old equipment, DNR staff)	10	3T, 4N	3 (30%)	5 (50%)	7 (70%)	0 (0%)
Private (pvt equipment)	19	13T, 4N	7 (37%)	12 (63%)	14 (74%)	8 (42%)
Total	71	34T, 10N	14 (20%)	21 (30%)	38 (54%)	12 (17%)

Table 4. Exposed seeds on the soil surface after planting by crop type in Minnesota in 2016 and 2017. We did not dig up seeds to determine whether they were treated, so if no seeds were on the surface, seed treatment was unknown.

Field type	# fields	Exposed seeds in center plots	Exposed seeds in corner plots	Exposed seeds outside plots	Spills
Corn treated	24	2	4	21	5
Corn untreated	1	0	0	0	0
Corn unknown if treated	26	0	0	0	0
Total (and %) corn fields	51	2 (4%)	4 (8%)	21 (41%)	5 (10%)
Soybean treated	9	5	8	8	4
Soybean untreated	8	6	8	8	1
Soybean unknown if treated	0	0	0	0	0
Total (and %) soybean fields	17	11 (65%)	16 (94%)	16 (94%)	5 (29%)
Wheat treated	1	1	0	0	1
Wheat untreated	1	0	1	1	1
Wheat unknown if treated	1	0	0	0	0
Total wheat fields ^a	3	1	1	1	2
Total (and %) all field types	71	14 (20%)	21 (30%)	38 (54%)	12 (17%)

^aDue to low numbers of sampled wheat fields, percentages are not provided.

Table 5. Summary of imidacloprid detections in domestic chicken blood and feces in each of 3 dose groups at University of Minnesota- College of Veterinary Medicine in 2015. Note that birds in the high dose group were euthanized early, which may have limited the ability to eliminate imidacloprid in feces.

	Dose (mg/kg/day)	N	Percent detects	Fold change	Median	Geometric mean	Minimum	Maximum
Blood (ng/ml)	1.04	6	20.0	4.2	1.7	1.4	0.5	2.1
	5.02	10	33.3	9.8	2.6	2.2	0.7	6.9
	20.80	8	61.5	2051.7	3270	805.6	4.2	8617
Feces (ng/g wet weight)	1.04	26	81.3	91.8	14.6	10.1	0.8	73.4
	5.02	39	97.5	278.9	19.1	14.1	0.7	195.2
	20.80	5	100.0	2.8	3.2	3.7	2.3	6.5

Table 6. Summary of tissue concentrations of imidacloprid in all laboratory-exposed domestic chickens for all dose groups combined at University of Minnesota- College of Veterinary Medicine in 2015.

Tissue	First detection (day)	Last detection (day)	Fold change	N	Percent detects	Min conc ^a	Max conc ^a	Median conc ^a	Geometric mean conc ^a	SD
Feces	1	21	279	70	90.9	0.7	195	14.6	11.3	35.9
Kidney	NA ^b	NA	1681	11	73.3	0.5	823	1.7	13.4	276.5
Liver	NA	NA	19882	11	73.3	0.3	5766	6.7	64.6	2473.6
Spleen	NA	NA	30413	11	73.3	0.2	6387	16.8	63.6	2320.8
Brain	NA	NA	10410	10	66.7	0.6	5725	1212.7	76.7	2295.8
Muscle	NA	NA	3469	10	66.7	0.8	2775	382.3	62.8	1128.5
Blood	1	8	17234	24	32.9	0.5	8617	4.1	14.1	2389.5

^a Conc = concentration (ng/g wet weight in tissues and ng/ml for blood).

^b NA = Not applicable because tissues were collected when chickens were killed the last day.

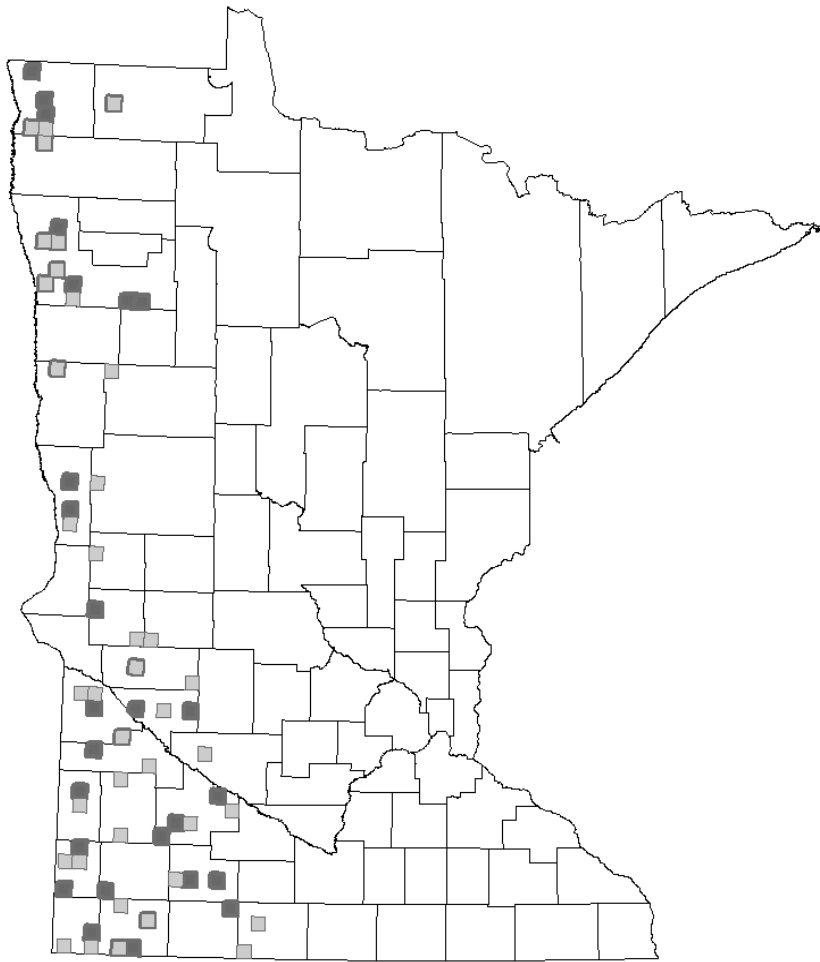


Figure 1a. Townships ($n = 76$) in Minnesota surveyed for seed spills during planting season in 2016 (dark gray), 2017 (light gray), and both years (light gray outlined with dark gray).

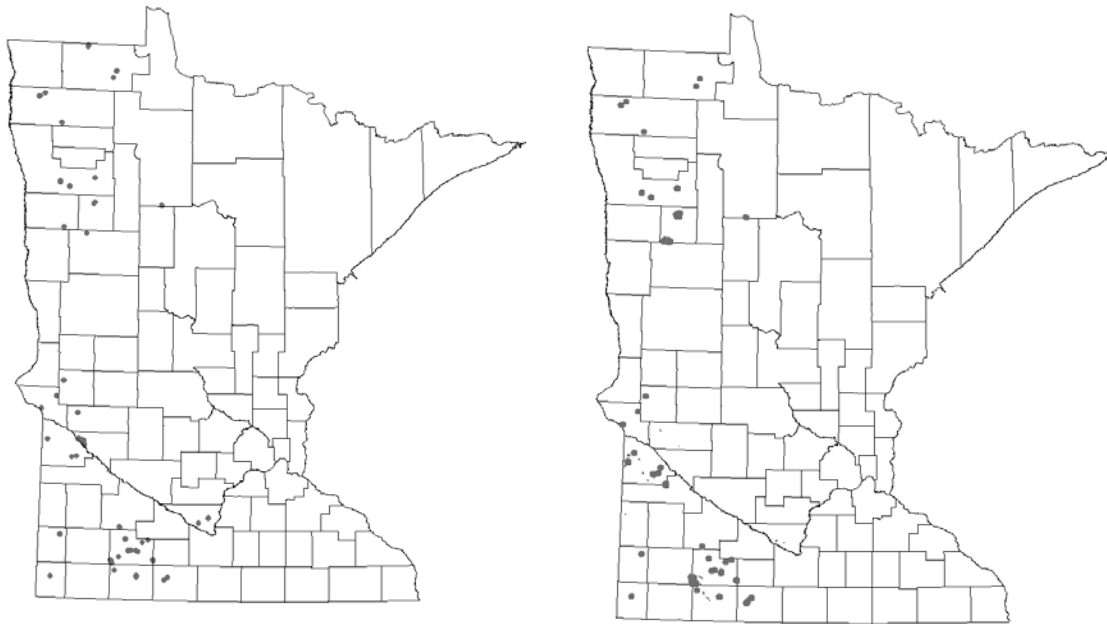


Figure 1b. Location of fields where seeds were measured on the soil surface after planting (left) and where cameras were placed at simulated spills (right) in Minnesota during 2016 and 2017. Fields are indicated as larger than their actual size to show their relative locations at a statewide scale; thus, some fields cannot be distinguished separately from other nearby fields (e.g., 17 fields on Lac Qui Parle Wildlife Management Area appear to be a single large site). Generally, the same sites were used, but some differences occurred related to the stage after planting during our visits and the ability to return to sites to remove cameras.

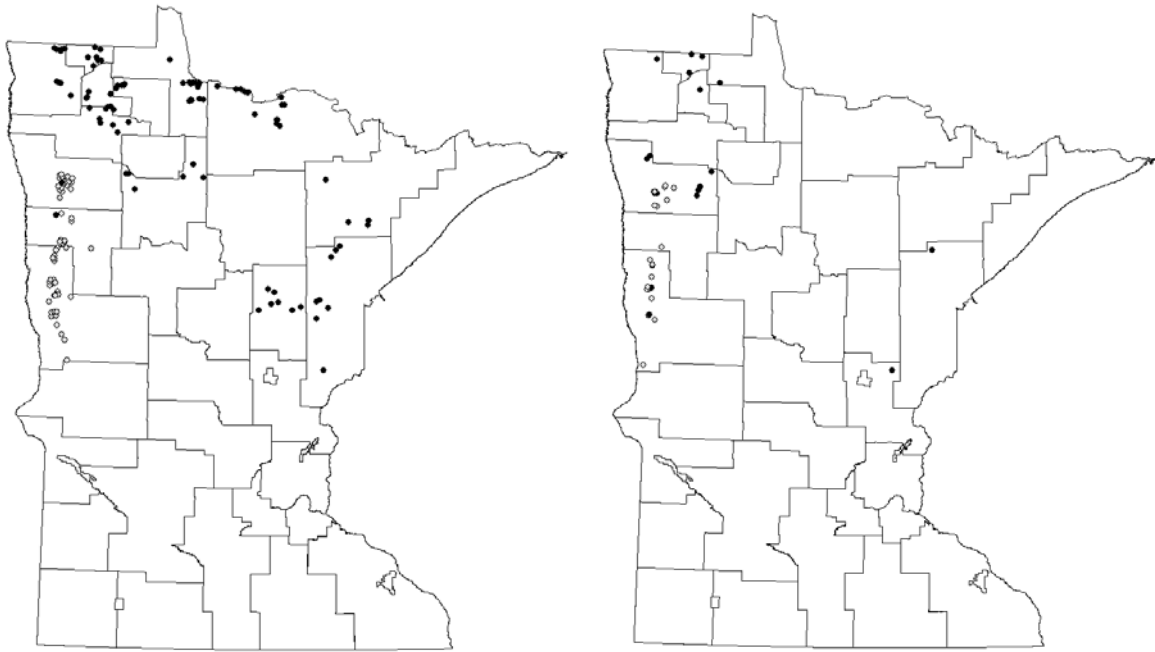


Figure 1c. Locations where sharp-tailed grouse (black) and greater prairie-chicken (gray) fecal pellet samples (left) and hunter-harvested birds (right) were collected in Minnesota during 2015, 2016, and 2017. No fecal pellet samples were collected during 2016.

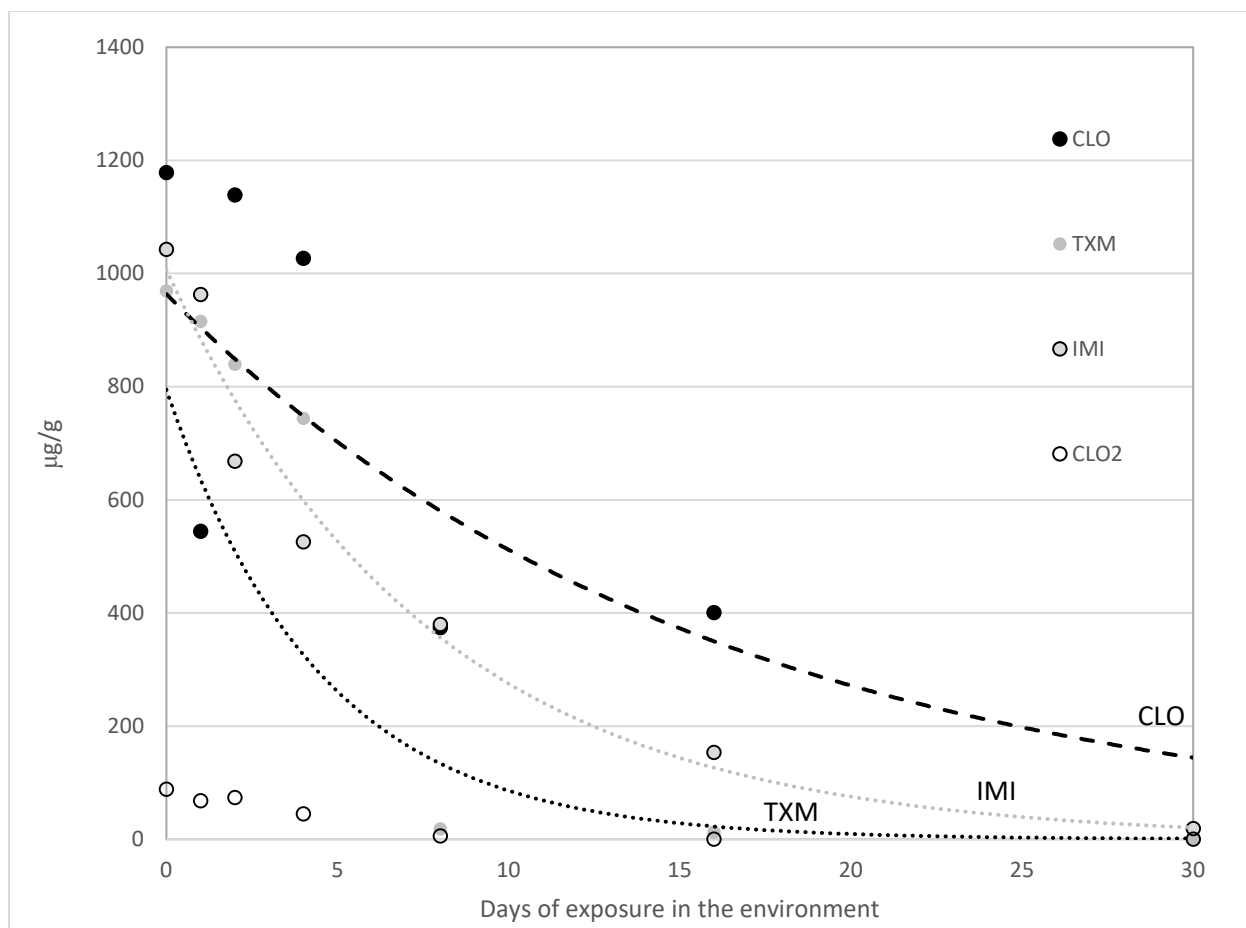


Figure 2. Concentrations of neonicotinoid seed treatments (Clothianidin -CLO, Imidacloprid -IMI, and Thiamethoxam -TXM) on corn and soybean seeds left on the soil surface for 0-30 days near Bemidji, Minnesota during 2016, according to an exponential decay model.

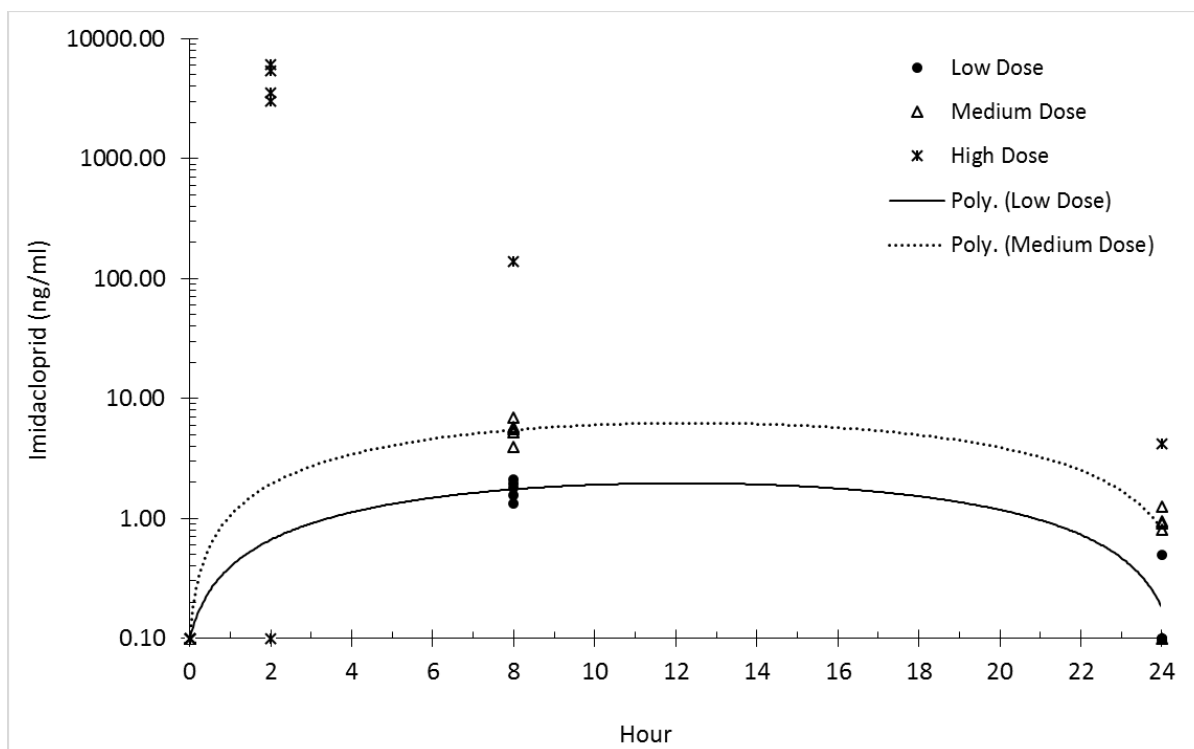


Figure 3. Changes in imidacloprid (IMI) concentrations in blood of dosed domestic chickens after 1 dose at the University of Minnesota - College of Veterinary Medicine in 2015. IMI doses were 1%, 5%, and 20% of a reported IMI LD_{50} for chickens (i.e., low, medium, and high dose groups, respectively). IMI detection limit is 0.10 or $-1.0 \log_{10}$ ng/ml in blood. Data points overlap when plotted on x-axis minimum value. A polynomial (Poly) trend line was fit for the low- and medium-dosed birds, but could not be fit to the data from high-dosed birds because chickens in this dose group were euthanized within 24 hours due to animal welfare concerns. Thus, the high dose group is not directly comparable to the other dose groups.

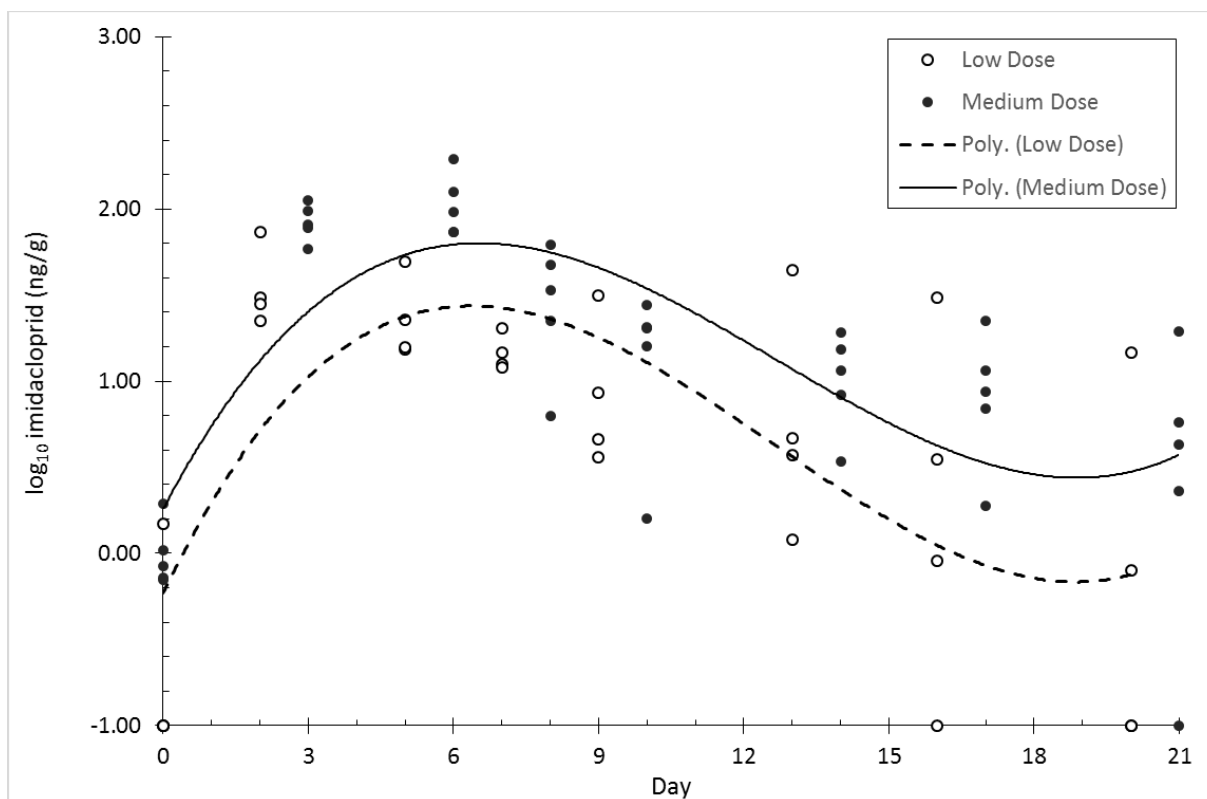


Figure 4. Changes in imidacloprid (IMI) concentrations in feces of dosed domestic chickens at University of Minnesota – College of Veterinary Medicine in 2015. Samples collected on day 0 were baseline samples, prior to exposure. Daily IMI dose for 7 days of 1% (low dose) and 5% (medium dose) of a reported IMI LD₅₀. The last day of dosing occurred on day 7 of the 21 day experiment. IMI detection limit is 0.10 or -1.0 log₁₀ ng/g in feces. The high dose group is not included because samples were collected only on day 0, so no temporal trends could be determined. Chickens in the high dose group were euthanized within 24 hrs after dosing due to animal welfare concerns. Thus, the high dose group is not directly comparable to the other dose groups. Polynomial (Poly) trend lines were fit to the data for the low and medium dose groups.

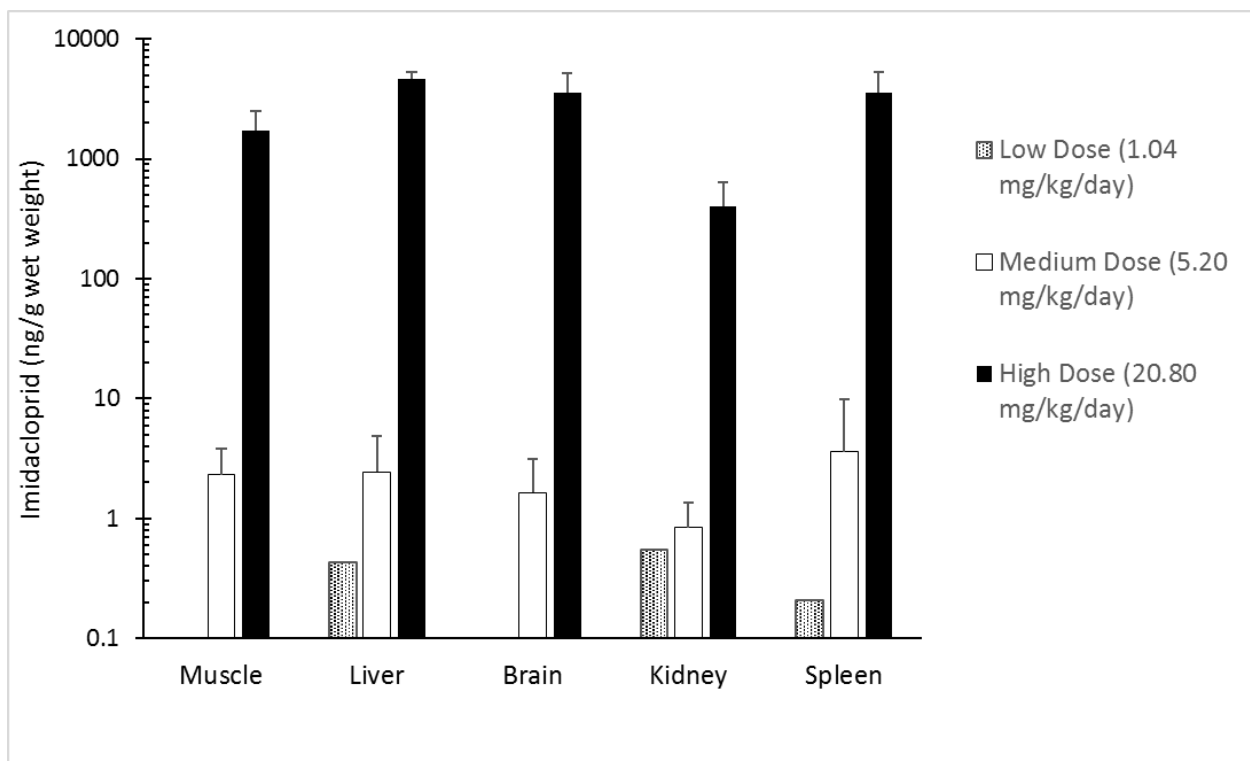


Figure 5. Concentrations of imidacloprid (geometric mean + SD ng/g wet tissue weight) in tissues of laboratory-exposed domestic chickens on experimental day 1 (high dose) or 21 (low and medium dose) at University of Minnesota - College of Veterinary Medicine in 2015. Data at the detection limit of 0.10 ng/g are not visible. Error bars represent the standard deviation of observations for a given group. No error bars are provided for the low dose group because bars represent only 1 individual with detectable concentrations.



EVALUATION OF DESIGN AND ANALYSIS OF A CAMERA-BASED MULTI-SPECIES OCCUPANCY SURVEY OF CARNIVORES IN MINNESOTA

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SUMMARY OF FINDINGS

Camera-based surveys are increasingly being used to monitor wildlife species across large areas and a diverse range of habitats. We initiated a study in a forested area of northern Minnesota to assess various design and analysis questions related to use of remotely-triggered cameras for simultaneously monitoring the occurrence of multiple species of carnivores. Starting in spring 2016, we deployed 100 cameras twice a year (spring and fall) in an area equivalent to 20 townships, with 5 cameras placed in each 9.65- x 9.65-km township. To test different lures and strategies for camera placement, we conducted a 2 x 2 factorial experiment following a randomized complete block design. Four cameras were placed at randomly selected locations within forested areas, and were assigned one of 2 lures (salmon oil or a liquid synthetic fatty-acid scent [FAS] oil) and one of two different placement strategies (on the closest suitable tree within 5 m from the randomly selected point, or at a user-chosen location within 90 m of the randomly selected point). We deployed an additional camera, without a lure, on a secondary road or trail within a forested area of each township. All cameras were active for a minimum of 6 weeks; cameras recorded more than 1,900,000 photos spread across 4 sampling sessions totalling 19,244 active trap-nights. The number of sites at which carnivore species were detected and the number of pictures taken varied greatly among seasons and by species. Visual inspection of preliminary data from fall 2016 and spring 2017 suggests that coyotes (*Canis latrans*), fishers (*Pekania pennanti*), and raccoons (*Procyon lotor*) preferred salmon oil over the FAS oil, whereas results were less conclusive for other species (e.g., bobcats (*Lynx rufus*)). Black bears (*Ursus americanus*), gray foxes (*Urocyon cinereoargenteus*), martens (*Martes americana*), and striped skunks (*Mephitis mephitis*) showed opposite preferences in these two sessions; pooling the data from these two sampling periods together indicates that bears may prefer FAS oil, whereas gray foxes, martens, and skunks may have a slight preference for salmon oil. Differences in detection rates were minimal for the two different random placement strategies (i.e., within 5m or 90m of a random point), whereas there were large differences in detection rates between randomly placed lured sites and unlured trail sites. In particular, we detected black bears, fishers, martens, and raccoons more often at lured, randomly-selected sites compared to unlured trails, whereas wolves (*C. lupus*), skunks, and red foxes (*Vulpes vulpes*) were more often detected at unlured trail sites. We also frequently detected several non-carnivore species, including white-tailed deer (*Odocoileus virginianus*), red squirrels (*Tamiasciurus hudsonicus*), snowshoe hares (*Lepus americanus*), and, more rarely, porcupines (*Erethizon dorsatum*) and moose (*Alces alces*). More detailed analysis of the data is pending.

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INTRODUCTION

Monitoring programs designed to track the distribution and actual or relative abundance of carnivores can be important for determining population status and for quantifying the effects of harvest, habitat change, and environmental variability on populations. The Minnesota Department of Natural Resources (MNDNR) currently relies on 2 track-based surveys (scent station and snow-track surveys) to monitor trends in a suite of 14 carnivores/furbearers. The data from these surveys have provided rough estimates of trend for many species, although interpretation must always be qualified with acknowledgement of 2 key, but untested, assumptions, namely that detection rates do not exhibit significant temporal or spatial trends and that road-based surveys adequately represent population-wide trends. Logistical challenges with conducting these surveys have also increased in the last decade due to loss of survey collaborators from other natural resource agencies, increased traffic or paving/plowing of roads, and less reliable snow in early winter. In the past decade, several key carnivore species had declined (e.g., fishers, martens, bears) and management intensity had increased on wolves. Given the importance of monitoring these species, statistical uncertainties with existing surveys, and increasing logistical challenges, we felt it was an opportune time to consider alternative ways to monitor carnivore populations. Camera surveys are an attractive option because they provide a means to estimate detection rates with little if any additional field effort, are less dependent on specific environmental conditions, and are more amenable to use of 'citizen scientists' with little formal training (photos can be verified by trained staff). Thus, remote cameras are increasingly being used or considered for large-scale multi-species occupancy surveys (e.g., O'Brien et al. 2010, Pettorelli et al. 2010, Ahumada et al. 2011, Kays et al. 2011, Fisher and Burton 2012).

Camera-based surveys are not new to wildlife monitoring (Kays and Slauson 2008, Kucera and Barrett 2011), but the simultaneous development of improved remotely-triggered cameras, rigorous analytical methods, and reduced costs have bolstered their applied value. As evidenced by their use in monitoring a wide array of carnivores in different landscapes (e.g., see Table 5.1 in Kays and Slauson 2008), cameras are a non-invasive tool well-suited to detect species that may be difficult to trap and handle, occur at low densities, or have nocturnal and secretive habits.

Occupancy models (*sensu* MacKenzie et al. 2002, MacKenzie et al. 2006) are commonly used in wildlife monitoring programs, often in conjunction with camera traps, due to their flexibility, sound statistical framework, and close connection to population estimation. Taking advantage of repeated sampling (in space or time), occupancy models can provide unbiased estimates of occupancy probabilities that adjust for imperfect detection (i.e., failure to detect a species when it is present in a certain area). Failing to account for imperfect detection can lead to misleading estimates of spatial and temporal trends in occurrence (Guillera-Arroita et al. 2014a), and as a result, poor management and conservation decisions. While there are several important assumptions that must be met to apply occupancy models, the approach is not dependent on a specific tool or method to detect animals.

General survey design guidance for occupancy surveys is available (e.g., MacKenzie and Royle 2005, MacKenzie et al. 2006, Bailey et al. 2007, Guillera-Arroita and Lahoz-Monfort 2012, Guillera-Arroita et al. 2014b), but ideally study designs should be tailored to features of the target species and study area to avoid violation of model assumptions (e.g., independent detections and constant occupancy status), which can lead to biased estimators of detection and occupancy rates or require complex modelling approaches for sound statistical inference. Not surprisingly, occupancy modelling is an emerging and fast-moving field, and we expect new methods to be developed and guidance on their use to continually evolve in the coming years (Rota et al, 2016; Broms et al, 2016; Tobler et al, 2015; Ovaskainen et al, 2016).

Implementing a camera-based occupancy survey requires consideration of a variety of design and analysis options. While we do not delve into the details of each here, we

highlight the following considerations: 1) camera selection and settings (Swann et al. 2004, Kays and Slauson 2008, Damm et al. 2010, Swann et al. 2011, Meek et al. 2012, Rovero et al. 2013, Weingarh et al. 2013, Wellington et al. 2013); 2) camera positioning; 3) whether to use baits/lures, and if so, which ones (Kays and Slauson 2008, Schlexer 2008, Du Preez et al. 2014); 4) time of year, which can affect species' behavior and 'availability' as well as likelihood of meeting methodological assumptions (e.g., Kendall and White 2009, Rota et al. 2009); 5) number of cameras; 6) camera spacing and consideration of spatial correlation among sites (e.g., Sargeant et al. 2005, Hines et al. 2010, Magoun et al. 2010, Aing et al. 2011, Guillera-Arroita et al. 2011, Dorazio and Rodriguez 2012, Johnson et al. 2013); 7) whether or how best to discretize (e.g., hours, days, weeks) the temporally-continuous data from cameras into multiple survey occasions (e.g., Guillera-Arroita et al. 2011, Bischof et al. 2014); 8) site selection (e.g., random, systematic, convenience) and whether to allow flexibility in micro-site selection; and 9) approach to data analysis (e.g., single-species versus hierarchical community models; Dorazio and Royle 2005, Dorazio et al. 2006, Kery and Royle 2008, Zipkin et al. 2009, 2010, 2012, Giovanini et al. 2013, Pacifici et al. 2014).

Optimizing survey design becomes more complicated when multiple species with varying abundance and detection rates are involved. Biological characteristics of the species, such as home range size, movement patterns, and habitat preferences show large variation among carnivores (Boitani and Powell 2012). Consequently, a sampling design optimal for one species can violate important model assumptions for another. In the case of MNDNR surveys, where the suite of target species ranges from small to medium-sized mammals, such as skunks and martens, to large, roaming species like wolves and bears, design and analysis options that best account for or address this variability will be preferred. Recent attention has been given to design of camera-based occupancy surveys targeting a community of carnivores (Hamel et al. 2013, Shannon et al. 2014), but their conclusions may not extend beyond the specifics of the biological system and analysis approaches they considered.

OBJECTIVES

The broad objectives of this project are to:

1. Compare effects of various survey design and analysis options on the magnitude and precision of estimates of detection and occupancy rate for multiple species.
2. Assess possible logistical constraints on implementing a large-scale multi-species camera survey in Minnesota; and
3. Compare the efficacy of camera surveys to the track surveys currently being used for monitoring carnivores in Minnesota.

As noted above, there is a large array of design and analysis questions to consider when conducting a multi-species occupancy survey with cameras. Hence, we decided to use an adaptive approach to survey design, focusing efforts on 4 specific design questions: 1) timing (spring versus fall survey; survey duration); 2) lure options (salmon oil versus synthetic fatty acid scent oil); 3) site selection (cameras on trails versus randomly selected sites); and 4) strategies for camera deployment (enhanced placement versus not enhanced). Our approach to analysis will also consider the effects of using daily versus weekly survey intervals and single- versus multi-species occupancy models.

STUDY AREA

Starting in spring 2016, we implemented the first camera survey in one study area located in Itasca County, north-eastern Minnesota (Figure 1). This 1872 km² (48 x 39 km) area is mainly covered by forests and lakes and includes a high percentage of public land, including a portion of the Chippewa National Forest (SW portion of the study area), George Washington State Forest (NE portion), Scenic State Park (NC portion) and other state and county lands interspersed throughout.

METHODS

Based on our minimum camera specifications (i.e., passive infrared (PIR) cameras with intermediate to fast trigger (<0.7 s) and recovery (<1.7 s) speeds, multi-picture capability (minimum 3) per trigger event, “no-glow” (black LED) infrared flash, and of moderate cost (maximum \$200 per camera)) and a competitive bid process, the camera model we deployed was the Bushnell Trophy Cam HD Aggressor No-Glow.

Survey timing and duration

We considered 4 objectives in selecting the timing of our camera surveys: 1) maximize the species richness of carnivores that would be ‘available’ for detection; 2) minimize the likelihood of violating the occupancy model assumption of species’ closure during the survey; 3) minimize logistic challenges with deploying cameras; and 4) maximize ‘biological relevancy’ and consistency with timing of existing surveys and annual management decisions. Although our experience has been that winter is a good time to conduct lure-based camera surveys for many carnivores, we concluded that several species would be undetectable (e.g., bears, skunks), ongoing harvest seasons for many species would increase risk of violating closure assumptions, and deep snow could pose logistic challenges. Although summer was a potential option, we believed that more rapid desiccation of lures and rapidly changing ‘availability’ of maturing offspring made it a less desirable option than spring and fall surveys. Hence, we chose to compare camera-based surveys conducted in the spring and fall, presumably reflecting spring ‘pre-breeding’ and fall ‘pre-harvest’ populations.

Our previous experience had been that few additional species are detected after 3–4 weeks of camera deployment. Although cameras can be left out indefinitely with only minimal additional financial cost related to personnel to review photos, long surveys increase risk of violating closure assumptions through mortality, immigration, or emigration. Hence, we chose to deploy cameras for 6 weeks during the first year, specifically May 1 to June 15 and September 1 to October 15. To reduce the occurrence of false triggers due to the growing vegetation or the interaction between insolation and lack of canopy cover (see below), in 2017 and 2018 we delayed the starting of the spring session until mid-May.

Lure Selection

We concluded that use of a bait or lure was likely necessary to produce sufficient detection probability for many carnivore species, especially if cameras are to be deployed using a more desirable probabilistic sampling scheme. Similar to conclusions by Fisher and Burton (2012), we believed that olfactory lures will be preferred over baits and that all species of interest in this study can likely be attracted, albeit to varying degrees, with a more logistically-practical olfactory lure.

We decided to test 2 lures the first year, limiting our consideration to attractants that were likely to be not only effective for a suite of carnivore species, but also ones that could be reasonably standardized and were expected to be commercially available into the foreseeable future, easily applied, resistant to variable weather conditions, and could be purchased and distributed without significant secondary processing. There was a vast array of potential lures to consider. Based on our goals, personal experience, examination of the literature (e.g., Schlexer 2008), and consultation with a trapping lure manufacturer, we chose to compare commercial salmon oil with a liquid version of the synthetic fatty acid scent (FAS) that has been used (in tablet form) on a long-term multi-species track survey in Minnesota (Erb 2015). Details of the lure placement protocol are discussed below; here we simply note that at each site selected for salmon oil, we deployed 473 ml (16 oz), whereas for sites selected for FAS oil, we deployed a 237 ml (8 oz) bottle that consisted of 80% mineral oil and 20% liquid FAS.

Macro-site selection

To identify forested locations for camera deployment, we used Light Detection and Ranging (LiDAR) data (e.g., see Merrick et al. 2013) collected by the State of Minnesota in 2011 (<http://www.mngeo.state.mn.us/chouse/elevation/lidar.html>) to identify pixels (~ 20 X 20 m) with mean tree height >3 m (10 ft) and canopy cover >50% (Figure 2). We divided the study area into 20 contiguous blocks the size of townships (9.65 x 9.65 km). To ensure a minimum distance of 1.6 km (1 mi) between cameras both within and across blocks, we constrained the randomly selected points to lie within 4 equally-spaced sub-quadrats within each block (Figure 2). We then intersected the desired forest locations (pixels) identified via LiDAR with the sub-quadrats and used the *Generate Random Points* tool in ArcGIS to select one random point falling within each of the 4 sub-quadrats in each block (Figure 2).

In addition, we deployed an un-lured camera placed on a secondary trail closest to the center of each township (hereafter, *trail camera*), provided the site was at least 400 m (0.25 mi) from all primary roads and at least 1.6 km (1 mi) from other cameras (Figure 2). We loosely defined secondary roads or trails as those that did not receive year-around maintenance and were accessed primarily on foot or with off-road vehicles. Our primary intent in deploying un-lured cameras along trails was to assess whether this type of convenience sampling was more likely to detect larger carnivores, such as wolves, that often use these trails and may be more wary of lured sites.

After selecting all locations and before deploying the cameras, each site was visualized on 2015 aerial photos to help ensure all requirements for deployment were likely met, including an additional requirement that each site was a minimum of 30 m (100 ft) from any non-forested edge. If a selected site later became unavailable (e.g., site was logged between sessions), a new location was chosen as close as possible to the previous site and in a similar forest type when possible.

Micro-site selection and covariates

Another important decision, after selecting the camera macro-sites, was how much flexibility should be allowed in determining the exact placement location of the camera. Although the use of lures effectively expands the area of camera 'coverage' well beyond the actual camera, within a given forest patch one can still potentially locate a microsite where the probability of carnivore use or detection will be higher. However, allowing flexibility in micro-site selection could introduce a source of heterogeneity in detection probabilities that may be difficult to quantify objectively. Using experienced biologists, we decided to test whether expert-based choices in fact increase detection rates. We accomplished this by dividing lured cameras into 2 camera placement strategies: 1) *not enhanced*, meaning the camera was placed on a tree within a 5-m (15-ft) radius from the randomly selected point; or 2) *enhanced*, meaning the operator actively looked for an optimal deployment location within a 90-m (300-ft) radius of the randomly selected point. For unlured trail sites, we allowed flexibility in the final deployment location of cameras due to the need to position the camera on a tree at the desired angle to the trail and within sufficient distance of the trail to ensure trigger activation by animals; from the original coordinate, users were allowed a distance of 45 m (150 ft) in either direction down the trail to place the camera.

At all camera stations, we recorded several vegetation characteristics (tree species diameter and dominance, shrub cover, canopy cover) and presence of game trails, natural 'bottlenecks', and other features within approximately 15 m of the final deployment location that could increase probability of detecting a carnivore. We also took a digital photo of angular (45°) canopy cover in 4 directions around the base of the camera tree, parallel and perpendicular to the camera-lure axis. While walking to each camera site (usually < 3 km), we also recorded presence of indirect carnivore sign (tracks, scats, dens). For trail cameras, we recorded trail width, ease of access (e.g., walk, ATV, vehicle), an initial index of frequency of use by humans (which we will corroborate based on human-detections by the cameras), and vegetative coverage and height on the trail surface. Other variables (e.g.,

distance to main roads or water, landscape configuration metrics) will be measured using GIS.

Experimental design

To test different lures and placement strategies, we conducted a 2 x 2 factorial experiment following a randomized complete block design. Along with the trail camera, 4 lured cameras were placed within each block at sites selected using the processes described above in the macro- and micro-site selection sections. Cameras at each randomly chosen site were randomly assigned 1 of 2 lure types (salmon oil or fatty acid scent oil) and 1 of 2 camera placement strategies (not enhanced or enhanced, Figure 3). During the second year of sampling, protocols remained the same with the exception that we employed a crossover design with respect to lure choice (i.e., a site with salmon oil in 2016 received a FAS lure in 2017).

Camera deployment and settings

In each camera session, we deployed 100 passive infrared Bushnell Trophy Cam HD Aggressor No-Glow cameras, 80 at lured sites and 20 at un-lured trail sites. The general settings for all the cameras were based on pre-deployment testing. All cameras were attached to sturdy trees with bungee straps and placed about 75 cm (30 in) above the ground. The detection area in front of the cameras was cleared of vegetation (ferns, branches, leaves) that could obstruct the viewing area or cause false triggers, especially on windy days. At lured sites, we poured the lure on a tree located 4.5 to 9 m (15 to 30 ft) from the camera tree, with a preferred distance of 6 to 7.5 m (20 to 25 ft). We aimed trail cameras at a 45° angle to the main axis of the trail to ensure more opportunity to capture images of faster moving animals. We also aimed all cameras north (ranging from northeast to northwest) when possible to reduce false triggers and blurred photos from direct sunlight. All the cameras were programmed to record 3 mega-pixel images (color during daylight and black/white during night), with 3 'rapid-fire' pictures per trigger event and a 2-second delay between subsequent triggers. Additionally, a set of 3 rapid-fire time-lapse pictures were taken twice a day (noon and midnight) to check the functioning of the cameras and to record regular measures of daily temperature at each site. Date, time, temperature and camera ID were printed on all the images and recorded in the image metadata.

Photo processing and analysis: Following the protocol described in Niedballa et al. 2016, we are using the open access photo manager software Digikam © to record information on the carnivore species detected, number of individuals, and other species-specific features (e.g., presence of bear cubs, or aggressive behavior towards the camera) in the EXIF metadata of each picture. In addition, we are also annotating information that might be important to model the presence and detection of carnivore species, such as detection of prey (deer and deer fawns, snowshoe hares and squirrels) or occurrence of humans and human related activities (e.g., hunting, ATVs, dog-walking). This information, along with date and time of each detection event, are then extracted and analysed using the *camtrapR* package (Niedballa et al. 2017) in Program R (R Core Team 2015).

For each species, we compared the proportion of sites with at least one detection for: a) different lure treatments (salmon vs fatty acid scented oil); b) small-scale deployment strategy (enhanced vs not enhanced); and c) large-scale deployment strategy (on trail vs random site selection). In future stages of the analysis process, we will tackle the same questions using more robust statistical methodologies (e.g., mixed effect models), and modelling occurrence and detection probabilities as functions of landscape features (e.g. bottlenecks, game trails) and forest characteristics (e.g. forest type, shrub cover) to provide information on species distribution and detectability. In addition, we will calculate cumulative species richness curves to address questions related to survey duration and timing. Further details of analysis methods will be presented in future reports.

RESULTS AND DISCUSSION

Camera Function

During the first 2 years of sampling, we recorded more than 1,900,000 pictures (Spring 2016: ~680,000 pictures; Fall 2016: ~370,000; Spring 2017: ~470,000; Fall 2017: ~385,000), across a total of 19,244 active trap-nights (Spring 2016: 4471 ; Fall 2016: 4789; Spring 2017: 5101; Fall 2017: 4883). In spring 2016, 75 of the 100 cameras deployed remained operational for the full session; one was missing (site was logged), 4 malfunctioned, and bears altered camera positioning on approximately 20 cameras, though only 9 of these were moved to an extent that the lure tree was no longer visible. Insolation due to lack of canopy cover during the first weeks of the spring survey along with growing vegetation (especially ferns) in the later weeks resulted in a large number of false triggers and, in some cases, cameras that were no longer operable (e.g., when growing vegetation filled the detection area). In fall 2016, 93 of the 100 cameras remained operational; canopy cover appeared to reduce sunlight-driven false triggering, all ground vegetation had sprouted and could be cut, and we added a second strap to secure the cameras and minimize bear disturbance to cameras. Bears were still the main reason for cameras becoming inoperable in the fall (5 out of 7), and the reduced number of bear-related problems could also be due to a decrease in the number of bear visits in the fall. However, the addition of a second strap seemed to be effective in reducing bear disturbance to cameras, decreasing the number of false triggers in the subsequent sampling sessions (including spring 2017, despite the higher number of bear detection events during spring than fall).

In addition, to partially avoid false triggers in spring 2017 and the current spring survey (2018), we decided to postpone the beginning of the sampling period for two weeks (from 1 May to 15 May) with the hope of allowing initial canopy growth (more shading) and initial growth of lower-growing herbaceous vegetation. The later start date allowed us to trim more emerging vegetation in the detection area at the time of camera deployment and reduced the number of false triggers caused by insolation and growing understory vegetation. Although reducing camera trigger sensitivity may also reduce false triggers, we were more concerned about potential loss of animal detections from reduced sensitivity.

Species Detections

The number of sites at which species were detected and the number of pictures taken varied greatly among sessions and by species. Black bears and bobcats were detected at a larger number of sites during the spring sessions compared to fall, whereas fishers and gray and red foxes were detected at more sites in the fall (Figure 4A). Coyotes, martens, and striped skunks were detected at ≥ 2 times as many sites during the fall 2016 and spring of 2017 compared to spring 2016 (Figure 4A). For gray wolves and raccoons, the number of sites with ≥ 1 detection increased from spring 2016 to fall 2016 to spring 2017 (Figure 4A). Badgers (*Taxidea taxus*) were detected at 4 and 2 sites during fall 2016 and spring 2017, respectively, whereas weasels were detected only at one site in fall 2016. We also frequently detected white-tailed deer, red squirrels, snowshoe hares, and on occasion, porcupines, moose, and several species of birds.

The number of pictures per species differed between spring and fall sessions, with higher numbers during fall for many of the species (bobcat, fisher, marten, skunk, and gray fox; Figure 4B). In spring 2017, we recorded an extremely high number of pictures of bears (Figure 4B). The number of pictures recorded at a site is heavily influenced by animal behavior, or the amount of time an individual spends in the detection area in front of the camera. We are currently exploring ways to quantify temporal dependence patterns in these data so that we can better interpret this metric (number of pictures) or develop alternative metrics that are less sensitive to changes in animal behavior.

Given the number of issues we ran into during spring 2016, we concentrate our summaries here on data from the fall 2016 and spring 2017 surveys. Fall cameras were active from

approximately September 1 to November 2, for a total of 4,789 'trap-nights' ($\bar{x} = 48$, $SD = 11$ trap-nights per camera). Most ($n=60$) cameras detected 1–3 carnivore species (1 species, $n=21$; 2 species, $n=20$; 3 species, $n=19$); the maximum number of species detected was 7 (Figure 5A). The spring 2017 session lasted from May 16 to July 13; cameras were operable for a total of 5,101 active trap-nights ($\bar{x} = 51$, $SD = 11$ trap-nights per camera). We detected 2–4 species at most of the sites ($n=70$; 2 species at 21 sites; 3 at 21; and 4 at 28) with a maximum of 6 carnivore species at 6 locations (Figure 5B).

Comparison of lures and site-selection strategies

The use of lures increased the time most carnivore species spent in the detection area in front of the camera (Figure 6). This, in turn, increased the number of pictures collected for each detection event, facilitating species identification, especially in challenging situations such as night-time pictures or for species of similar shape and body size (e.g., coyote-wolf, marten-fisher). Differences in visit duration between lure types, however, were small.

Across seasons (i.e., fall 2016 and spring 2017), only coyotes, fishers, and raccoons appeared to show consistent lure preferences, with visitation higher at salmon oil sites. Gray wolves, red foxes, and bobcats did not appear to show any lure preference in either season (Figure 7A). Black bears, gray foxes, martens, and striped skunks showed opposite inclinations in the two sessions; pooling the data from the two sessions suggests a slight preference for FAS oil by bears and a slight to moderate preference for salmon oil for gray foxes, martens, and striped skunks (Figure 7A, bottom row).

Differences between micro-site deployment strategies (enhanced vs not enhanced) were small. Two conspicuous exceptions were gray wolves in fall 2016 and gray foxes in spring 2017; both canids were detected more often at sites with cameras deployed using the non-enhanced placement strategy, though these observations did not hold for either species in both sessions (Figure 7B). Pooling sessions, there are indications that gray wolves, gray foxes, raccoons, and martens may have slightly preferred non-enhanced locations, whereas fishers may have slightly preferred enhanced locations (Figure 7B, bottom row).

Macro-site selection strategies resulted in strong differences in detection rates at unlured on-trail sites versus lured random sites for some species (Figure 8). In particular, black bears, fishers, martens, and raccoons were consistently (i.e., both sessions) more often detected at lured, randomly-selected sites compared to unlured trails. Conversely, wolves, skunks, red foxes, and to lesser degree, coyotes, were consistently detected more often at unlured trail sites (Figure 8). Gray fox detections were not consistent across sessions, but pooled data suggests preference for unlured trail sites as well.

A sample of the pictures collected during spring 2016 session is shown in Figure 9. Although many preliminary findings are generally consistent with expectations, more complete and formal analyses will be conducted and presented in future reports.

ACKNOWLEDGMENTS

Special thanks to Barry Sampson and Carolin Humpal for assistance with preparing, programming, and deploying cameras. We would also like to thank Michael Joyce for his assistance with processing and analysis of LiDAR data, and Jordan Schaefer, Perry Loegering, Mark Spoden, Jeff Hines, and Kevin Carlisle for logistical support during camera deployment. This project was funded in part by the Wildlife Restoration Program (Pittman-Robertson).

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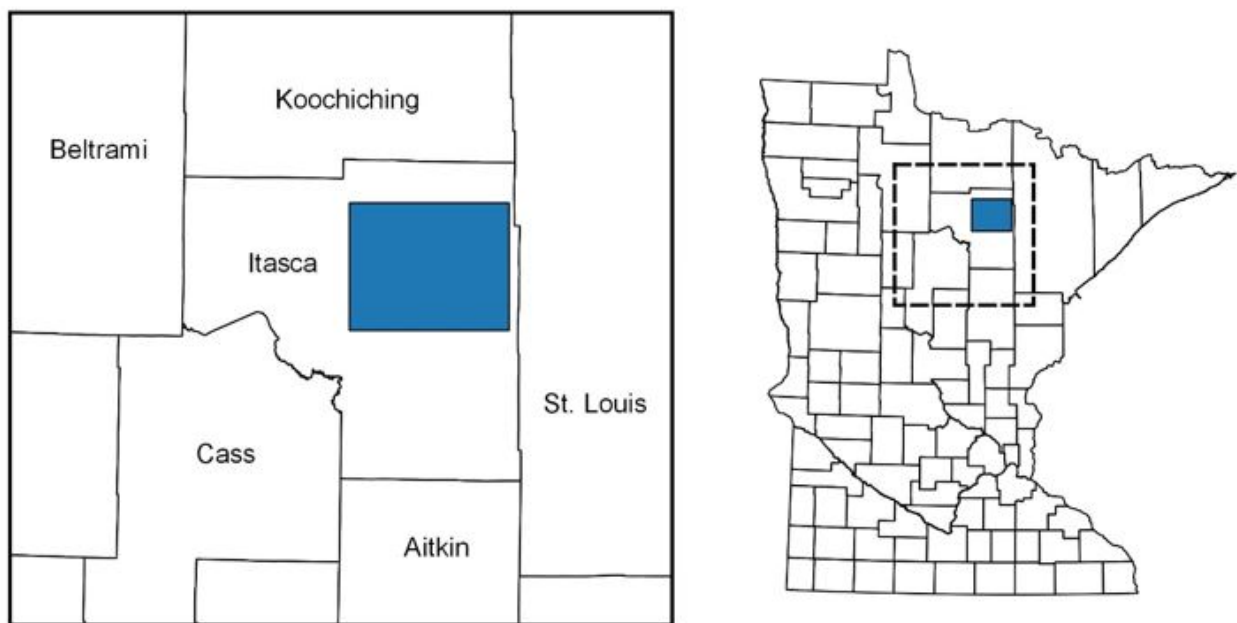
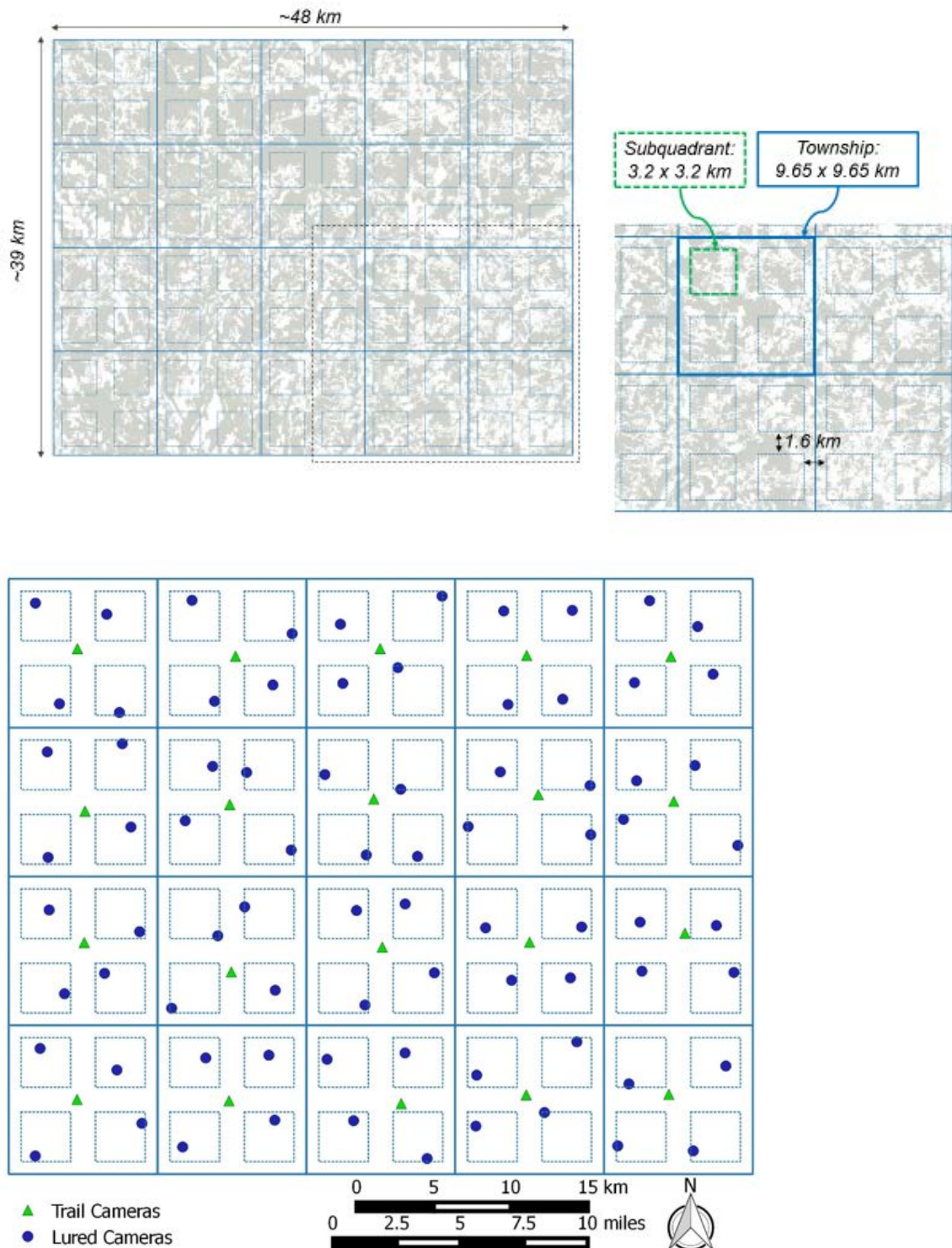


Figure 1. Location of the 2016-2018 carnivore camera survey in the northeastern portion of Itasca County, Minnesota.



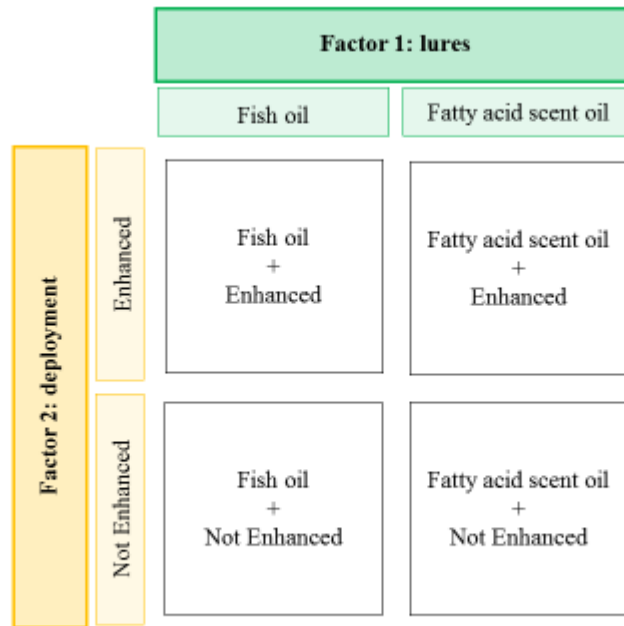
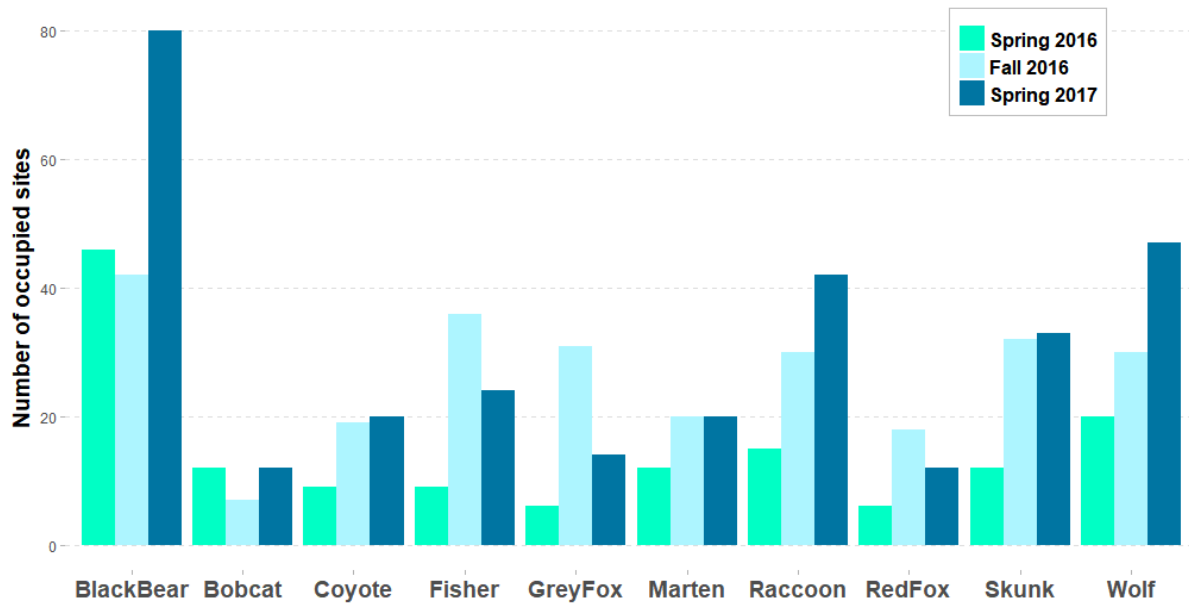


Figure 3. Factorial sampling design, 2016-2018. In each of 20 townships in Itasca County, MN, 4 cameras were randomly assigned to one of 4 different treatments given by the intersection between two factors: lure type and small-scale camera deployment strategy. The lure factor had 2 levels: *fatty acid scent oil* (FAS) and *fish oil* (salmon oil) and we used a crossover design (i.e., lures were switched) in the second year; the second factor, small-scale camera deployment strategy, also had 2 levels: *not enhanced* (i.e., camera placed on nearest tree to the randomly selected UTM location) and *enhanced* (i.e., camera placed at a presumably optimal location within 90 m of the randomly selected point to increase carnivore detection).

A)



B)

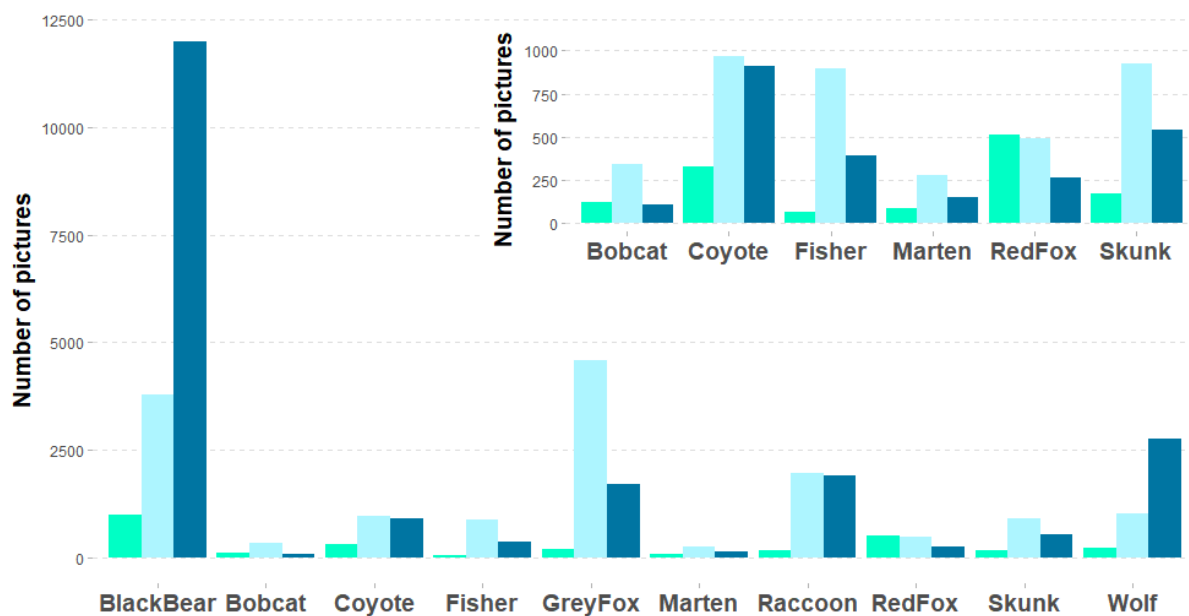
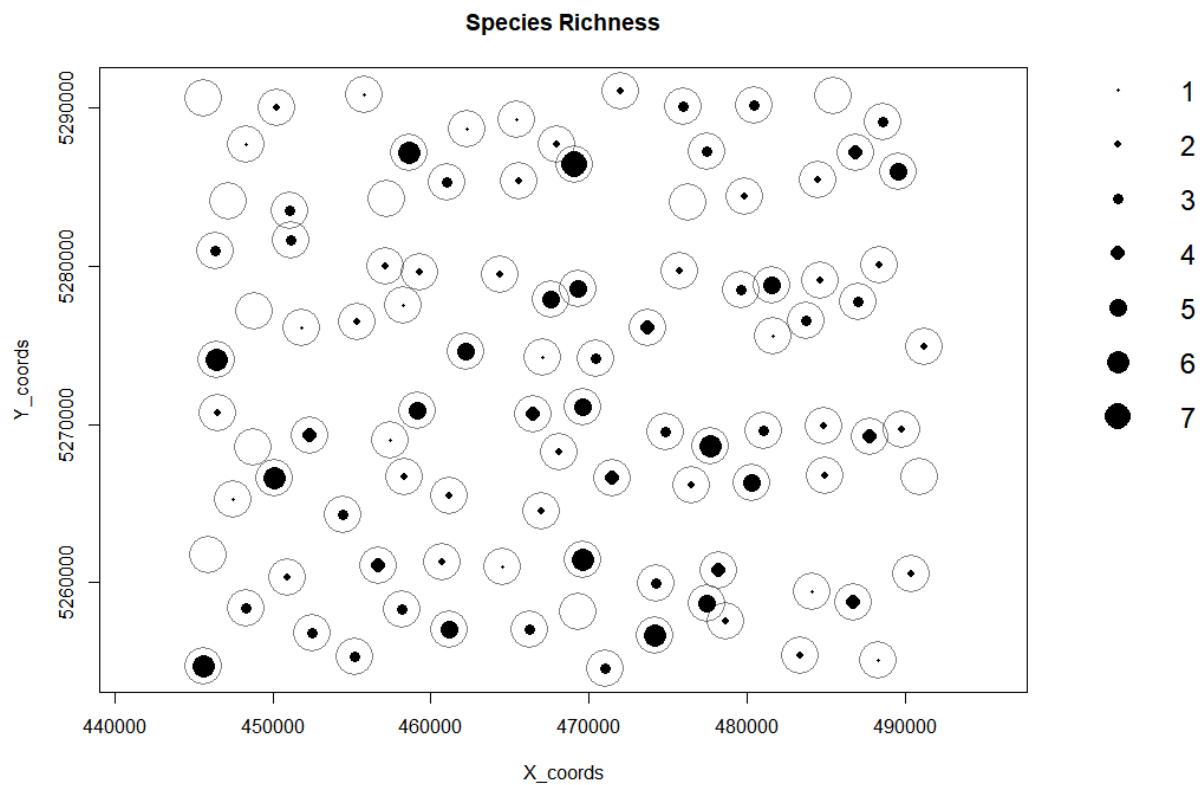


Figure 4. (A) Number of sites at which each species was detected during spring 2016 (green; 4,471 active trap-nights), fall 2016 (turquoise; 4,789 active trap-nights), and spring 2017 (blue; 5,101 active trap-nights) of this Minnesota study. (B) Number of pictures per species during the three sampling sessions; the inset (top-right) expands the y axis for species with <1,000 pictures per session.

A)



B)

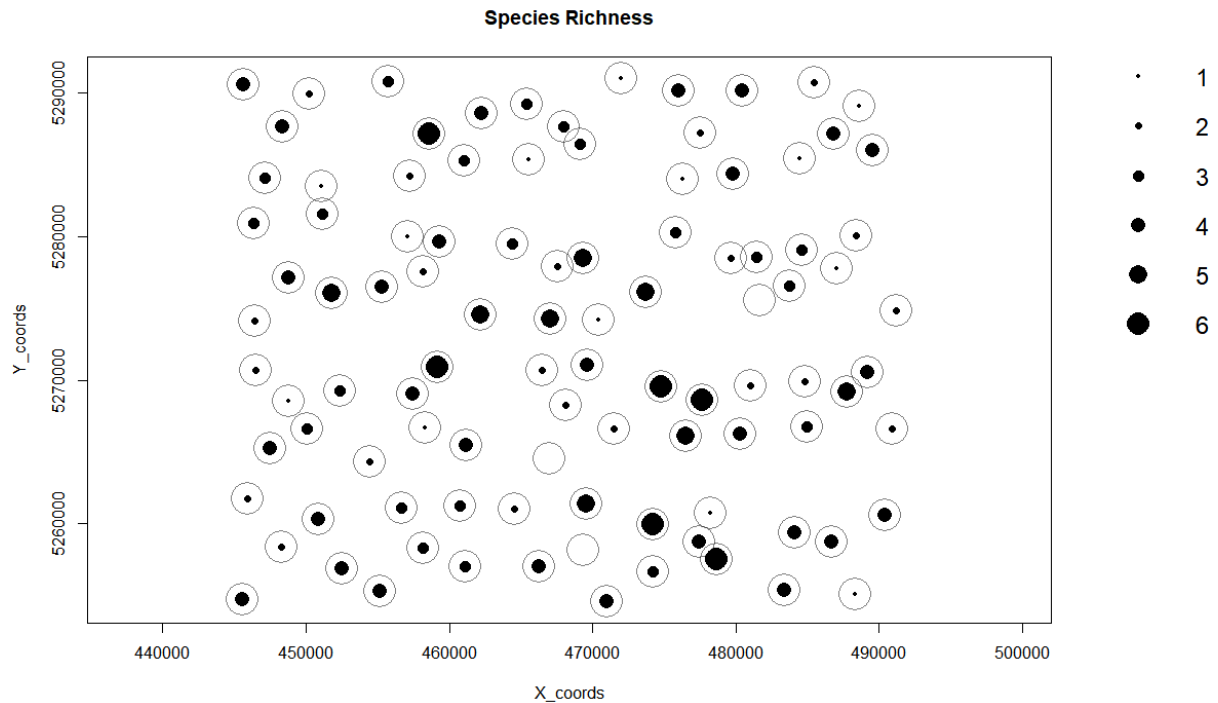


Figure 5. Species richness at each camera location during A) fall 2016 and B) Spring 2017, at 100 camera sites deployed in Itasca County, MN. Most of the cameras detected 1–3 carnivore species during a 6-week period in fall 2016 ($\bar{x} = 48$, $SD = 11$ trap-nights per camera), and 2–4 in spring 2017 ($\bar{x} = 51$, $SD = 11$ trap-nights per camera). The number of species detected at each site varied greatly between these two sampling sessions.

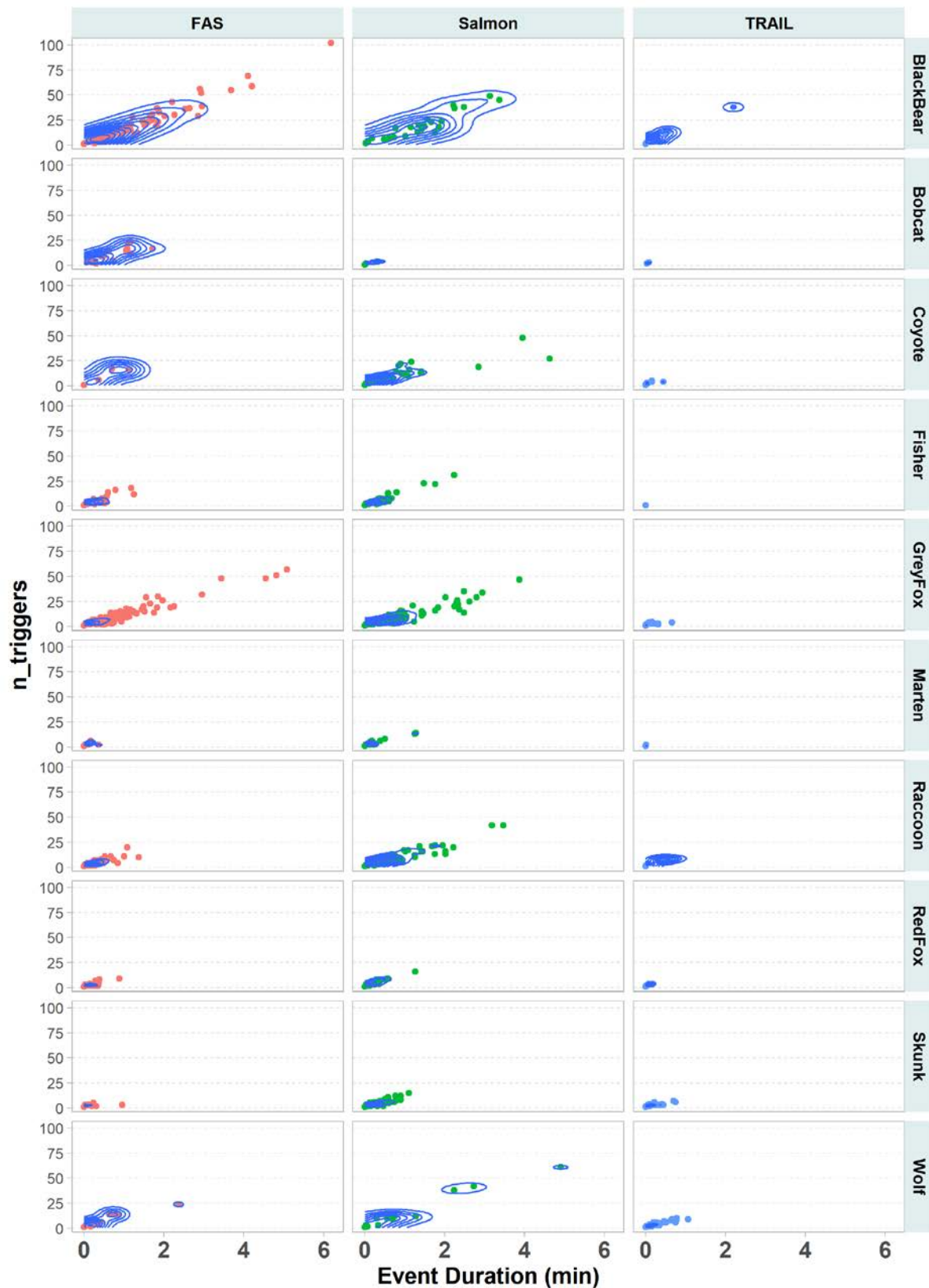


Figure 6. Event duration and number of trigger events by lure type and large-scale deployment strategy (random (with FAS or salmon oil) versus unlured trail camera) for each species. The use of a lure increased the time spent in the detection area, and, in turn, the number of pictures collected per event, facilitating species identification. Blue contours show density of dots; orange, green, and light-blue dots indicate events at FAS, salmon oil, and unlured trail cameras, respectively.

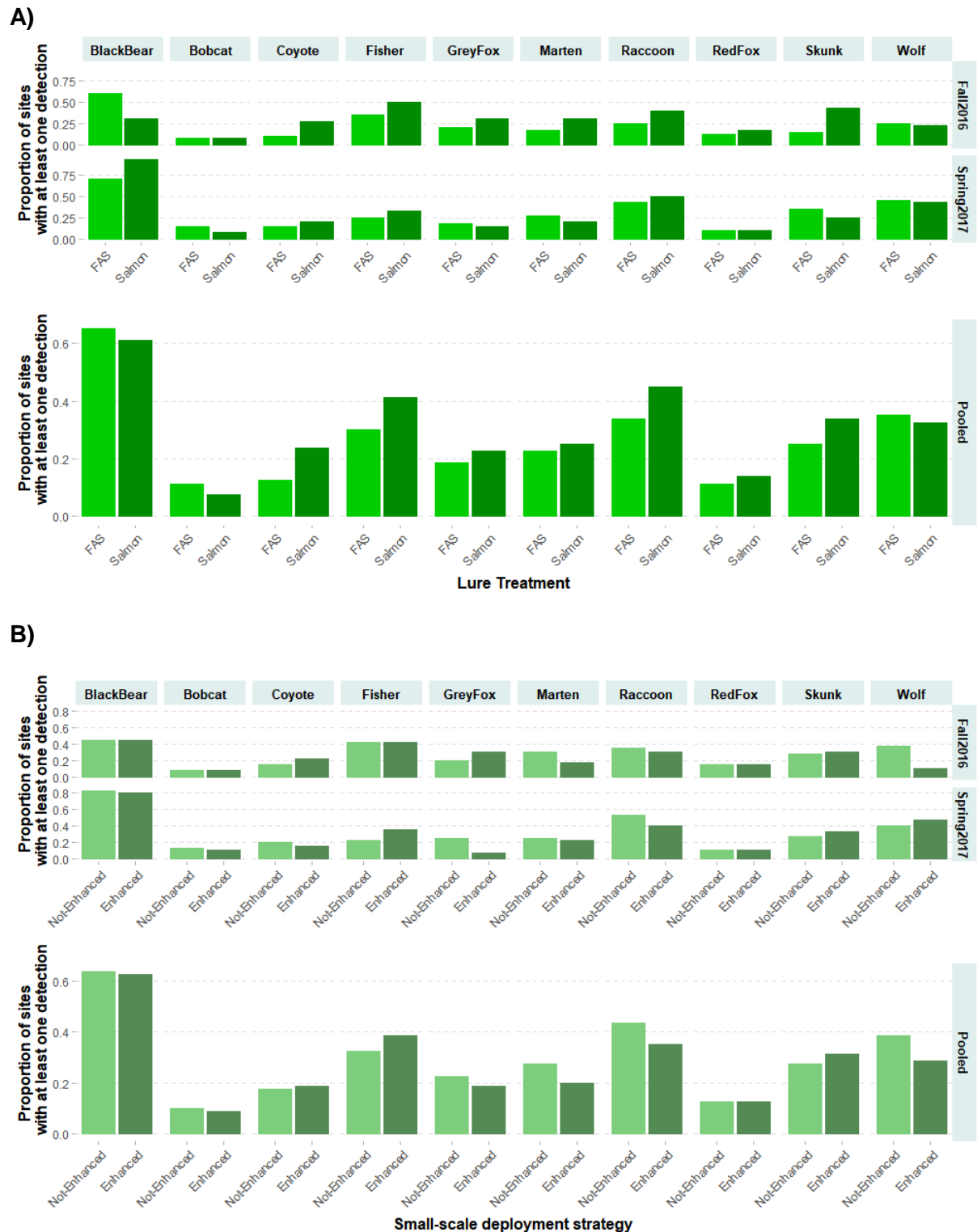


Figure 7. Proportion of camera sites in Itasca County, MN, at which each species was detected based on A) lure type (FAS: $n=40$ sites, 1,897 and 2,000 active trap-nights in fall 2016 and spring 2017, respectively; Salmon oil: $n=40$ sites, 1,886 and 2,058 active trap-nights in fall 2016 and spring 2017); and B) small-scale deployment strategy (not-enhanced: $n=40$ sites, 1,949 and 2,142 active trap-nights in fall 2016 and spring 2017; enhanced: $n=40$ sites, 1,834 and 1,916 trap-nights in fall 2016 and spring 2017). Each graph reports data for fall 2016, spring 2017, and for the two sessions pooled together (upper, middle, and bottom row of each graph, respectively).

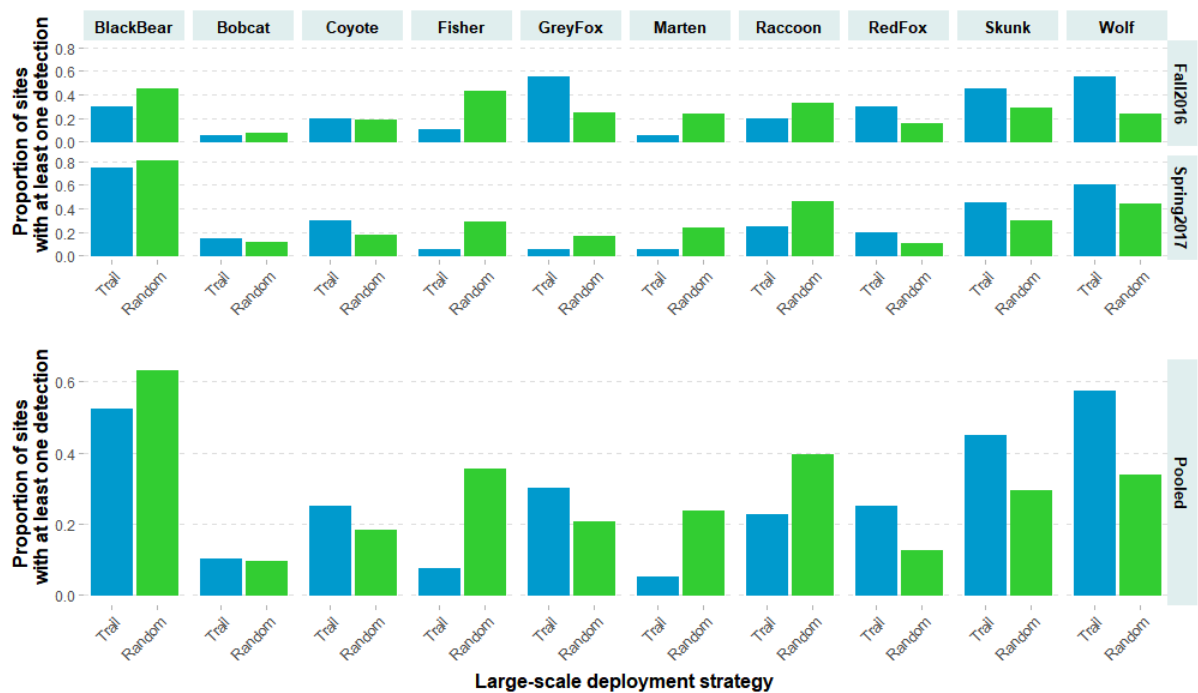


Figure 8. Proportion of camera sites in Itasca County, MN, at which each species was detected in fall 2016 and spring 2017 (top panel) and both sessions pooled (bottom panel) based on macro-site selection strategy (unlured trail cameras: n=20 sites, 1,006 and 1,043 active trap-nights in fall 2016 and spring 2017, respectively; lured randomly placed cameras: n=80 sites, 3,783 and 4,058 active trap-nights in fall 2016 and spring 2017, respectively).



Figure 9. Example of images collected during the spring 2016 survey, Itasca County, MN. From top-left to bottom-right: gray wolf, red fox, bobcat, black bear with two cubs, fisher, raccoon, striped skunk, and coyote.



ECOLOGY AND POPULATION DYNAMICS OF BLACK BEARS IN MINNESOTA

David L. Garshelis, Andrew Tri, Spencer J. Rettler¹, and Brian J. Dirks²

SUMMARY OF FINDINGS

During April 2017–March 2018, we monitored 16 American black bears (*Ursus americanus*) previously radiocollared (mostly with GPS collars) 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. During summer, we captured and collared 8 more bears. We did not collar young males, which are likely to disperse; however, most of the capture sample was young males — indicative of heavy hunting pressure. Hunting has been the primary source of mortality in all areas, although vehicle collisions have been a significant source of mortality for bears wandering off Camp Ripley, which is flanked by highways. In the 2017 hunting season, 22% of collared bears on the CNF were legally shot, even though hunters were asked to avoid killing collared bears, and each was marked with conspicuously large, colorful ear tags.

Reproduction was strongly affected by food supply. The NW area had the highest reproductive rate, due to early maturity, large litters, and litter intervals rarely exceeding 2 years. Camp Ripley bears matured early but had the highest proportion of 3-year litter intervals. Litter sizes of 3 were most common in NW and CNF, whereas litter sizes of 2 were most common in VNP; in Camp Ripley, 3-year-old mothers all had litters of 2, whereas older mothers had an equal proportion of 2- and 3-cub litters.

Camera traps set outside den sites revealed widely varying dates of initial den emergence (22 Feb–18 April) and final departure from the den site 0–42 days later. Much of the bears' activity outside the den, before leaving the den site, involved collecting dry bedding material. Photos showed that bears often got wet in their dens from melting snow, resulting in the death of 1 cub.

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 continuously 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

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southern periphery of the bear range, was added as a second study site in 1991. Camp Ripley is un hunted, 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 un hunted, 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 hunting licenses).

We used these 4 study sites to examine spatial variation in bear population dynamics and ecology to help inform bear management.

OBJECTIVES

1. Compare sources of bear mortality in different parts of the bear range.
2. Quantify temporal and spatial variation in cub production and survival.
3. Understand factors affecting emergence and departure from dens.

METHODS

During May–August, 2016, we captured bears in the CNF with barrel traps and immobilized them with ketamine-xylazine. During December–March, we visited all radiocollared bears once or twice at their den site and immobilized them with Telazol. For all handling, we measured and weighed bears, assessed body condition, took blood and hair samples, and extracted a vestigial first premolar to estimate age on all bears whose age was unknown (i.e., first handling of bears older than cubs). We changed or refit the collar, as necessary. We collared all new females and larger males that we thought would not disperse from the study area.

This year we used mainly GPS-Iridium collars (Telonics Inc., Mesa, AZ) or VHF collars with an attached GPS pod (Telemetry Solutions, Concord, CA), except in VNP where we used only VHF collars. All collared bears had brightly-colored, cattle-size ear tags (7x6 cm; Dalton Ltd., UK) that would be plainly visible to hunters. Bears that were not collared had small inconspicuous ear tags.

We 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. Licensed hunters could legally shoot collared bears, although they were asked not to. 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. Requests to hunters to voluntarily not shoot collared bears have been made through the news media and MNDNR hunting regulations and website since 2001, although the individual letters to hunters was not initiated until 2011.

We assessed reproduction by observing cubs in March dens. We sexed and weighed cubs without drugging them. We quantified 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 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 (see Laske et al. 2014). These data are not presented in this report, but will be used to inform our research questions about factors affecting den emergence.

We set remote cameras (camera traps; Reconyx, Inc., Holmen, WI) outside bear dens to gain information about dates and behaviors of bears emerging from dens and departing from the den site. Bears that emerged from dens <48 hours after our den visit were excluded from the analysis.

RESULTS AND DISCUSSION

Radiocollaring and Monitoring

As of April 2017, the start of the current year's work, we were monitoring 25 radiocollared bears: 16 in the CNF, 5 at Camp Ripley, 2 in VNP, and 2 in the NW (Table 1).

In early spring we collared and released in the CNF 1 previously orphaned cub that was raised in a rehabilitation center until it was 1 year old and self-sufficient. We were interested in how this bear used the habitat. Normally we do not collar rehabbed bears and do not release them in a study site.

During May–July we captured 11 new bears (9M; 2F) in the CNF, and collared 6. We also caught and collared 2 bears that had previously been caught in 2016 but were deemed too young to collar then. We avoided collaring small males, supposing they would disperse. Our capture sample, though, over the past 3 years was heavily skewed toward males (31 of 40 = 76%) and young bears (80% 1–4 years old); only 4 captured bears were >6 years old (Figure 2). The heavy skew toward males and young age structure of the captured bears suggests that this area has been subjected to heavy hunting, and many of the bears had immigrated from elsewhere.

At Camp Ripley we collared 2 adult females that were found in den sites, and also collared 3 female yearlings that were denned with 2 collared mothers.

Mortality

Since 1981 we have recorded the cause of death for 383 radiocollared bears, 76% of which died (or likely died) from legal hunting (Table 2). In all 4 study sites, legal hunting was the primary cause of mortality (Figure 3), despite (a) Camp Ripley and VNP being unhunted (but bears wander outside during fall on foraging trips, and (b) hunters being asked to not shoot collared bears with large ear tags for the past 17 years (spanning the full period of the NW study).

During the 2017 bear hunting season, 5 collared bears were shot in the CNF (Table 1). This represented 22% of the collared bears monitored at the time, and included 3 of 7 collared adult females. This high (unsustainable) harvest rate among collared bears was similar to last year, and is further evidence that the CNF study site is a population sink (where mortality exceeds reproduction, and there is a high rate of immigration of young males).

Vehicle collisions are another significant source of mortality. We found one CNF adult male dead in July 2017, but he was too decayed to ascertain cause of death directly; however, his GPS locations and mortality sensor showed that he died Memorial Day weekend, 2 days after crossing a busy highway (Highway 38), and he died within 0.25 miles of the road, so we strongly suspect he was hit by a vehicle. Vehicle collisions are most common at Camp Ripley (Figure 3), which is flanked by 2 highways. One collared adult female, who had been monitored since 2004 and had produced 15 cubs since then, was hit and killed on a highway just outside Camp Ripley (4-lane U.S. Highway 10) in July 2017. We had previously implanted a heart monitor in this bear, and observed that her heart rate increased dramatically as she crossed the road (based on matching the data from the heart monitor with GPS collar; Ditmer et al. 2018, Supplement). This bear had crossed busy roads frequently in other years, because she lived mainly outside Camp Ripley; she had crossed this highway at least 10 times in the 2 months between emerging from her den and being killed.

Reproduction

Since 1982, within the 4 study areas, we have checked 297 litters with 766 cubs (mean = 2.6 cubs/litter; range = 2.2–2.8 by study site), of which 50.3% were male (Tables 3–6). All bears that were expected to have cubs this year, based on a 2-year reproductive cycle, did so. Overall, bears at Camp Ripley, despite being large, have had a higher rate of missed litters (3-year litter intervals) than bears in the other study sites (Table 7).

We observed 2 bears that produced their first litters this year in the CNF, 1 at 4 years old and 1 at 5. Two 4 year-old females in the VNP did not produce cubs. Since the beginning of the study, 38% of females in the CNF produced cubs by 4 and none in the VNP produced cubs by 4, whereas 85% of females in the other 2 study sites produced cubs by 4 years old (Figure 4).

Bears in the CNF and NW produced more 3-cub litters than 2-cub litters, whereas 2-cub litters were most common at Camp Ripley and VNP (Figure 5). The relatively small litter sizes at Camp Ripley were due to many of those bears producing cubs when only 3 years old (all 3-year-old mothers had litters of 2 cubs). Eliminating these bears, litter sizes of 2 and 3 cubs were about equal at Camp Ripley (Figure 5). Among all study sites combined, 35% of bears had litters of 2 cubs, and 54% had litters of 3.

Camp Ripley bears apparently sacrificed litter size for earlier age of reproduction (Figures 5 and 4, respectively). NW bears had both large litters and early age of first reproduction, so were most prolific of all the sites.

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 6). 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). In all areas except the NW, 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 (fewer cubs). The most striking differences among study sites were in the reproductive rates of these 4–6 year-olds (Figure 6).

Mortality of cubs during their first year of life averaged 19% (annual range 0–31% for years with at least 10 cubs monitored), with mortality of male cubs (24%) exceeding that of females (16%; $\chi^2 = 6.15$, $P = 0.01$). However, in the most recent 15 years on the CNF (2004–2018), female cub mortality equaled that of males, and so overall cub mortality increased from 17% (1982–

2003) to 29% ($\chi^2 = 4.1$, $P = 0.04$). The timing and causes of cub mortality are unknown (but see below for 1 case this year where it was observed)

Camera Trap Photos at Dens

We obtained camera-trap photos of bears that yielded dates of natural emergence and departure from 30 dens: 1 in 2015, 6 in 2016, 7 in 2017, and 16 in 2018. Dates of first emergence ranged from 22 February to 18 April (Figure 7). After first emergence (which we defined as completely exiting the den, not just poking their head out), bears remained at the den site for 0–42 days. This span of time is similar to that reported by Miller et al. (2016; 0–47 days) for 21 black bear dens monitored with camera traps in Utah. In our study, mean date of first emergence was highly variable, and widely overlapping for solitary bears (of either sex), females with yearlings, and females with cubs. However, females with cubs left the den site about 1 week later (mean = April 6) than other bears (mean = March 30; although the 2 oldest and largest males [>400 lbs] left on April 18 and 26).

We observed no relationship between the dates that bears first emerged from dens and when they eventually left the site (Figure 7). We suspect that bears employed different thresholds for leaving. Bears with young cubs tended to stay until cubs were mobile and able to climb trees. Solitary bears or mothers with yearlings often waited for most of the snow to melt; however, some did not. This year we deployed temperature sensors inside dens, just outside dens, and on each bear's collar, hoping to better understand the effect of temperature (and especially, increasingly warming springs) on bear denning habits. These data have not yet been analyzed.

Between the time of emergence and eventual departure from the den site, bears moved back and forth between their den and outside the den. When outside the den, but before leaving the vicinity of the den (defined as beyond the detection of the remote cameras), bears were involved in the following principal behaviors: raking more bedding material into the den, stretching/walking, laying in the sun, eating snow or drinking water, monitoring cubs playing and climbing trees (Figure 8). We thus interpret the period between den emergence and departure to be a time when bears: (1) attempt to stay dry in the den while snow is melting and causing some discomfort; (2) regain muscle strength; (3) warm body temperature; and (4) rehydrate. Often, in March, we observed bears poking their head outside the den to eat snow while not coming completely out of the den (Figure 8). Hibernating bears do not eat or drink through the winter, but in the month before leaving the den site, they sought to rehydrate.

Although we have not yet quantified time by activity, the photographs in our study indicate a substantial investment in gathering more bedding material. In fact, it often appeared that the primary reason for coming out of the den was to get more bedding, apparently because the den had gotten wet; in some cases, the photos clearly showed that bears had gotten wet in their dens. In one case, a female with 4 cubs in the NW study site had an excavated den that became saturated when a nearby river thawed and rose; she carried her wet cubs individually out of the flooded den and placed them in a nest of grass that she built behind her den (Figure 9). The last cub that she removed was limp and appeared to have died — we later found a dead cub in the nest. In another case, a female with cubs left her flooded den site on April 12; just 11 days later our remote camera photographed a female wood duck (*Aix sponsa*) on the ephemeral pond just outside the former den (Figure 10).

Camera traps and GPS collars were not available when we began our research >30 years ago, so we cannot ascertain whether spring flooding of dens was as common then as it is now. However, our long-term data seem to show a trend toward increased selection of above-ground dens, which are drier than excavated dens, but expose bears to other potential environmental hazards.

ACKNOWLEDGMENTS

We thank Paul Iaizzo, Tim Laske, and Tinen Iles (University of Minnesota), who greatly assisted with fieldwork and led the associated work on heart monitoring (not covered in this report). Michael McMahon, Stefan Nelson, and Brent Hemly also provided valuable field assistance. Agassiz NWR kindly provided use of their bunkhouse. This project was funded in part by the Wildlife Restoration (Pittman-Robertson) Program grant W-68-D-15.

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Table 1. Fates of radiocollared black bears in Chippewa National Forest (CNF), Camp Ripley, Voyageurs National Park (VNP), and northwestern Minnesota (NW) study sites, April 2017–March 2018.

	CNF	Camp ripley	VNP	NW
Collared sample April 2017	16	5	2	2
Trapped and collared	8			
Collared in den		5		
Released from rehab	1			
Killed in vehicle collision	1 ^b	1		
Killed by Minnesota hunter ^a	5			
Died from unknown causes				
Removed radiocollar	1			
Dropped radiocollar	1			
Collared sample April 2018	17	9	2	2

^a Hunters were asked not to shoot collared bears (although it was still legal).

^b Uncertain cause of death, but crossed a busy highway 2 days before dying close to the road.

Table 2. Causes of mortality of radiocollared black bears ≥ 1 year old in 4 Minnesota study sites, 1981–2018. 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 ^a	235	13	16	14	278
Likely shot by hunter ^b	9	1	0	4	14
Shot as nuisance	22	2	1	3	28
Vehicle collision	13	10	1	3	27
Other human-caused death	9	1	0	0	10
Natural mortality	8 ^c	3	5	0	16 ^c
Died from unknown causes	5	2	0	3	10
Total deaths	301	32	23	27	383

^a Since 2001, the MNDNR has asked hunters not to shoot collared bears, so the proportion killed due to this cause is no longer representative of the population at large.

^b Lost track of during the bear hunting season, or collar seemingly removed by a hunter.

^c 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, Minnesota, during March, 1982–2018. 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	—	—	—
2016	0	0	—	—	—
2017	1	3	3.0	—	0%
2018	4	12	3.0	42%	—
Overall	184	487	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, 2007–2018.

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%	0%
2016	2	6	3.0	50%	0%
2017	1	3	3.0	0%	0%
2018	1	4	4.0	50%	
Overall	32	90	2.8	44%	16% ^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, Minnesota, during March, 1992–2018.

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	2.5	20%	10%
2016	0	0	—	—	—
2017	4	10	2.5	60%	0%
2018	2	5	2.5	—	—
Overall	52	124	2.4	48%	18%

^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, Minnesota, during March, 1999–2018. 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%	0%
2015	0	0	—	—	—
2016	0 ^b	0	—	—	—
2017	0	0	—	—	—
2018	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.

^b One bear that likely had cubs was not checked because access to her den was precluded by poor ice conditions.

Table 7. Intervals between surviving litters for black bears within 4 study sites in Minnesota (see Figure 1) through March 2018 (CNF since 1981, Camp Ripley since 1991, VNP since 1997, NW since 2007). Cubs are generally born in January and remain with their mother for about 17 months, so the normal reproductive interval is 2 years. Reproductive intervals here include only litters where at least 1 cub survived through the next denning period (1 year), so intervals <2 years are impossible.

Study area	2-year reproductive intervals	≥3-year reproductive intervals	% intervals ≥3 years
CNF	111	8 ^a	7%
Camp Ripley	32	5	14%
VNP	15	1	6%
NW	18	0 ^b	0%

^a Including the only case of an interval spanning >3 years, due to whole litter loss followed by a non-reproductive year.

^b Excluding 1 missed litter (3-year interval) that was due to the bear leaving the den after disturbance and aborting the litter.

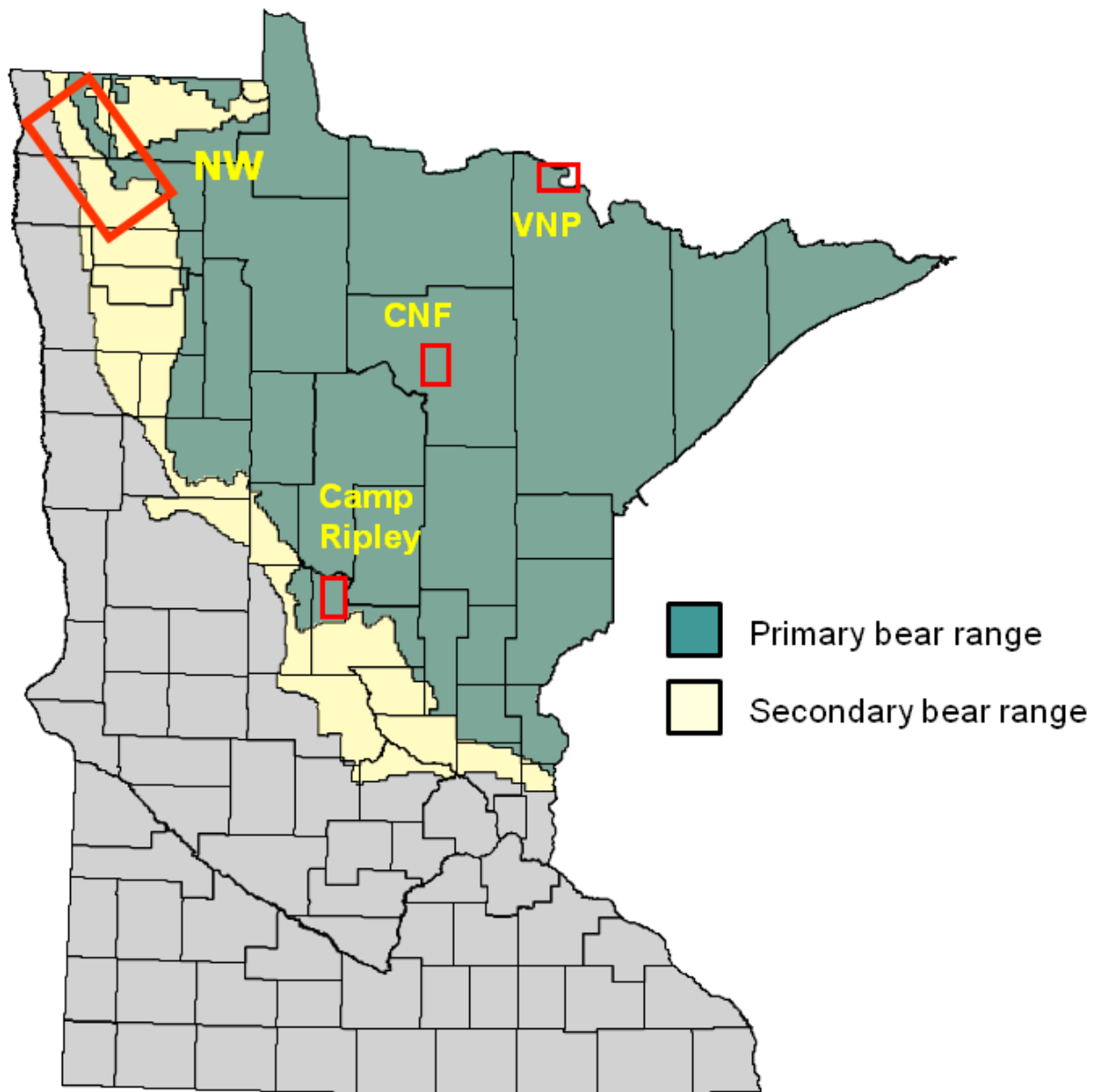


Figure 1. Location of 4 study sites within Minnesota's bear range: CNF (Chippewa National Forest, central bear range; 1981–2018); VNP (Voyageurs National Park, northern fringe of range; 1997–2018); Camp Ripley Military Reserve (near southern edge of range; 1991–2018); NW (northwestern fringe of range; 2007–2018).

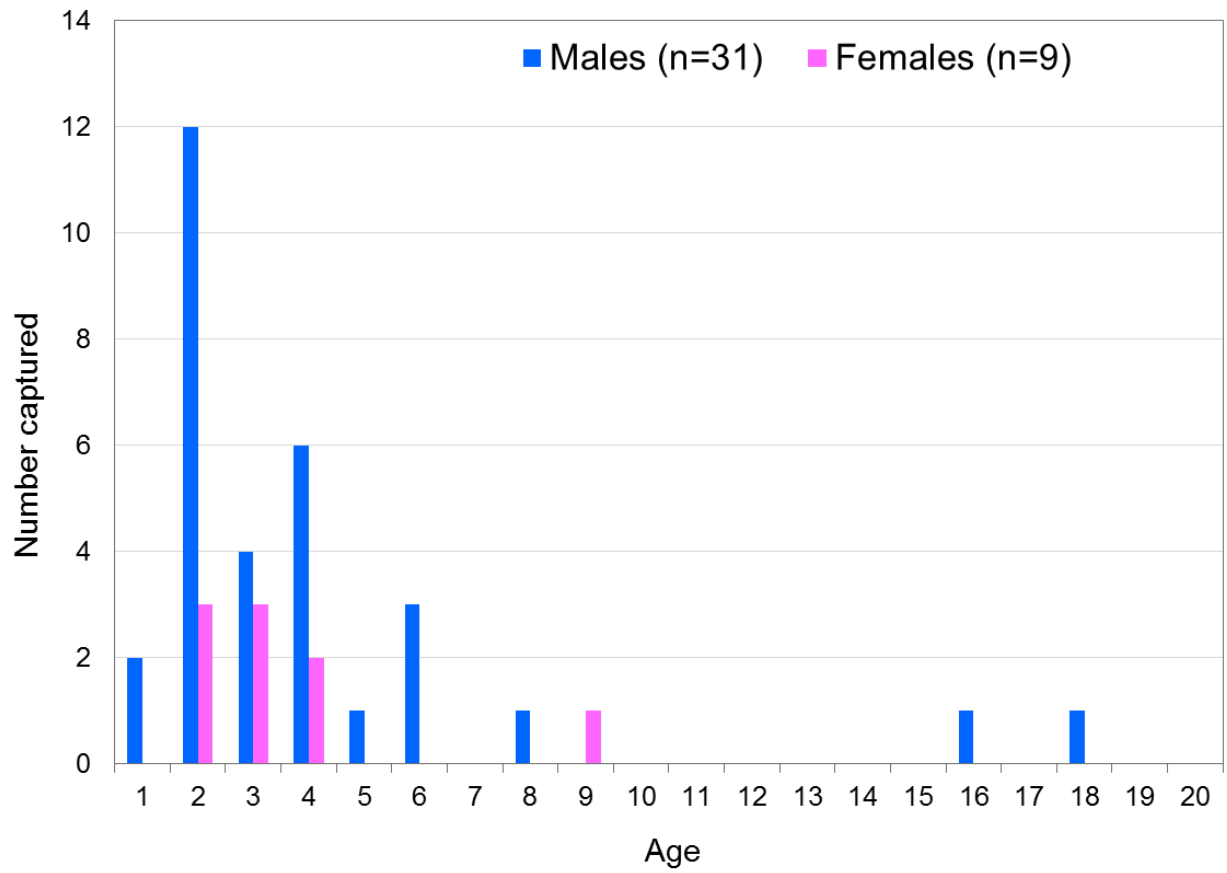


Figure 2. Bears captured by sex and age in the CNF, Minnesota, May–Aug, 2015–2017.

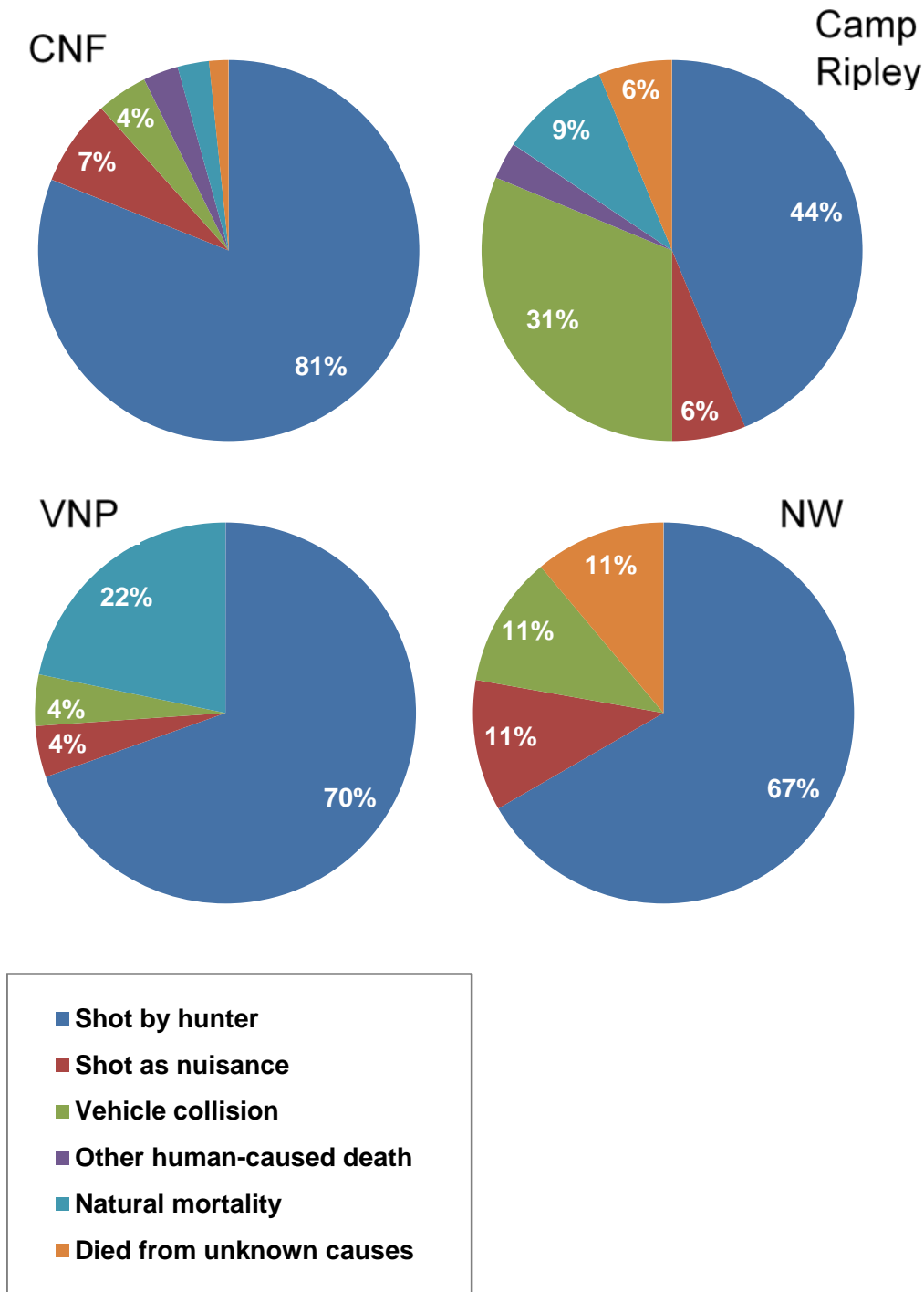


Figure 3. Proportional causes of death of radiocollared bears in each of 4 study sites in Minnesota. CNF expectedly had the highest proportion of bears killed by hunters because this is primarily public land that is heavily hunted. Camp Ripley and VNP are unhunted but bears are vulnerable when they leave on foraging forays. Hunters were asked not to shoot collared bears during the entire span of the NW study, so the proportion killed by hunters does not reflect the population at large. See map and dates for each study site in Figure 1.

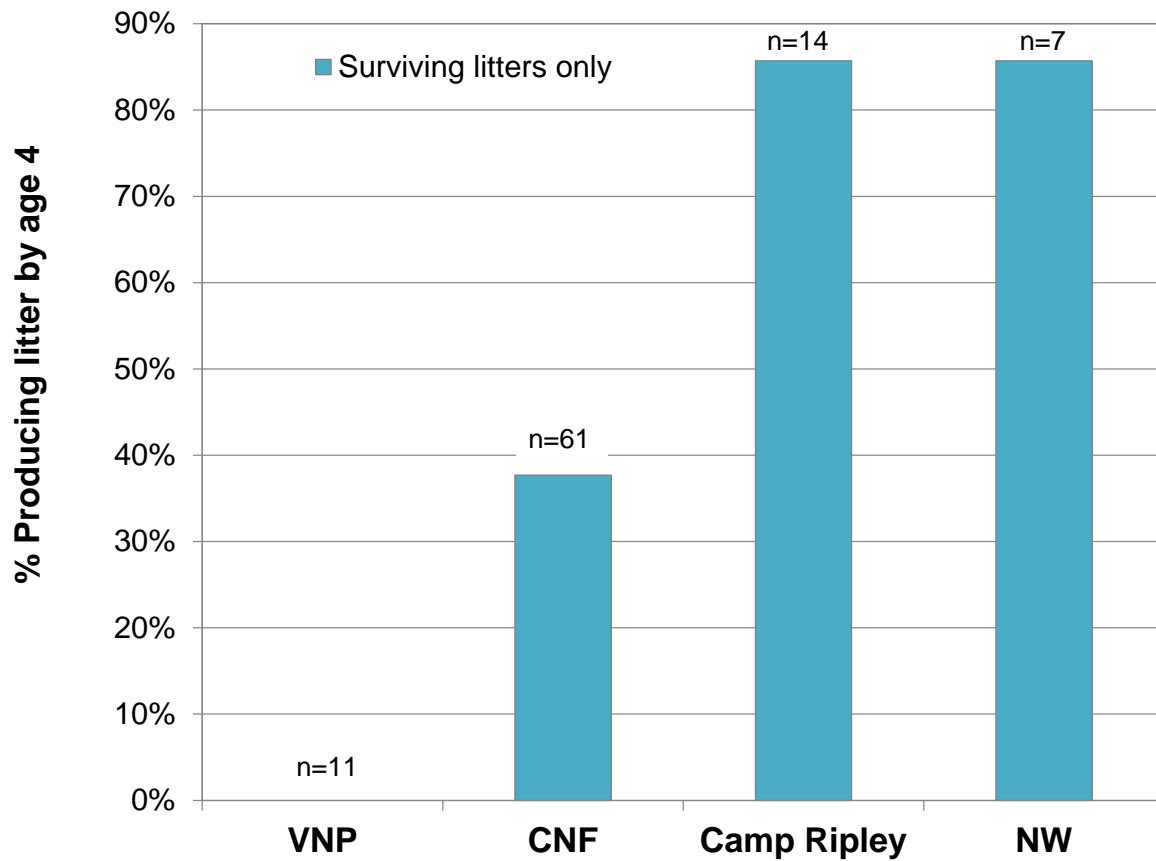


Figure 4. Percent of radiocollared female bears on each Minnesota study site that produced a surviving litter of cubs by 4 years old. Births of cubs were detected in natal dens in March each year (through March 2018). A surviving litter was one in which at least one yearling was present in the mother's den the next winter. Note that no females in VNP produced cubs by 4 years of age.

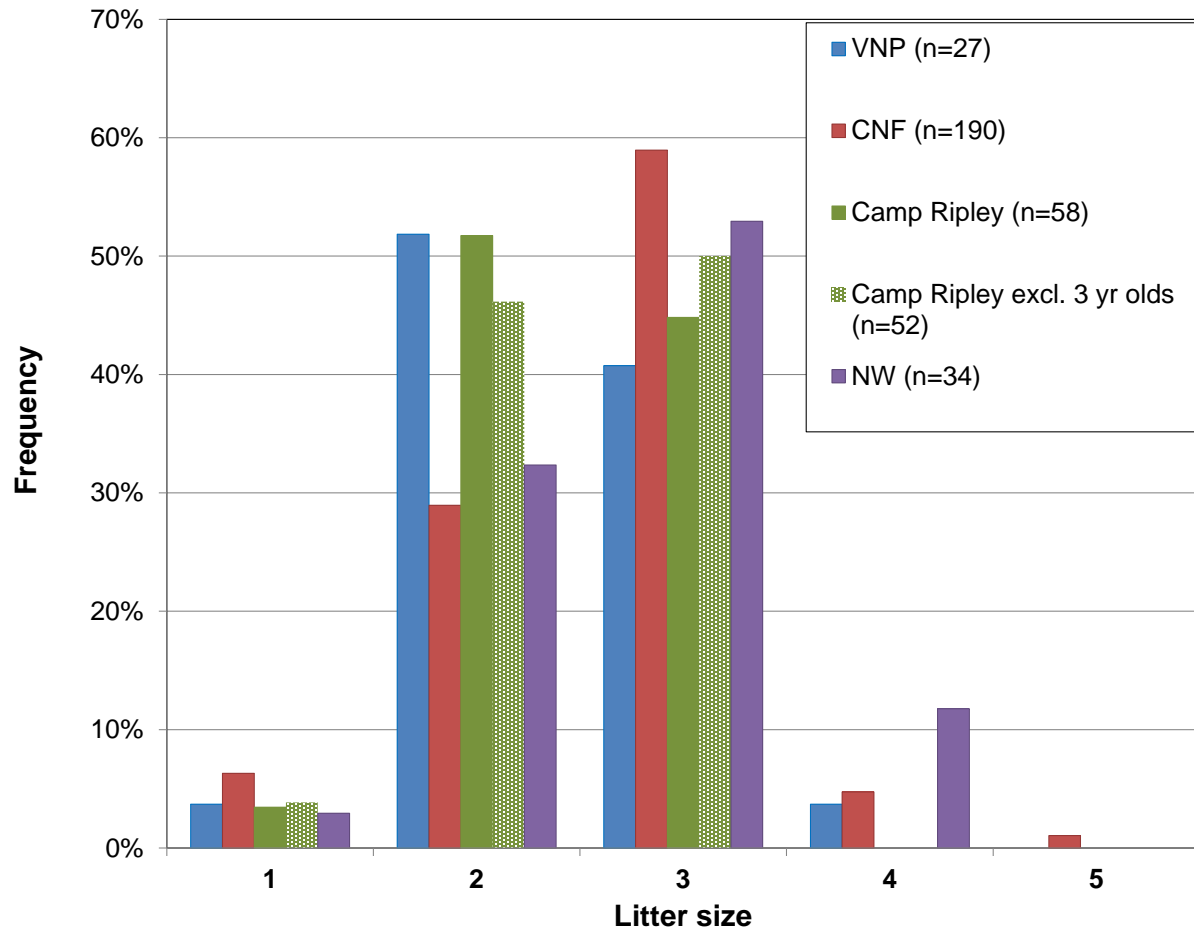


Figure 5. Frequency of cub litter sizes (examined in natal dens in March) within 4 Minnesota study sites (see Figure 1) through March 2018. Data include only litters that survived 1 year (even if some cubs in the litter died). Camp Ripley data are shown for mothers of all ages, as well as excluding 3-year-old mothers. For the other sites, elimination of 3-year-olds did not make a difference.

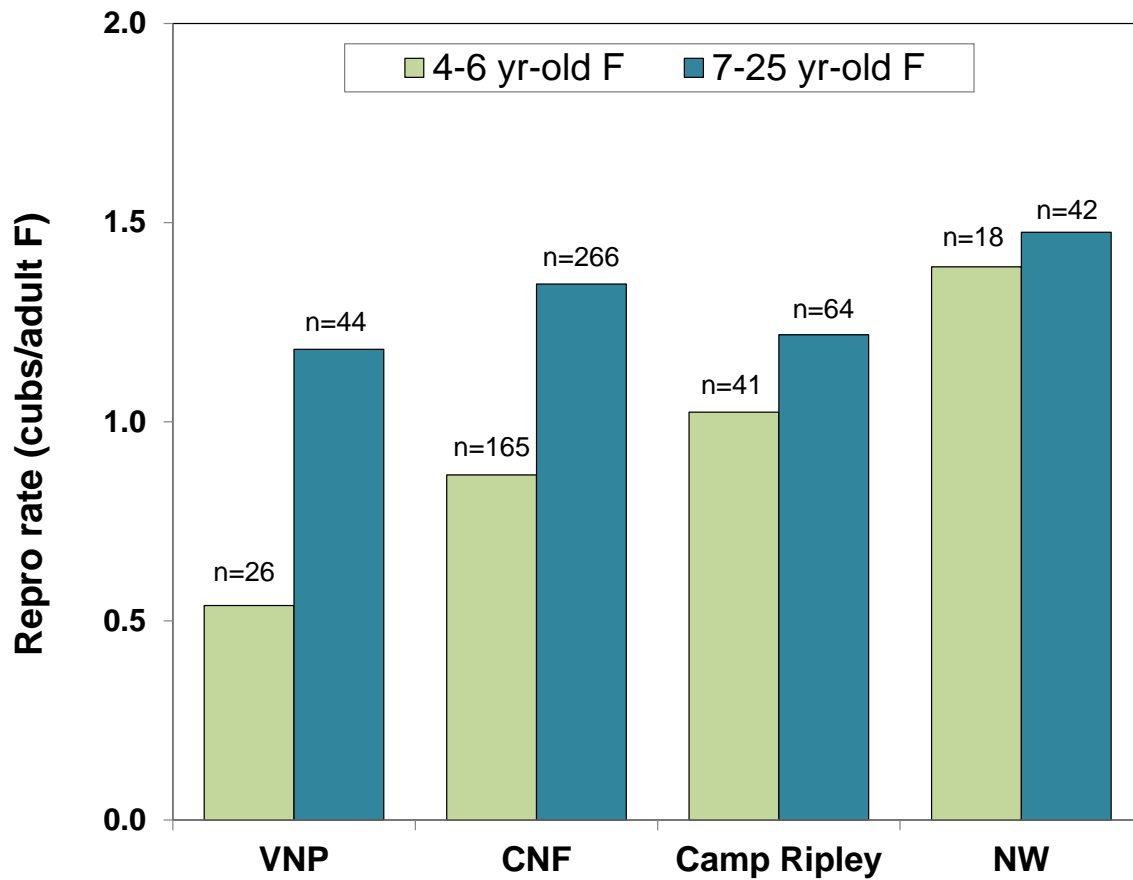


Figure 6. Reproductive rates of radiocollared bears within 4 Minnesota study sites (see Figure 1) through March 2018 (VNP since 1997, CNF since 1981, Camp Ripley since 1991, NW since 2007). Data include only litters that survived 1 year (even if some cubs in the litter died). Sample sizes refer to the 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.

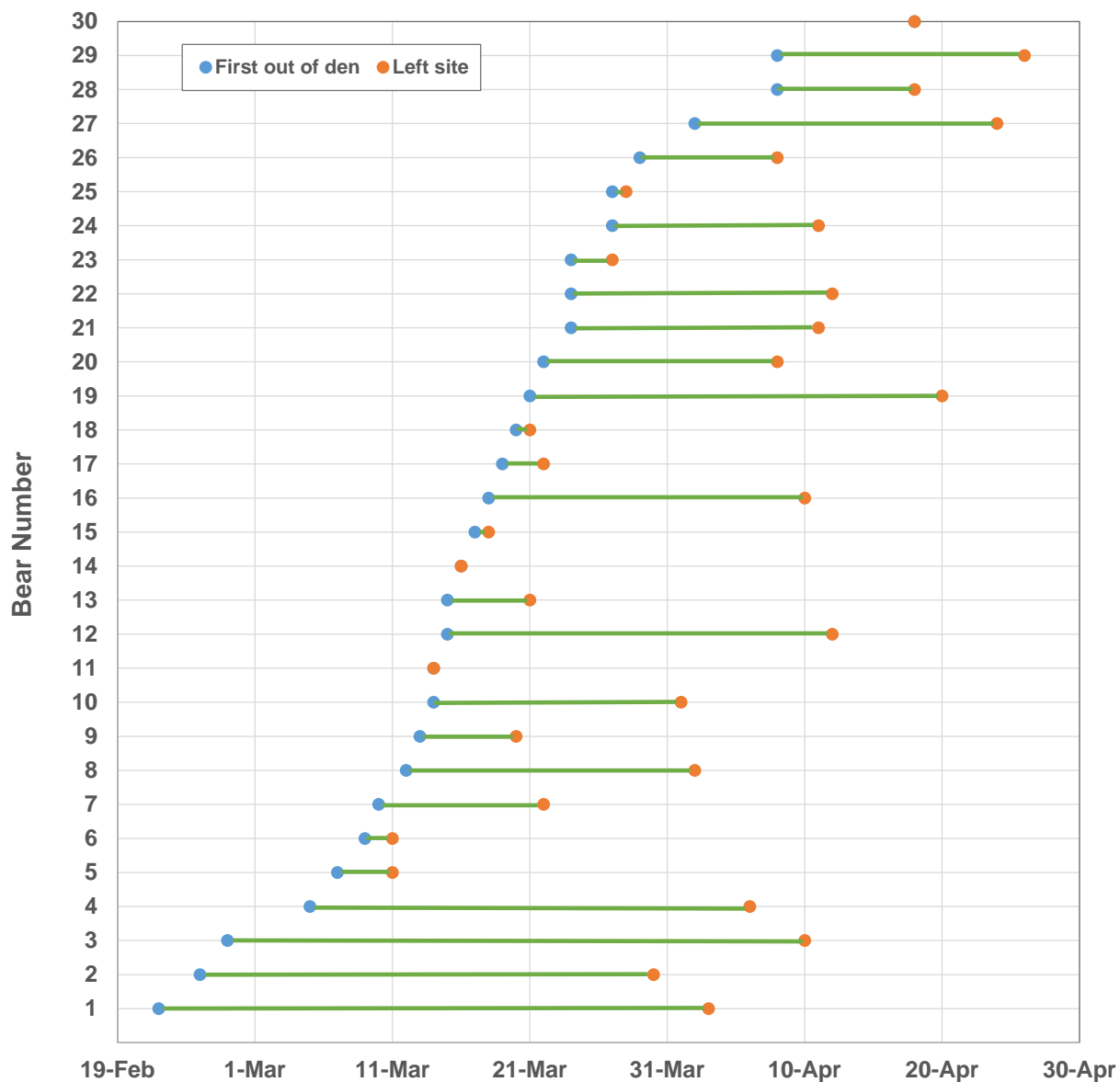


Figure 7. Dates of first emergence from dens, and eventual departure from the den site, for 30 radiocollared black bears monitored with remote cameras in Minnesota, 2015–2018. Cases are arranged in order of first emergence (blue dots). Green lines show the time period (0–42 days) that the bear remained at the den site following initial emergence. In 3 cases (bear 11, 14, 30), the bear left the same day it emerged.

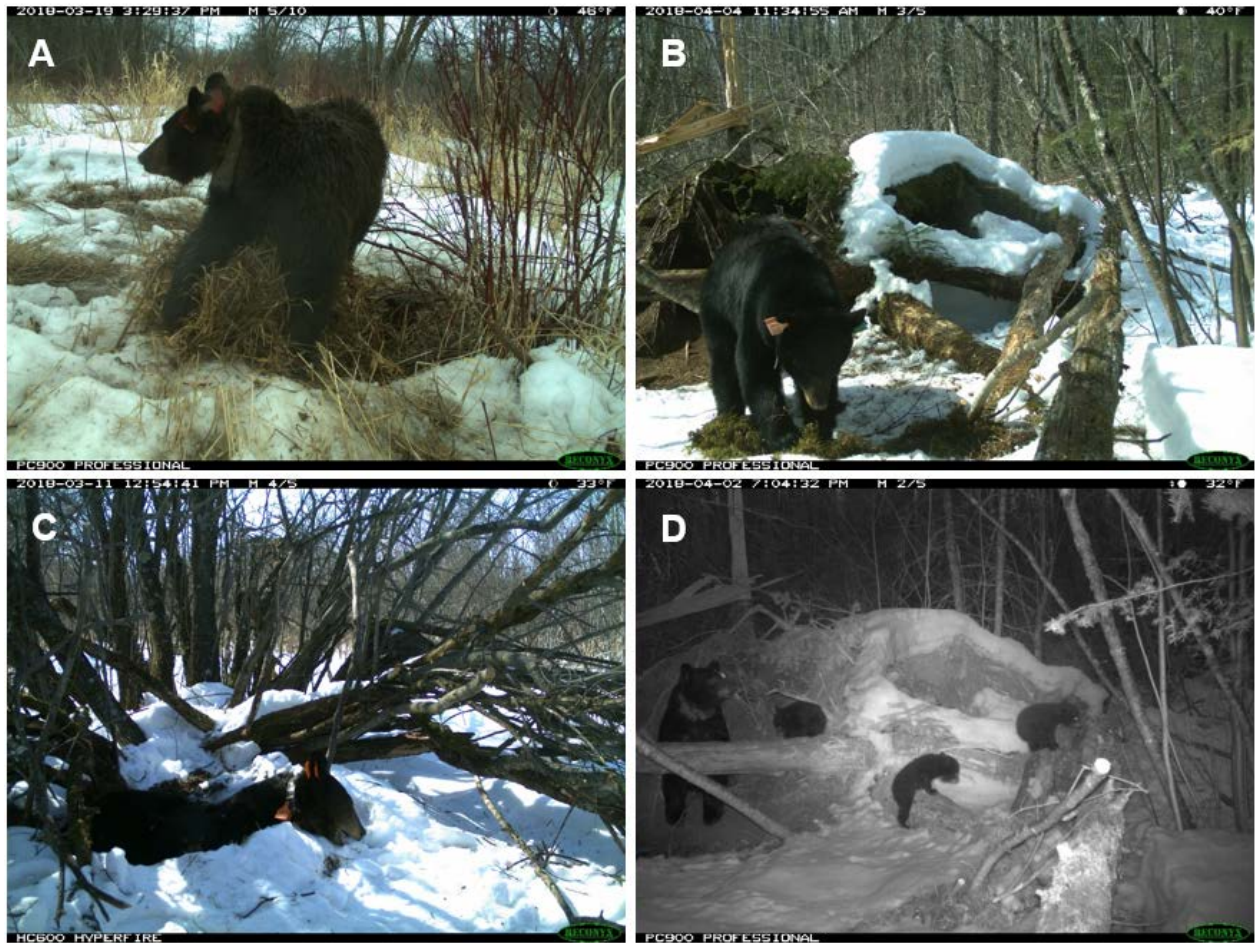


Figure 8. Camera-trap photos of activities around bear dens in Minnesota, March–April, 2018 (exact dates shown on photos), as bears emerged but did not yet leave the site. Common activities included: (panels A,B) raking in dry bedding material as melting snow leaked into their dens; (C) consuming snow; and (D) providing an opportunity for 2.5-month-old cubs to gain strength before abandoning the protection of the den site.

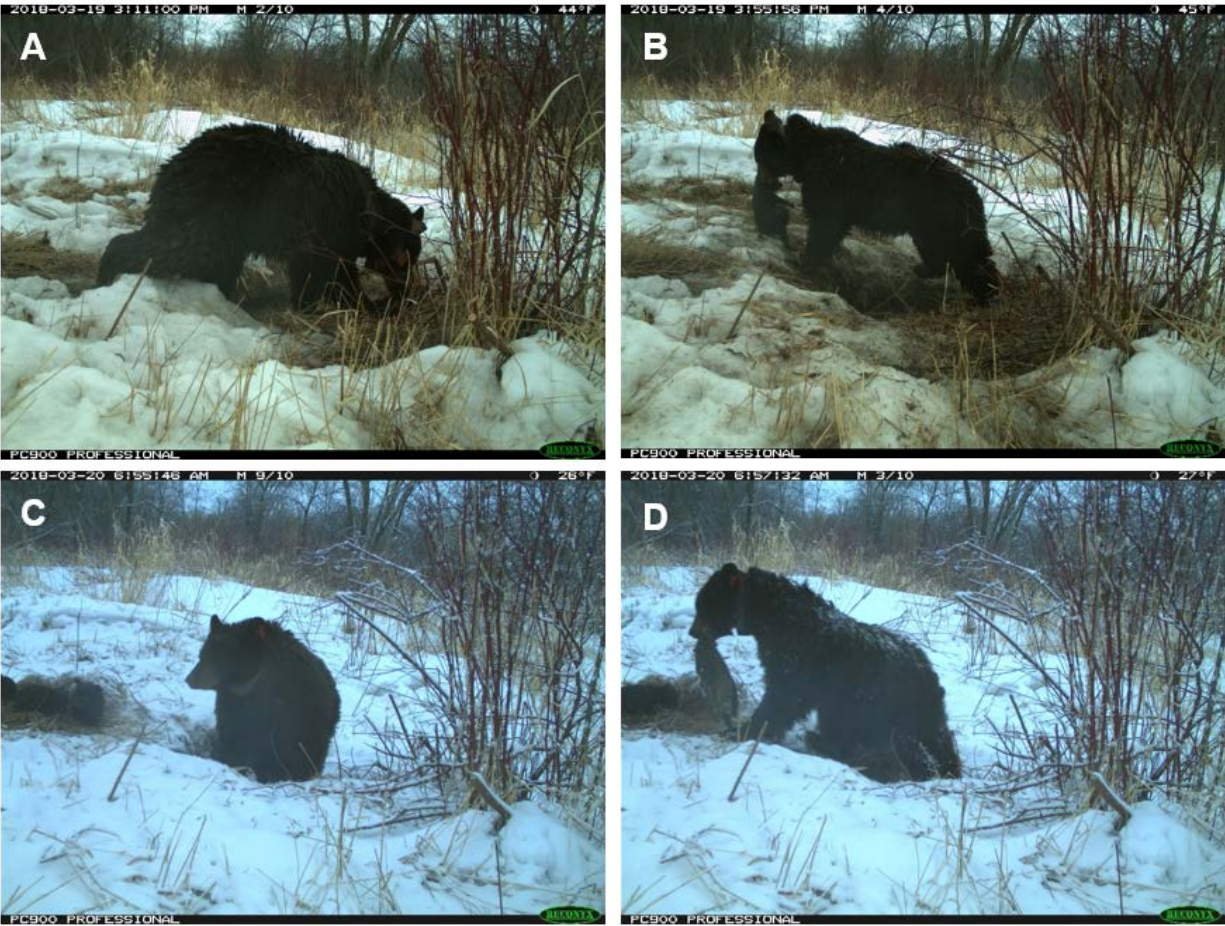


Figure 9. Camera trap photos (March 19–20, 2018) of a female bear in NW Minnesota (A) exiting an excavated den that flooded (note wet, matted hair). (B) She carried her wet cubs out of the den to a dry nest that she built outside the den. (C) This bear had a litter of 4 cubs, 3 of which survived (visible in nest). (D) The 4th cub died in the den and was carried out; its remains were found in the nest.

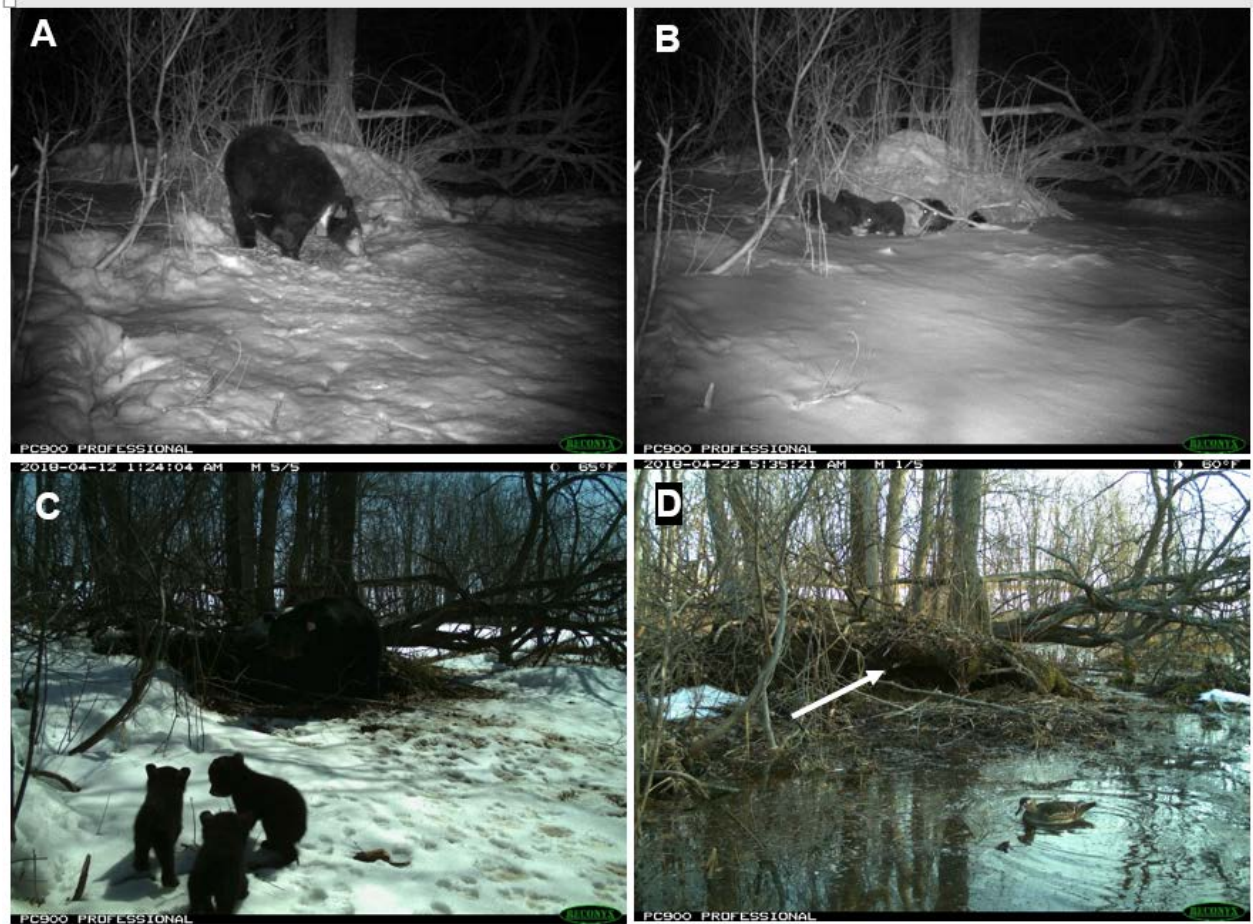


Figure 10. Camera trap photos of (A) a female bear in the CNF in Minnesota first emerging from her den on March 15, 2018; (B) the cubs emerging after a fresh snowfall; (C) the family leaving the densite on April 12 (note numerous tracks from the bears' frequent activities outside the den prior to leaving the site), and (D) 11 days later the site becoming a pond inhabited by a wood duck (den entrance shown by arrow). All photos are from the same camera position.



REDUCED NATURAL FOODS ALTER BOTTOM-UP PRESSURES ON AMERICAN BLACK BEARS

Spencer J. Rettler^{1,2}, David L. Garshelis, Andrew Tri, Véronique St-Louis, and James Forester¹

SUMMARY OF FINDINGS

For bear management in North America, food availability (bottom-up) and hunting pressure (top-down) are the primary factors that limit population growth. Whereas seasonal and year-to-year variation in production of fruits and nuts (i.e., primary bear foods) have been widely recognized, long-term trends in natural food production have rarely been reported. Here we compared the current (2015–2017) availability of 18 fruits and nuts constituting the main foods of American black bears (*Ursus americanus*) in north-central Minnesota to what was available during the 1980s. The study area was the same in both time frames, constituting primarily national forest, where timber harvesting was routine in the 1980s but much less so in recent decades. We hypothesized that forests matured over the 25-year period and consequently produced less fruit for bears. Within each of 12 forest types, we measured the abundance, productivity and biomass (kg/ha) of each fruit-bearing plant species using the same methodology as used in the 1980s. For all groups of species, the probability of forest stands producing any fruit at all in the recent time period was half (~40%) what it was in the 1980s (~80%). At the landscape scale, we estimated a ~70% decline in biomass availability due to a reduction in young forest types that produce the most fruit, and also a ubiquitous decline in fruit production across most forest types. Our hypothesis that this decline was due to reduced timber harvesting was only partially correct, as we observed the same decline in fruit production within mature forest of the same type and canopy closure, and also along edges of stands with high light penetration. Earlier springs, with potentially greater vulnerability of flowers to frost, and an ongoing invasion of invasive earthworms, which are known to radically affect the soil, may be additional causes for diminished fruit production. Bears will likely need to alter their foraging habits to compensate for reduced availability of natural foods as the landscape continues to change. This study demonstrates the complexity of forest management and its unintended effects on wildlife.

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² This project served as an M.S. thesis, completed in June 2018,

INTRODUCTION

In forested environments, different combinations of overstory and understory species compositions, tree densities, and canopy closures will provide distributions of food and cover that favor different wildlife species. Here we focus on how these forest attributes can affect the production of food for an omnivorous, but largely frugivorous big game species, the American black bear (*Ursus americanus*; henceforth black bear). This forest-dependent species was historically over-exploited by hunters; however, it is now increasing numerically and expanding geographically across most of its range in the USA, Canada, and northern Mexico (Garshelis et al. 2016). The widespread increase in this species is mainly attributable to purposeful control of top-down forces, specifically reductions in legal and illegal hunting and killing of nuisance animals. Also, in some areas, forest cover has expanded, generally promoting increased vitality of this species.

Food availability affects bear body mass and condition, which greatly influences reproduction (Noyce and Garshelis 1994, Samson and Huot 1995, Costello et al. 2003); this can significantly affect the resilience of the population to hunting (Kontio et al. 1998). Black bears in Minnesota rely on over a dozen key fruits, which are produced on bushes or small trees in the forest understory from June through October (Garshelis and Noyce 2008). Habitat-specific abundance (areal coverage) and productivity (fruit production) of the key bear foods was measured on one study site near the center of the Minnesota bear range from the mid-1980s through the early 1990s. A notable finding was that fruit biomass for bears was especially high in young, regenerating or planted forests (Noyce and Coy 1990). Annual timber harvest on this study site declined from roughly 70,000–100,000 board-feet during the mid-1980s to mid-1990s to 20,000–60,000 during the early-2000s to 2015 (United States Forest Service 2017). Because of this reduced forest cutting, forest composition changed and food-rich stands of young aspen and young pine plantations, which were common in the 1980s, matured and appeared to shade out some of the fruit-producing understory. We hypothesized that fruit production declined, and that this could negatively affect the bear population.

OBJECTIVES

1. Determine whether declines in bear food production occurred since the 1980s.
2. Quantify the magnitude of change in bear food production since the 1980s, and identify probable causes for change.

STUDY AREA

Located in northcentral Minnesota, the Chippewa National Forest (CNF) falls in the transition zone between the boreal forests to the northeast and the temperate forests in the central part of the state. Our study area covered 62,200 ha, dominated by the eastern extent of the CNF (42% of study area); the remainder included some of the George Washington State Forest (11%), county land (6%), private land (18%), commercial timber industry land (8%) and open water (15%) (Figure 1). Within this area were both lowland and upland forests. Lowlands were dominated by one or more of these species: speckled alder (*Alnus incana*), black spruce (*Picea marina*), tamarack (*Larix laricina*), black ash (*Fraxinus nigra*), northern white-cedar (*Thuja occidentalis*), quaking aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*). Upland forests were dominated by some of these species: quaking and bigtooth aspen (*Populus grandidentata*), maple (*Acer spp.*), red pine (*Pinus resinosa*), paper birch (*Betula papyrifera*), and balsam fir (*Abies balsamea*). From 1990 to 2009, 2% of federal land, 4% of state land and 6% of county land was disturbed (e.g., timber harvest, natural blowdown from storms; Garner et al. 2016). From 2010 to 2015, timber harvest activity on state and county forests (9%) remained significantly higher than in the CNF (3%). Many lakes, forest roads, and recreational trails can

be found throughout the public land. This area was heavily hunted for bears throughout the scope of this study (1980s to present) due to the large extent of easily-accessible public land.

METHODS

We estimated abundance and production of 18 species of wild fruits (or groups of related species; Table 1) during early July to late August, the peak fruiting season. The fruit sampling methodology we used during 2015–2017 ($n=126$ unique forest stands) was patterned after that used by Noyce and Coy (1990), who sampled the same study area during 1984–1989 ($n=142$ unique stands). We designated 12 forest stand types based on the species composition and age category of dominant trees: mature aspen (>30 years old), aspen regeneration (5–15 years), lowland aspen (mixed with balsam poplar), mature red pine (>35 years), young pine plantations (8–20 years), maple, paper birch, recent timber harvests/upland brush (<5 years), black ash, lowland brush, northern white-cedar, and black spruce/tamarack. Initially, we chose stands to sample based on forest stand inventory data derived from public land spatial databases. Before sampling, however, we confirmed the stand type on the ground. We attempted to sample 10 stands of each forest type, but in some cases we could not locate that many. Furthermore, we attempted to resample the same stands in different years, but had to find alternate ones if the target stand had been harvested since the first sample.

Within each sampled stand, we temporarily marked the boundaries of 12 circular plots, each with a radius of 3 m. Plots were spaced 30 m apart in a rectangular grid (3 x 4 plots). For small stands, we used closer spacing. For stands with an obvious edge (e.g., abutting a forest road or trail), we positioned one row of plots along the edge in order to discern whether fruiting was enhanced by the increased sunlight. During the 1980s, only interior plots were sampled with the exception of a few stands during the 1988 field season. We measured canopy cover (%) within each plot, including all vegetation >2 m tall and above the fruit-bearing species, using spherical convex densitometers or by visual inspection. We calibrated visual inspections among observers and with densitometers.

We ranked abundance of fruit-producing species on a 0–4 scale based on areal coverage within the plot: 0 = 0%, Trace = 0.1–0.9%, 1 = 1–5%, 2 = 6–33%, 3 = 34–67%, 4 = 68–100%. We used the mid-point percent value for further treatment of these data (e.g., abundance rating of 2 was converted to 19%). We separately ranked fruit production, also on a 0–4 scale: 0 = little to no fruit, 1 = below average production, 2 = average, 3 = above average, and 4 = bumper crop. We considered only the number of fruits produced (per plant or per area), not their size or ripeness. To ensure that our ratings matched those of Noyce and Coy (1990), we developed our subjective scale using their fruit yield data (range of values for fruits/m² corresponding to each subjective rating). We also used their estimate of mass of each fruit (when fully ripe) to convert our subjective ratings to biomass in grams per m².

A description of the statistical procedures for analyzing data and results of statistical tests are provided in Rettler (2018). For some analyses we compared species that ripened in summer versus fall, or whether they were produced on a short versus tall shrub (Table 1).

We could not directly compare landscape level changes in fruit availability across decadal periods because forest type composition in the 1980s was not available, and forest type information for 2015–2017 was available only for public lands (federal, state, and county, comprising 69% of the land area of the study area). For the 1980s, we used visual estimates of forest composition within 500 m of telemetry flight locations of radiocollared bears, and for the 2010s we used the same set of locations, but instead of visually identifying forest types from an airplane, we used stand inventory layers for public lands. More details are provided by Rettler (2018).

RESULTS

Plant Abundance

Five fruiting species declined in abundance between the 1980s and 2010s. The most notable decline was for sarsaparilla in aspen regeneration (12% decline) and paper birch (11% decline). Abundance of beaked hazel and downy arrowwood increased between the sampling periods. The largest increase was for beaked hazel in mature aspen (13%). Notwithstanding these species-specific changes, the overall abundance of plant species groups (Table 1) that produced fruit or nuts (during the summer, fall and on short or tall shrubs) did not change between the 1980s and 2010s.

Fruit Production

Ten species declined in fruit production between the 2 decadal sampling periods; no species showed an increase. All species groups had a higher probability of producing some fruit in the 1980s (~80%) than in the 2010s (~40%; Figure 2). However, when fruits were present, there was no difference in production ratings. Increased canopy cover reduced the probability of short shrubs and summer food species producing fruit.

Edges and interior parts of stands had similar probabilities of producing some (non-zero) fruit. However, when fruits were present, edges produced more fruit. The edges of stands in the 2010s had a lower probability of producing fruit than the interior of stands in the 1980s, but there was no difference in fruit production ratings on the edges of stands in the 2010s versus the edges in the 1980s.

Fruit Biomass

Half (9 of 18) of the fruiting species sampled declined in biomass from the 1980s to 2010s (Figure 3). The difference in biomass of fruits between the 1980s and 2010s was due to the higher probability of stands producing no fruit during the 2010s, not the biomass of fruits that were present. As canopy cover increased, fruiting biomass declined. Canopy cover significantly increased from the 1980s to 2010s in black ash, mature aspen, lowland aspen–balsam poplar, and mature red pine, and declined in upland brush (Figure 4).

Landscape Biomass Availability

The availability of mature aspen on public lands increased from 28% of the study area in the 1980s to 49% in the 2010s, whereas young aspen correspondingly declined from 18% to <1% over these 3 decades. Pine plantations showed the same trend—young plantations comprised 3.4% of the landscape in the 1980s versus <1% in the 2010s, whereas mature pine increased from 4% to 8%. The reduction of young aspen and young pine on the landscape yielded a loss of >900,000 kg of bear foods on the study area, and the reduction in birch caused an additional loss of >300,000 kg from 1980s to 2010s (Figure 5). Combining all forest types, the total loss of biomass was estimated at >1.5 million kg for public lands on the study area (~37,000 ha). We estimated that state and county lands produced a higher biomass of bear foods due to shorter timber cutting cycles producing more regenerating aspen, upland brush, and pine plantations.

DISCUSSION

We observed a large (~70%) reduction in summer and fall fruit availability within forest types across the landscape from the 1980s to 2010s, which would certainly be sufficient to affect the biology and behavior of bears. The best fruit production years in the summer and fall of the 2010s were similar to food failures in the 1980s. This decline in food availability over 3 decades occurred across many forest types and was driven by 2 factors: (1) changes in composition of the landscape, with a reduction in young, productive forest types, and (2) reduced productivity in

stands of the same age and type. For the most part, it was not driven by a decline in abundance of fruit-producing plants. Most fruit-producing plants in northern Minnesota were large, woody shrubs or small trees, which would not change much in abundance without a significant disturbance. Conversely, short herb and shrub species whose vegetative structures die back and regrow each year showed a greater change in abundance. Most notable of these were sarsaparilla and raspberry.

Sarsaparilla is typically the first fruit-bearing species to ripen in the summer and was one of the most common berries found in bear scats during the 1980s (Garshelis and Noyce 2008). Since then, its abundance, production and fruit biomass have declined precipitously in many of the forest types in which it was found. Likewise, raspberry was the single largest contributor to overall fruit biomass in younger forests in the 1980s (Noyce and Coy 1990), but it has drastically declined over the past few decades. Thus, bears in this area have lost 2 early-summer staples in their diet. Additionally, several important late-summer and fall foods have declined, notably blackberry, chokecherry, and beaked hazel. During the 1980s, blackberry and chokecherry contributed the most late-summer fruit biomass in upland and lowland forests, respectively (Noyce and Coy 1990). Now these species barely contribute to the food availability in aspen regeneration, pine plantations and lowland aspen. The reduction in beaked hazel production is especially striking because this is the most important fall food across much of Minnesota, and so its abundance can strongly affect bears' vulnerability to hunting (which is done using bait) and their condition before entering dens (Garshelis and Noyce 2008, Noyce and Coy 1990).

Our results indicate that a prolonged reduction in timber harvest activity in the CNF from the mid-1990s to 2010 led to a change in forest structure (i.e., increased canopy cover), an increase in mature forest types on the landscape, and consequently a reduction in food availability. Most of the mature forest stands were >40 years old; therefore, they were in the understory reinitiation phase of stand development after timber harvest. Competition for sunlight is high during this time and understory tree species begin to fill in new gaps as the dominant tree species naturally thins. Maple was often present in the understory and mid-canopy of mature aspen, paper birch and red pine stands, and new cohorts of black ash filled in the mid-canopy in lowland aspen and black ash stands. This likely explained the increase in canopy cover of these mature forest types since the 1980s, which lowered the productivity of many of the shade-intolerant fruiting species. However, this relationship does not explain the large reduction in food availability in young forests, as well as why most of the stands in the 2010s did not produce any fruit or nuts (Figure 2). Furthermore, while the edge of forests did have higher productivity than the interior during the 2010s, due to greater light penetration, the probability of any fruit production on the edges of forests during the 2010s was still much lower than the interior of forests during the 1980s.

To investigate why many young forests and forest edges with plentiful light did not produce any food in the 2010s we considered the hypothesis that climate warming shifted the phenology of flowering to earlier in the spring and increased their exposure to damage from a later frost. While we know that killing frosts occasionally destroy berry crops in Minnesota, we did not find any evidence from the available climate and phenology data (NOAA 2018, NPN 2018) to indicate that this was more common in the 2010s than the 1980s. However, without detailed historical and contemporary information on plant phenology in relation to weather, we cannot conclusively determine whether the flowering dates have changed or whether the frequency of frost events affected fruit production.

Another hypothesis is that invasive European earthworms may be affecting fruit production. There is clear evidence that earthworms have increased in the CNF, and their infestation of the forest was still in process decades ago; because the worms were introduced from fishing bait, their colonization is strongly associated with the many fishing lakes, cabins, and road access in

the CNF (Holdsworth et al. 2007a). In fact, the CNF is the most road-dense national forest in the country, and our study site has a particularly high density of small- to medium-sized lakes accessible for fishing (Figure 1). It is known that earthworms have a major effect on the physical, chemical, microbial, and mycorrhizal composition of the soil, and this significantly reduced abundance and diversity of understory plant species in the CNF and other northern temperate forests (Bohlen et al. 2004, Hale et al. 2006, Holdsworth et al. 2007b). The large decline in abundance of wild sarsaparilla in the CNF may be explained by the rarity of this species in areas heavily infested by earthworms (Frelich et al. 2006, Holdsworth et al. 2007b). We have not seen studies relating abundance of invasive earthworms to fruit production, but this seems like a logical extension given the reduction in soil nutrients such as nitrogen and phosphorous, and consequent stress to rooting systems following an earthworm invasion (Dobson et al. 2017, Frelich et al. 2006). If this hypothesis is correct, it may mean that the decline in bear foods coincides with earthworm infestations, which are much more widespread in Minnesota forests than just the CNF. Also, as noted by Frelich et al. (2006), the relationships between earthworms and forest productivity may be exacerbated by other agents, such as deer browsing and climate change, and climate warming is likely to exacerbate the earthworm invasion in closed canopy forests (Eisenhauer et al. 2014).

This reduction in food resources could have profound impacts on bears' body condition, reproduction, cub mortality (starvation), adult mortality (hunting vulnerability), and ultimately the carrying capacity of the landscape. In an analogous case on an island in Washington state, heavy logging promoted a rich pulse of fruiting, which supported a vigorous black bear population; when logging ceased, the bear population crashed (Lindzey et al. 1986).

When natural foods are scarce, bears often seek calorically-dense anthropogenic foods such as garbage and crops, bringing them into proximity to humans and increasing their chance of being killed. For bear populations that are harvested, there is strong evidence that years of poor food availability results in more visitations to hunters' baits and, consequently, increased hunter success (Noyce and Garshelis 1997; Obbard et al. 2014). In Minnesota, hunting success has increased dramatically in recent years, which may in part be due to bears' increased reliance on hunters' baits (Garshelis and Tri 2018). Thus, there is an interaction between top-down and bottom-up pressures, which may amplify the negative impacts on the population.

ACKNOWLEDGMENTS

For assistance with fieldwork we gratefully acknowledge Daniel Dewey, Charles Fortier, Sean KonKolics, Michael McMahon, and Stefan Nelson. This project was funded in part by the Wildlife Restoration (Pittman-Robertson) Program grant W-68-D-15.

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Table 1. Fruits commonly consumed by bears in north-central Minnesota and sampled in this study (1980s and 2010s), grouped by season of ripening and structure of plant.

Common name	Scientific name	Summer food	Fall food	Short shrub	Tall shrub
Wild sarsaparilla	<i>Aralia nudicaulis</i>	X		X	
Currant	<i>Ribes spp.</i>	X		X	
Gooseberry	<i>Ribes spp.</i>	X		X	
Blueberry	<i>Vaccinium spp.</i>	X		X	
Red raspberry	<i>Rubus idaeus</i>	X		X	
Alder-leaved buckthorn	<i>Rhamnus alnifolia</i>	X		X	
Juneberry	<i>Amelanchier spp.</i>	X			X
Pin cherry	<i>Prunus pensylvanica</i>	X			X
Choke-cherry	<i>Prunus virginiana</i>	X			X
Blackberry	<i>Rubus allegheniensis</i>		X	X	
High-bush cranberry	<i>Viburnum trilobum</i>		X		X
Downy arrow-wood	<i>Viburnum rafinesquianum</i>		X		X
American hazel	<i>Corylus americana</i>		X		X
Beaked hazel	<i>Corylus cornuta</i>		X		X
Pagoda dogwood	<i>Cornus alternifolia</i>		X		X
Red-osier dogwood	<i>Cornus sericea</i>		X		X
Round-leaved dogwood	<i>Cornus rugosa</i>		X		X
Wild plum	<i>Prunus americana</i>		X		X

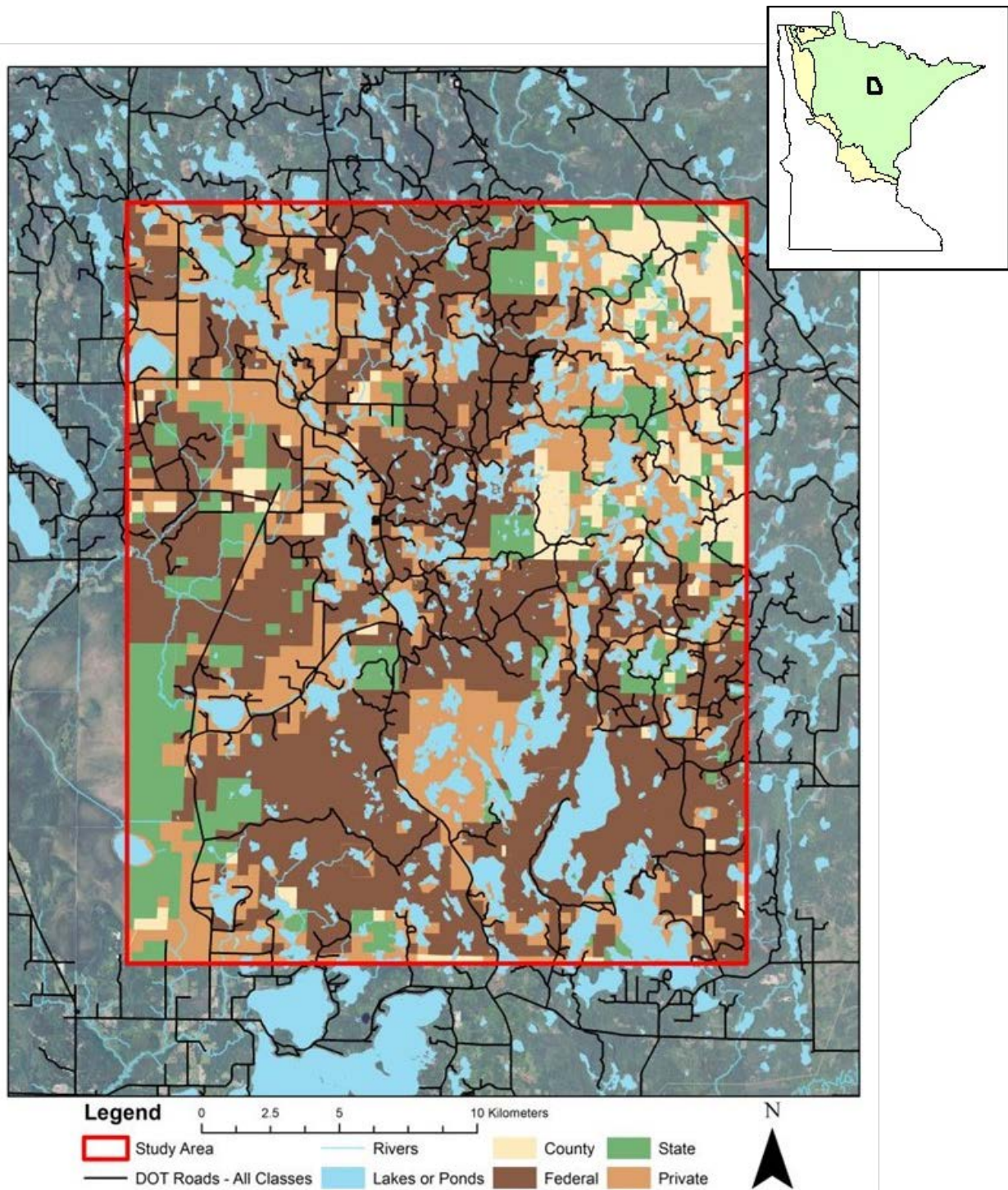


Figure 1. The study area, located in the center of primary bear range in Minnesota (green area in upper right inset) is largely public land (dominated by the Chippewa National Forest), with a high density of roads and lakes.

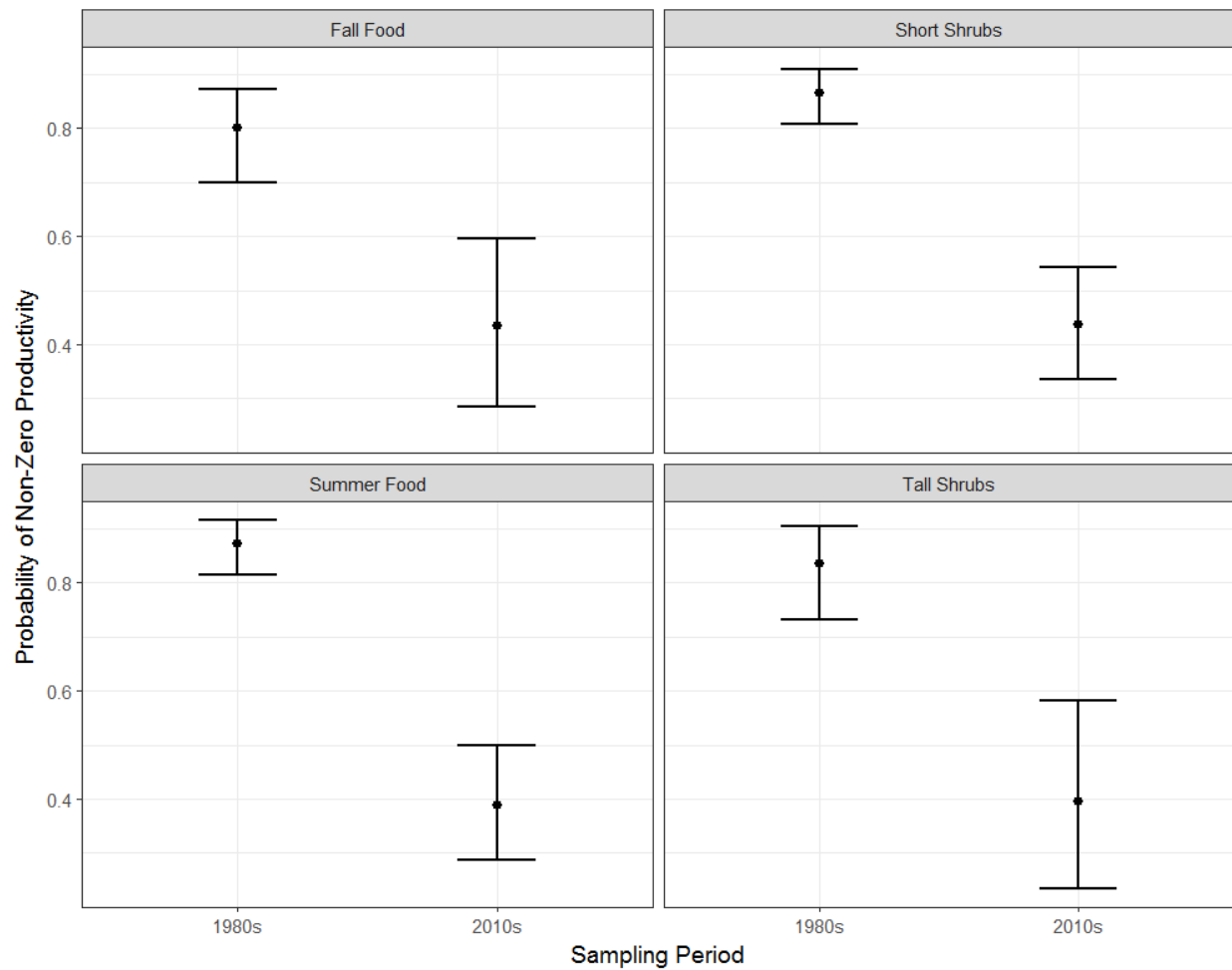


Figure 2. Probability of some fruit production (i.e., non-zero production) declined from $\geq 80\%$ in forest stands in the 1980s to $\sim 40\%$ in the 2010s for summer and fall bear foods, and short and tall shrubs (or small trees) producing bear foods (i.e., results were the same seasonally and for all plant types) in the study area in Minnesota.



Figure 3. Mean biomass of bear foods produced in different types of forest stands in the CNF study area in Minnesota during the 1980s versus 2010s. ASH=black ash, ASP=mature aspen, BIR=birch, CED=white cedar, LAS=lowland aspen, LBR=lowland brush, MAP=maple, PIN=red pine, PLA=pine plantation (8–20 years old), REG=aspen regeneration (5–15 years old), SPR=spruce-tamarack, UBR=upland brush/clearcuts.

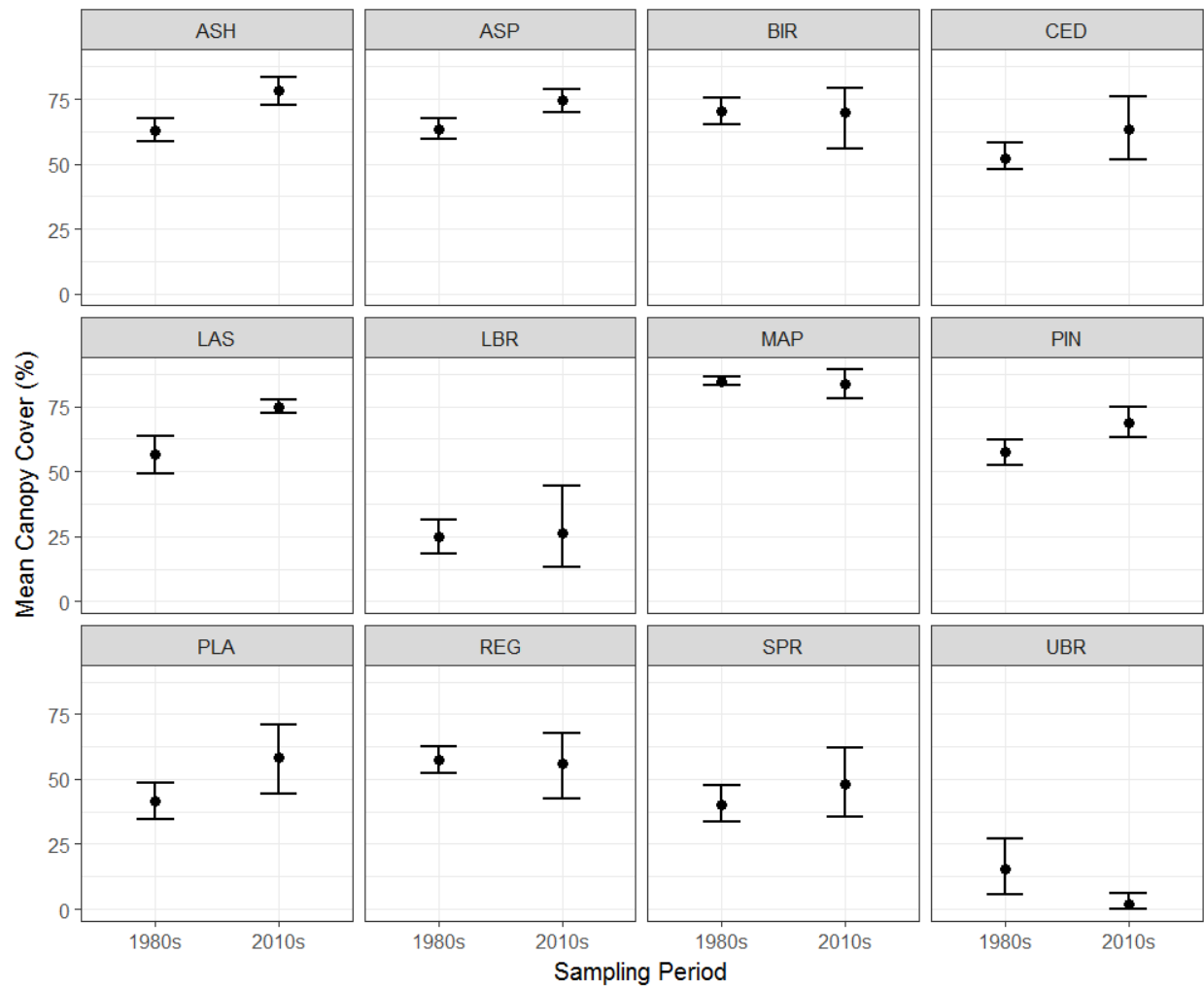


Figure 4. Mean canopy cover in different forest types (abbreviations as in Figure 3) in the CNF study area in Minnesota during the 1980s versus 2010s. Error bars depict 95% bootstrapped confidence intervals.

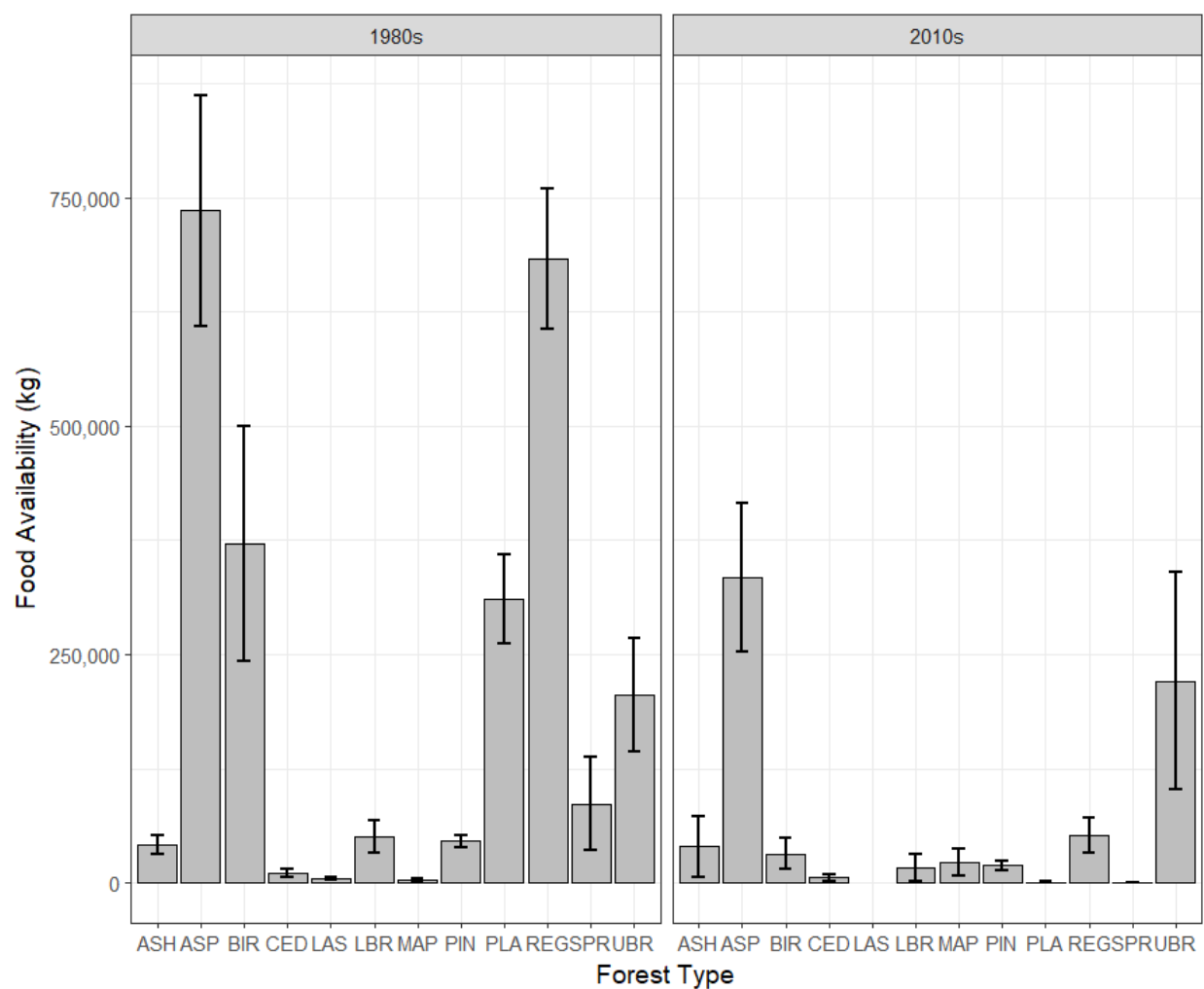


Figure 5. Estimated biomass of fruits important in the diet of bears in the CNF study site in Minnesota (estimated for public lands: 37,000 ha) during the 1980s versus 2010s in different types of forest stands (abbreviations as in Figure 3). The change from the 1980s to 2010s reflect a combination of (a) changes in amount of each type of forest in the study area, (b) changes in amount of fruit-producing plant species, and (c) changes in fruit production.



USING LIDAR DATA TO QUANTIFY FOREST STRUCTURAL HABITAT VARIABLES IMPORTANT TO FISHERS AND MARTENS

Michael Joyce¹, John Erb, Barry Sampson, and Ron Moen²

SUMMARY OF FINDINGS

Fishers (*Pekania pennanti*), martens (*Martes americana*), and many other wildlife species rely on three-dimensional structural habitat characteristics to provide essential resources. Spatially-continuous data on fine-scale structural habitat features are generally not available across large landscapes because passive remote sensing systems are not capable of measuring three-dimensional characteristics and because it is financially and logistically challenging to collect field-data continuously across the landscape. Light detection and ranging (LiDAR) is an active remote sensing technology capable of providing accurate, high-resolution data on three-dimensional vegetation structure across large spatial extents. Many past studies have demonstrated that LiDAR data can be used to map coarse- and fine-scale habitat characteristics at the scale of individual trees, field plots, or forest stands. However, most research has focused on forestry applications, and relatively few studies have focused on modeling structural variables that serve as basic wildlife habitat indicators.

We were interested in using LiDAR to supplement field data collected as part of a long-term project on fisher and marten ecology in Minnesota. Our objectives were to evaluate the potential of LiDAR technology to quantify both coarse- and fine-scale forest habitat metrics and to evaluate the effect of pulse density on prediction accuracy. We acquired high-density LiDAR data (8 pulses/m²) for a portion of our marten study area and selected 200 random locations within that portion to collect detailed vegetation measurements. Random sites were selected using a LiDAR-informed stratified random sampling design. We measured vegetation on 189 of the 200 plots during summer 2015 and 2016; the remaining plots could not be sampled due to wind disturbances that altered forest structure after LiDAR data collection. Statistical analyses are ongoing, and we defer reporting results until final analyses are completed.

INTRODUCTION

To create and implement effective habitat management plans, wildlife managers depend on reliable knowledge of species-specific habitat requirements, accurate information on the current abundance and distribution of suitable habitat features, and an understanding of how management actions influence habitat suitability over a range of spatio-temporal scales. In many situations, having accurate information on abundance and distribution of habitat characteristics is necessary for understanding species-specific habitat requirements and evaluating how management actions influence habitat use. Forest wildlife species vary in their dependence on specific habitat characteristics. For some species, habitat requirements may be adequately

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described using coarse-resolution data such as forest cover type, stand age or successional stage, or proximity to permanent water or other specific landscape features. For these species, broad-scale forest inventory data and GIS layers derived from passive remote sensing technologies (e.g., satellite imagery, aerial photographs) are often adequate to map and monitor changes in habitat quality. However, other wildlife species, including fishers, martens, and many forest songbirds, respond to three-dimensional, structural habitat features at fine spatial scales. Spatially-continuous data on fine-scale structural features generally are not available because passive remote-sensing systems are not capable of measuring three-dimensional characteristics and because it is financially and logistically challenging to collect fine-scale, field-based measurements continuously across large areas. Instead, habitat models for these species typically incorporate information gathered from detailed field-sampling at sites used by the species of interest, often for specific purposes (e.g., foraging, nesting, or denning sites). While site-level habitat models created from field data provide informative and mechanistic insights into a species' habitat requirements, they are often difficult to apply to larger scales at which forest management decisions are generally made. Regardless of whether a species relies on coarse- or fine-scale characteristics, having data on forest characteristics at continuous spatial scales is critical for sound habitat management and assessment.

Light detection and ranging (LiDAR) is an active remote sensing technology capable of providing accurate, high-resolution (<1 to >20 laser pulses/m²) data on three-dimensional physiographic and vegetative structure over large spatial extents (e.g., entire study areas or wildlife management units up to statewide coverage; Merrick et al. 2013, Vierling et al. 2008). LiDAR data are collected from a scanner that emits frequent, short-duration laser pulses and records the reflected signal returning to the sensor. As the emitted laser pulse is intercepted by an object or surface (e.g., vegetation, building, terrain), a portion of the laser energy is reflected and returned to the sensor. Discrete-return LiDAR systems record the spatial coordinates where the laser pulse intercepted an object or surface, resulting in a three-dimensional "cloud" of interception points or "returns". Modern discrete-return LiDAR systems are capable of recording ≥ 4 returns per laser pulse (Vierling et al. 2008).

High pulse density, multiple-return LiDAR data provide the detail necessary to accurately map a variety of forest structural attributes including both fine-scale attributes (e.g., canopy height [Means et al. 2000], canopy cover [Lefsky et al. 2002], shrub-density [Martinuzzi et al. 2009]) and coarse-scale attributes (e.g., forest successional stage [Falkowski et al. 2009]) continuously and with high precision across the landscape. Because of these capabilities, LiDAR is increasingly used to analyze forest structure and is becoming an integral part of operational forest management (White et al. 2013). LiDAR can be used to measure biophysical variables at the level of individual trees, forest inventory plots, and forest stands (Falkowski et al. 2006, White et al. 2013). Forest inventory metrics that have been successfully predicted at the plot and stand level using LiDAR include canopy height (Hawbaker et al. 2009, Thomas et al. 2006), canopy density or volume (Lefsky et al. 2002, Martinuzzi et al. 2009), basal area (Means et al. 2000, Woods et al. 2011), average diameter at breast height (Hawbaker et al. 2009, Jakubowski et al. 2013), tree density (Treitz et al. 2012), and forest biomass (Thomas et al. 2006, Treitz et al. 2012, Woods et al. 2011). LiDAR data can be used to make direct estimates for some attributes such as canopy cover, canopy height, and canopy volume (Graf et al. 2009, Lefsky et al. 2002, Merrick et al. 2013). However, many structural metrics require accurate field-plot data that can be used to build predictive models from LiDAR-derived explanatory variables. Overall, studies have focused on forestry-specific metrics and there has been less work focused on predicting structural attributes important to wildlife (but see Goetz et al. 2010, Graf et al. 2009, Hagar et al. 2014, Martinuzzi et al. 2009).

The potential for LiDAR to improve wildlife research and management has been recognized for some time. LiDAR data can be used to improve wildlife-habitat modeling in 2 different ways (Merrick et al. 2013, Vierling et al. 2008). First, it provides a tool that can be used with telemetry data or known species distributions to better understand resource selection. Forest attributes can be measured at fine spatial scales with LiDAR, allowing researchers to assess resource use at scales near those at which animals respond to structural attributes (Vierling et al. 2008). By providing spatially-continuous data, LiDAR data allows researchers to directly address how both landscape composition and configuration influence habitat selection. Furthermore, LiDAR can be used to investigate resource selection across a wide range of spatial scales including sites used for specific behaviors, individual home ranges, and entire wildlife management units or other regional units. Second, LiDAR can be used to predict habitat suitability or species distributions based on prior knowledge of habitat requirements or life-history characteristics. The ability to translate habitat models into spatially-explicit maps is particularly useful for wildlife management, for example, by providing accurate predictions of the distribution and abundance of suitable habitat or by allowing managers to monitor changes in habitat suitability through time with repeated LiDAR acquisitions.

Fishers and martens are two species that could benefit from LiDAR-based habitat modeling because they respond to both coarse- and fine-scale forest attributes (Joyce 2013, Raley et al. 2012, Thompson et al. 2012), habitat loss from human land use is thought to be a major threat to population persistence for both species (Proulx et al. 2004), and continuous data on fine-scale attributes required by fishers and martens are not currently available. At coarse scales, fishers and martens show strong selection for mature and old-growth forest conditions (Buskirk and Powell 1994), although both species have been documented using a variety of seral stages (Joyce 2013, Raley et al. 2012, Thompson et al. 2012). Fine-scale attributes, however, appear to drive fisher and marten habitat selection at multiple spatial scales. Both species depend on large-diameter cavity trees and other specific forest structures that serve as rest sites and reproductive dens (Joyce 2013, Raley et al. 2012, Thompson et al. 2012). Sites used for resting and denning typically have dense overhead cover, abundant coarse woody debris (CWD), and large-diameter trees (Aubry et al. 2013, Joyce 2013, Thompson et al. 2012). CWD provides subnivean access (Corn and Raphael 1992) and is a critical component of marten winter foraging behavior in the boreal forest (Andruskiw et al. 2008). At landscape scales, shrub cover (Slauson et al. 2007) and canopy cover (Cushman et al. 2011, Shirk et al. 2014) are associated with home ranges selected by martens. Furthermore, canopy cover is one of the strongest and most consistent predictors of fisher habitat use across spatial scales (Raley et al. 2012).

Despite the amount of research focused on understanding fisher and marten habitat requirements, there are critical aspects of habitat ecology that are not well understood. For example, several studies have suggested that availability of suitable denning habitat could limit fisher and marten populations (e.g., Ruggiero et al. 1998), but few studies have investigated distribution of suitable denning habitat, in part because continuous fine-scale data are needed to apply den-site habitat models across the landscape but are generally not available. Furthermore, most studies have focused on landscape composition, but landscape configuration likely also drives habitat use (Sauder and Rachlow 2014), and landscape configuration is strongly influenced by ownership and management history (Cohen et al. 2002, Kennedy et al. 2012, Spies et al. 1994). Because of their dependence on structural features that have been accurately predicted using LiDAR, LiDAR data has the potential to provide novel insights into fisher and marten habitat ecology and improve habitat management for these species.

Many of the resources exist for LiDAR data to be incorporated into natural resource management in Minnesota. Minnesota is one of a growing number of states for which statewide LiDAR data have already been acquired. One important question that still needs to be

addressed to use the statewide data or direct future LiDAR acquisitions is what pulse density is required to accurately quantify forest structural attributes at plot and stand levels. LiDAR acquisition costs increase with increasing pulse density (Jakubowski et al. 2013). Therefore, acquiring LiDAR data at the minimum pulse density necessary for accurate predictions will enable researchers and managers to maximize gain from finite resources. Previous research has shown that many forest metrics can be accurately predicted at fairly low pulse densities and that higher pulse density does not necessarily improve model accuracy, but the effect of pulse density on model accuracy depends on the variable of interest (Thomas et al. 2006, Treitz et al. 2012, Jakubowski et al. 2013). In general, the structural variables measured in these studies are strongly biased toward forestry applications. Although some of the biophysical variables evaluated are important indicators of wildlife habitat, a better assessment of how pulse density affects wildlife-specific forest attributes (e.g., canopy structure, CWD, shrub cover) is necessary before LiDAR can be used in the same operational capacity for wildlife management as it is currently being used for forestry.

Our objective was to evaluate the potential of LiDAR technology to quantify both coarse- and fine-scale forest habitat variables and to create applied GIS tools that can be used in day-to-day decision-making by forest and wildlife managers. Additionally, we will evaluate the effect of pulse density on prediction accuracy. This project will provide new information and tools for applied habitat management for fishers and martens, and will also increase the value of data already collected in ongoing research on fisher and marten ecology. Combining LiDAR-derived estimates of forest structural attributes with location data from radiocollared fishers and martens will enable us to address important research questions aimed at improving management of these species in Minnesota.

STUDY AREA

Marten research has taken place in portions of east-central St. Louis and west-central Lake counties in northeastern Minnesota (Figure 1). The marten study area (~1250 km²) is composed of a variety of forest types including upland mixed coniferous-deciduous forest, lowland conifer or bog, upland coniferous forest, and regenerating forest, as well as marshes, fens, shrublands, and anthropogenic cover types. We acquired high-density LiDAR data for a 65 km² portion within the larger marten study area during spring 2014 (Figure 1). The location of the high-density LiDAR acquisition was chosen because it included a large number of locations from radiocollared fishers and martens (i.e., rest sites, dens, and aerial telemetry locations), it encompassed ~100 ground-based vegetation survey sites measured previously as part of the larger fisher/marten research project, and it contained almost all of the forest types and successional stages available throughout the larger marten study area. Both the marten and embedded LiDAR study areas are predominantly public ownership including portions of the Superior National Forest, state, and county lands.

METHODS

There are two LiDAR datasets available that provide variable coverage of our study area (Table 1). Both datasets are discrete, multiple-return LiDAR data acquired from fixed wing aircraft during leaf-off conditions. The first dataset (hereafter, statewide data) was collected during spring 2011 as part of the Minnesota elevation mapping project (<http://www.mngeo.state.mn.us/chouse/elevation/lidar.html>) and provides complete coverage for Carlton, Cook, Lake, and St. Louis counties. The second dataset (hereafter, high-density data) was acquired in spring 2014 over a 25 square-mile portion of the marten study area. In general, specifications from both datasets (Table 1) match recommendations for forest inventory analysis (White et al. 2013). Those that do not (e.g., scan angle) are consistent with published studies

that have successfully modeled forest structure using LiDAR (e.g., Treitz et al. 2012 used a scan angle of $\pm 20^\circ$).

Several pre-processing steps are necessary prior to vegetative analysis. Raw LiDAR return points must be classified as ground or non-ground (e.g., vegetation, water, buildings) returns and manual quality assurance/quality control (QA/QC) steps must be taken to verify data conform to desired specifications. Digital elevation models (DEMs) are then created from ground returns and converted to digital terrain models (DTMs). Pre-processing steps have been completed for statewide data. For the high-density LiDAR data, we are using LP360 (QCoherent Software, LLC) for LiDAR point classification and DEM construction.

We are using the area-based approach to create predictive models of forest structural attributes that relate to habitat quality for marten. The area-based approach combines field-plot and LiDAR data to create predictive statistical models that can be projected across an entire landscape (White et al. 2013). The area-based approach has 4 main steps: 1) collect and summarize field-plot data; 2) extract and summarize LiDAR data corresponding to field sampling locations; 3) create and evaluate predictive models; and 4) apply models across the area of interest. Additionally, we are evaluating whether LiDAR can be used to directly detect individual pieces of CWD.

We measured forest inventory plots at random sites distributed throughout the high-density LiDAR acquisition area. We used a stratified random sampling design to ensure field sampling covers a large range of the forest conditions present on our study area (Hawbaker et al. 2009, White et al. 2013). We calculated mean LiDAR return height (m above ground) and standard deviation of return height for each 20- x 20-m cell in the study area to represent the range of structural conditions present throughout the landscape (Figure 2). Each cell in forest condition represented a potential sample location. Sample locations were further stratified into upland and lowland soil types using ecological landtype classifications from the Superior National Forest's terrestrial ecological unit data to ensure sampling covered a variety of soil types. For each broad soil type category, the available sampling space defined by the two LiDAR metrics was divided into 8 quantiles for mean return height and 2-3 quantiles for the standard deviation of return height to form 23 sample strata per soil type (Hawbaker et al. 2009). We selected a total of 200 random locations to sample. The number of locations selected per stratum was proportional to the total number of available cells in each stratum throughout the entire study area.

At each randomly-selected location, we measured structural variables within a 400-m² (11.3-m radius) circular plot. Plot size was selected to match recommendations for LiDAR-based forest inventory modeling (Laes et al. 2011, White et al. 2013) and corresponds to a 20-m pixel for landscape-level application of predictive models. Structural attributes were selected based on their importance to marten habitat from published literature (e.g., Andruskiw et al. 2008, Allen 1982, Raphael and Jones 1997, Slauson et al. 2007) and previous research in Minnesota (Joyce 2013; Table 2). Sampling protocols were largely based on United States Department of Agriculture (USDA) Forest Inventory and Analysis program protocols to maintain consistency with previous data collected at rest sites and reproductive dens used by radiocollared marten in Minnesota (Joyce 2013). All field measurements were taken in full leaf-on condition, although canopy cover and understory density also were sampled during leaf-off condition for a subset of field plots. During field sampling, locations of field plots were recorded using both consumer-grade (Garmin eTrex 30) and mapping-grade GPS receivers (Garmin SXBlueII+GNSS). The mapping-grade receiver communicated with both GPS and GLONASS satellites and utilized a combination of space-based augmentation system (SBAS) and real-time differential correction to obtain precise locations without post-processing. When using the mapping-grade GPS, we collected points for ≥ 30 minutes at a rate of ~ 20 points/min. Preliminary data at geo-referenced

survey markers suggested mapping-grade GPS locations collected this way provided sub-meter accuracy under full forest canopy (Joyce, unpublished). For the consumer-grade GPS, we used location averaging for ≥ 30 minutes.

LiDAR can be used to directly measure a subset of the forest attributes being measured at field plots (e.g., canopy height, canopy cover/closure, canopy structure metrics; Merrick et al. 2013, White et al. 2013), and we are currently evaluating whether LiDAR data can be used to detect individual pieces of CWD. For remaining attributes, we will create predictive statistical models using LiDAR metrics as explanatory variables and attributes summarized from field plot data as response variables. We will use FUSION software (McGaughey 2013) to extract LiDAR point clouds corresponding to field plots and summarize statistical properties of individual point clouds based on return height, return intensity, or point density for use as explanatory variables in statistical modeling.

The type of statistical model we used depended on the structural characteristic. We used multiple linear regression for continuous variables (e.g., average diameter at breast height). We used Poisson or negative binomial GLM count models for count variables (e.g., tree density). Snags were not present at a large number of plots. Consequently, Poisson GLM count models and multiple linear regression could not account for inflated zeros, and use of these types of statistical models could produce biased estimates of snag characteristics (Russell 2015, Zuur and Ieno 2016). We used zero-altered (hurdle) models for snag density (zero-altered Poisson), snag volume (zero-altered gamma), and average snag diameter (zero-altered gamma). Zero-altered models have 2 components (Zuur and Ieno 2016). The first component accounts for presence/absence of snags, while the second component accounts for snag density, volume, or diameter if snags were present.

Despite differences in model type, we used the same statistical framework for all forest structural variables. There are 3 steps in the statistical framework: 1) model-fitting and model selection, 2) model evaluation using cross-validation, and 3) model re-calibration. First, for each response variable, we created a set of candidate models using individual predictor variables or combinations of non-collinear predictor variables. The number of predictor variables included in multi-variate models did not exceed sample-size-based recommendations to avoid over-fitting data (Babyak 2004, Guidice et al. 2012). Models were fit in Program R (R Development Core Team, 2013) using techniques and packages best-suited to the type of model being fit. Candidate models were compared using an information-theoretic approach to select the best-supported model(s) from the candidate set (Burnham and Anderson 2002). Candidate models were chosen based on expected relationships between response variable and individual predictor variables. Second, we evaluated how well best-supported models predicted new data using a five-fold cross-validation procedure. We evaluated each cross-validation set using root mean squared error (RMSE), R^2 , and bias. Finally, we used a bootstrapping procedure to re-calibrate model coefficients in an effort to reduce the effect of over-fitting and therefore improve prediction accuracy (Harrell 2001, Giudice et al. 2012, Fieberg and Johnson 2015).

To evaluate the effect of LiDAR pulse density on accuracy of predictive models we will subsample LiDAR data to obtain 7 different pulse densities (8, 6, 4, 2, 1, 0.5, and 0.25 pulses/m²) using FUSION software. Subsampling will be performed in a way that accurately simulates data acquired at specific pulse densities (i.e., we wish to thin the density of laser pulses rather than the number of returns per pulse). Predictive models will be created at each pulse density, and prediction accuracy will be plotted as a function of pulse density (Jakubowski et al. 2013). Prediction accuracy will be assessed using R^2 , RMSE, and bias. From these plots we will determine the minimum pulse density necessary to create accurate predictive models (turning point, *sensu* Jakubowski et al. 2013) as well as the pulse density corresponding to the most accurate predictive model (best accuracy *sensu* Jakubowski et al. 2013). Results from

this analysis will determine which forest attributes can be predicted throughout the entire marten study area using statewide LiDAR data (0.45 pulses/m²).

RESULTS AND DISCUSSION

Pre-processing steps (QA/QC, point classification, DEM creation and conversion) have been completed for the statewide LiDAR data. High-density LiDAR data were collected during spring 2014 and delivered from the vendor during fall 2014. We have completed QA/QC on the high-density data and classified returns for large portions of the dataset. We are still refining point classification protocols, and final point classification should be complete during summer 2018. DEMs will be created and converted to DTMs once we complete point classification. Additional information about point classification and DEM construction is not provided here because methodology is still being refined.

Our 200 randomly-selected field plots included 115 plots in upland soil types and 85 plots in lowland soil types. During summer 2015, we measured 100 forest inventory plots. Data from these plots have been entered and checked for errors. We measured 89 additional plots during summer 2016, and completed data entry for all plots. We were not able to measure all 100 remaining plots in 2016 because wind storms altered some of the pre-selected plots before we could measure them. The final set of 189 field plots includes 110 plots in upland soil types and 79 plots in lowland soil types. We have started preliminary statistical analyses, but we defer results until all statistical analyses are completed.

ACKNOWLEDGEMENTS

We thank Josh Swanson, Stefan Nelson, Luke O'Neil, Emma Licht, Megan Gorder, Nathan Ose, Rachel Voorhorst, Andrew Leider, Brian Houck, Kelcy Huston, Peter Kienzler, Michael Jaksa, and Josh Kircher for assistance collecting field data. This project was funded in part by the Wildlife Restoration Program (Pittman-Robertson).

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Table 1. Specifications for statewide (2011-12) and high-density (2014; portion of St. Louis County) LiDAR datasets collected in Minnesota.

Specifications	Statewide	High-resolution
Acquisition Date(s)	Spring 2011 & Spring 2012	Spring 2014
Vendor	Wolpert, Inc.	AeroMetric, Inc.
Laser System(s)	ALS60, ALS70, and Optech GEMINI	ALS70
Altitude	2000-2300 m	1050 m
Flight Speed	240 - 278 km/h	278 km/h
Scan Angle	± 20°	± 20°
Side Overlap	25%	50%
Nominal Point Spacing	≤ 1.5 m	≤ 0.35 m
Pulse Density	0.45 pulses/m ²	8.0 pulses/m ²
Vertical Accuracy	5.0 cm (RMSE)	6.7 cm (RMSE)
Horizontal Accuracy	1.16 m (95% confidence)	100 cm

Table 2. Partial list of forest attributes that will be estimated using LiDAR data collected in Minnesota from 2011-14. Attributes were selected because of their biological significance to martens.

Forest attribute	Biological significance	Citation(s) ^a
Coarse woody debris density/volume	Prey habitat, facilitates prey capture, subnivean access, rest and den site characteristic	Andruskiw et al. (2008), Corn & Raphael (1992), Joyce (2013)
Tree diameter at breast height (dbh)	Indicator of stand age, related to arboreal denning and resting structures	Raphael & Jones (1997), Slauson & Zielinski (2009)
Basal area	Indicator of stand age, related to arboreal denning and resting structures	Payer & Harrison (2003,2004)
Canopy closure	Open canopy forests and non-forested habitat associated with predation risk and low prey availability	Slauson et al. (2007), Moriarty et al. (2015)
Canopy structure/heterogeneity	Associated with structural diversity of stands	Zielinski et al. (2006), Weir et al. (2012)
Stand height	Indicator of developmental stage	Bowman & Robitaille (1997)
Sapling density	Provides habitat for prey species (snowshoe hare) and may serve as escape cover	Carreker (1985), Slauson et al. (2007), Joyce (2013)
Shrub density	Provides habitat for prey species (snowshoe hare) and may serve as escape cover	Carreker (1985), Slauson et al. (2007)
Snag density/volume	Indicator of stand age and vertical complexity	Gilbert et al. (1997); Slauson & Zielinski (2009)
Horizontal cover	Related to sapling and shrub density; may serve as escape cover or provide habitat for prey species (snowshoe hares)	Carreker (1985), Slauson et al. (2007)

^aCitation for biological significance of attribute to martens.

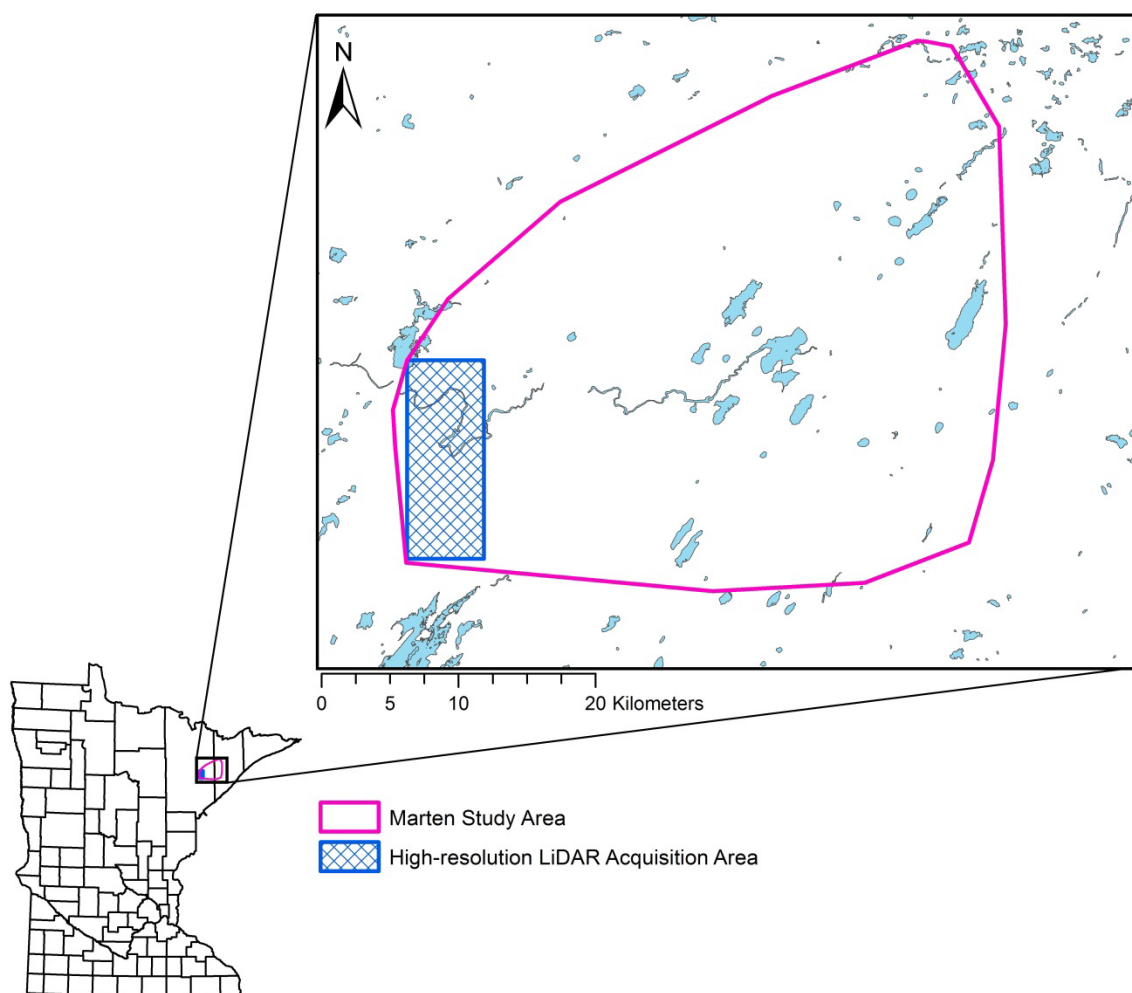


Figure 1. Map of primary marten study area in northeastern Minnesota with the location where high-density LiDAR data were acquired in 2014.

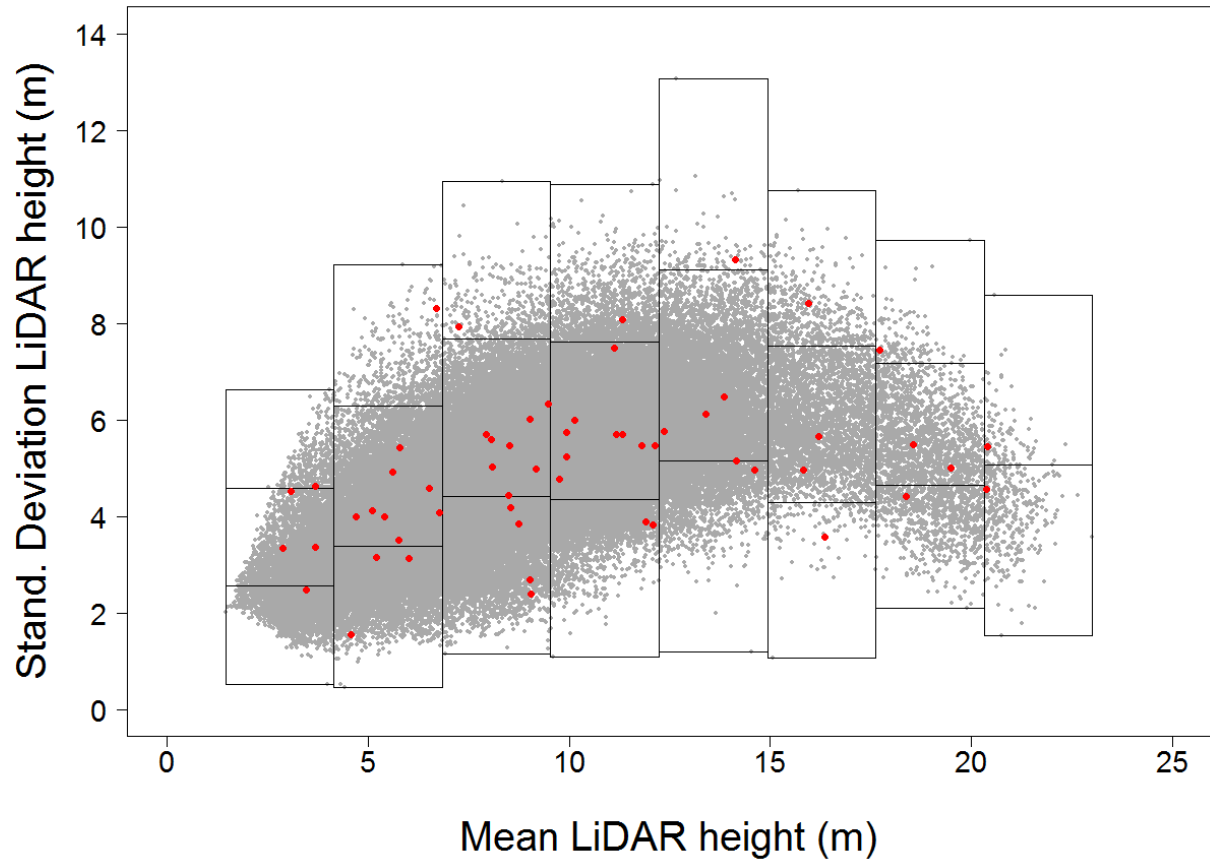


Figure 2. Sampling space for LiDAR-informed stratified random sampling design in a 25 mile² portion of St. Louis County, Minnesota. Structural variability within the study area is represented by mean and standard deviation in LiDAR return height for each 20 m pixel in the study area (gray circles). Black squares represent strata from which a random sample of plots was selected (red circles) and surveyed from 2014-16. Stratification was performed separately for areas with upland and lowland soil types.



ESTABLISHING THE FEASIBILITY OF MAKING FINE-SCALE MEASUREMENTS OF HABITAT USE BY WHITE-TAILED DEER IN NORTHERN MINNESOTA, WINTER 2017–2018

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SUMMARY OF FINDINGS

The Minnesota Department of Natural Resources (MNDNR) began a 2-year pilot study of white-tailed deer (*Odocoileus virginianus*) habitat in northcentral and northeastern Minnesota during winter 2017–2018. This study is using cutting-edge global positioning system (GPS) collar, remote sensing, and geographical information system (GIS) technologies to monitor and assess deer habitat use on 2 winter ranges. Prior to capturing and handling deer, we tested the spatial location-fix accuracy, and transmission- and fix-success rates for 12 GPS collars placed in 4 different (3 in each) cover types. We documented a 93% GPS-fix transmission-success rate and 100% overall fix-success rate. Overall, the mean location error using transmitted data was 6.2 m (± 0.68 [standard error]). Collars in dense conifer cover had a greater mean location error ($F_{3,798} = 33.2$; $P < 0.001$) than collars located in the other 3 cover types (hardwood, browse, and open). There was no difference in transmission- or fix-success rates among collars in the 4 cover types ($P > 0.05$). Of the 1,008 locations downloaded from the test collars, 89% (897) had a location error < 10 m and 98% (988) had a location error < 25 m. During 10 March–19 May 2018, we recovered 6 of 20 collars that had been fitted to free-ranging deer. These collars stored 3,093 locations on-board (100% fix-success) and successfully transmitted 2,165 (80%) GPS locations. The mean horizontal error was 15 m (± 0.22). We classified a total of 465 cover types within the Inguadona Lake study area over 3,969 ha. Dense conifer stands (473 ha) and forage types (488 ha) each accounted for about 12% of the site. Using locations of GPS-collared deer in dense conifer stands, they were a mean 162 m (± 44) to the nearest forage opening and 107 m (± 26) to the center of the stand being used. For deer locations in forage openings, they were a mean of 87 m (± 22) to the nearest dense conifer stand and 162 m (± 42) to the center of the forage opening in use. The mean area of dense conifer stands and forage openings being used was 12 ha (± 2.55) and 13 ha (± 3.55), respectively. The ability to make fine-scale measurements of available habitat and habitat use employing GPS collars, remote sensing tools, and GIS will allow us to assess the area, shape, juxtaposition, and arrangement of dense conifer cover and forage openings on winter ranges and provide more useful information to support forest management prescriptions.

INTRODUCTION

Based on recommendations from the Office of the Legislative Auditor (OLA), the Minnesota Department of Natural Resources (MNDNR) is developing a statewide white-tailed deer (*Odocoileus virginianus*) management plan to maintain deer numbers within management units

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and near population goals for improved hunting and wildlife viewing (MNDNR 2018). Habitat management is a key component of this plan. Because winter is the nutritional bottleneck for northern deer, has the greatest impact on their natural survival rates, and may have a pronounced impact on spring fawning, wildlife managers focus most of their efforts on improving winter habitat as a means of positively influencing population performance (DelGiudice et al. 2002, 2006, 2013a). During winter 2017–2018, the MNDNR initiated a deer habitat study in northcentral and northeastern Minnesota, which is using a combination of global positioning system (GPS) collar, remote sensing, and geographic information system (GIS) technologies to better understand deer use of cover types and how area, shape, juxtaposition, and arrangement of conifer stands, forage openings, and other cover types influence their use (DelGiudice et al. 2017).

Previous studies of deer use of winter habitat and requirements in northern Minnesota, Canada, and the Great Lakes region that relied primarily on very high frequency (VHF) telemetry collars were restricted by inherent constraints compared to the use of more advanced GPS collars (Morrison et al. 2003; Potvin et al. 2003; DelGiudice et al. 2013a,b, 2017). Constraints included lower location-fix accuracy (≥ 95 m), limited temporal distribution of location-fixes (i.e., daytime locations only), fair weather (i.e., safe flying conditions for fixed-wing aircraft), relatively infrequent location-fixes (i.e., small numbers of seasonal locations per individual deer), and greater costs (time and monetary) required to collect the data (Pellerin et al. 2008, Kochanny et al. 2009). Due to these limitations, more precise information regarding winter habitat use is essential to a more thorough understanding of seasonal habitat requirements of deer and to accurately prescribe management strategies.

Advancements in technology have allowed for notable enhancements in performance of GPS collars. With improved accuracy and precision of location-fixes and higher fix-success and transmission-success rates, GPS collars facilitate collection of a plethora of near real time data, including habitat use and selection, movement rates, and interspecific interactions. Before collar deployment and assessing winter habitat use by deer, the influence of canopy closure and cover type on their performance requires testing (Rempel et al. 1995, Dussault et al. 1999). Studies have shown that different habitats have diverse, adverse effects on GPS collar performance (e.g., accuracy, fix-success), associated specifically with varied canopy cover, stem density, basal area, and topography (Moen et al. 1996, Rempel and Rogers 1997, Dussault et al. 1999). However, recently, Telonics, Inc., a GPS collar manufacturer in Mesa, Arizona, incorporated programming for Quick Fix Pseudorange (QFP) into their Globalstar Recon collars, which enhances their ability to obtain accurate location-fixes with as little as a 3–5-second view of a satellite constellation, compared to the 30–90 seconds required for a normal GPS location-fix; this is particularly valuable to studies of habitat use by deer and other ungulates.

Use of improved GPS collar technology has the potential to maximize accurate location data not obtainable in studies using VHF telemetry or less sophisticated GPS collars, and to facilitate fine-scale measurements of habitat use. These data permit 24-hour monitoring of habitat use to better understand (1) individual variability associated with selection of forest cover types, and (2) how structure, size, shape, arrangement, interspersions, and perimeter (edge):area influence habitat use at the stand level (DelGiudice et al. 2017).

OBJECTIVES

1. To assess GPS collar performance prior to deployment, including spatial location error and transmission- and fix-success rates relative to different cover types
2. To classify and inventory cover types for the Inguadona Lake study site, 1 of the 2 sites in this study

3. To provide examples of fine-scale measurements of winter habitat use by deer

STUDY AREA

The study includes 2 deer winter range sites located in northern Minnesota's forest zone (Figure 1). The Inguadona Lake (ING) site is located in the northcentral part of the state in Cass County, 2 km south of the Chippewa National Forest border. This site is 76 km² and is a mosaic of state, county, and private land, with most of the latter occurring along lake shores. Reported pre-fawning deer densities in this area were 7–9 deer/km² (D'Angelo and Giudice 2016), and included both residential deer (year-round) and seasonal migrators (Fieberg et al. 2008). Topography is undulant with elevations of 400–425 m above sea level. The area is classified as part of the Pine Moraines region (MNDNR 2015), and includes uplands dominated by deciduous and mixed deciduous-conifer stands and lowlands dominated by mixed conifers. The uplands included red (*Pinus resinosa*), white (*P. strobus*) and jack pine (*P. banksiana*); paper birch (*Betula papyrifera*); black ash (*Fraxinus nigra*); red maple (*Acer rubrum*); balsam fir (*Abies balsamea*); and trembling aspen (*Populus tremuloides*; DelGiudice 2013a.). Lowlands included northern white cedar (*Thuja occidentalis*), black spruce (*Picea mariana*), balsam fir, and tamarack (*Larix laricina*).

The MNDNR calculates an annual Winter Severity Index (WSI) by accumulating 1 point for each day with an ambient temperature $\leq -17.7^{\circ}\text{C}$ and an additional point for each day with a snow depth ≥ 38 cm during November–May. During 1981–2010, mean January temperature was -13°C and mean annual snowfall was 110 cm (MNDNR Climatology 2018). Over the past 8 years, WSI in the ING study area indicated moderately severe or severe conditions in just 1 winter (2013–2014; WSI ≥ 140 ; MNDNR Climatology 2018).

The Elephant Lake (EL) site, located in St. Louis County, is representative of the forest zone in northeastern Minnesota. The EL site is 120 km² and includes state, federal, county, and private land. Pre-fawning deer densities are lower than at the ING site and remain below management's goal of 3–5 deer/km² since the 2 severe winters of 2010–2011 and 2013–2014 (D'Angelo and Giudice 2016). Topography is undulant with elevations ranging from 400 to 450 m above sea level. The area is part of the Northern Superior Upland region (MNDNR 2015) with lowland conifer stands and upland conifer and mixed deciduous-conifer stands. The lowlands included northern white cedar, black spruce, and tamarack. The uplands included northern white cedar; balsam fir; red, white and jack pine; aspen; and paper birch (MNDNR 2015). Mean January temperature was -15°C and mean annual snowfall was 165 cm during 1981–2010 (MNDNR Climatology 2018). Since 2011, WSI reflected moderately severe to severe winters in 3 years (2010–2011, 2012–2013, 2013–2014; MNDNR Climatology 2018).

The primary source of natural mortality of adult deer at both study sites was wolf (*Canis lupus*) predation (DelGiudice et al. 2002). The most recent wolf population estimate (2017) in northern Minnesota was 2,856, or 4 wolves/km² (Erb et al. 2017). Black bear (*Ursus americanus*) and wolf predation have been major causes of fawn mortality (Kunkel and Mech 1994, Carstensen et al. 2009). As of 2014, the bear population of northern Minnesota was estimated at about 15,000 (Garshelis and Tri 2017).

METHODS

Prior to capture and handling of free-ranging deer, we tested performance of the GPS collars that would be deployed. Specifically, we tested location-fix spatial accuracy and success rate, and transmission rate of 12 Globalstar Recon GPS units (Model IGW-4660-4; Telonics, Inc., Mesa, Arizona) in 4 cover types at the Carlos Avery Wildlife Management Area (WMA, Forest Lake, Minnesota): dense conifer ($>70\%$ canopy closure), mixed hardwoods, browse (willow [*Salix* spp.], aspen, and beaked hazel [*Corylus cornuta*]), and open field. These cover types

were similar to types that occur on the winter range study sites in northern Minnesota. We placed 3 collars 10 m apart and 1 m above ground in each cover type and programmed them to collect a GPS-fix every 2 hours. A differentially corrected GPS location (averaged >60 waypoints) was recorded at the location of each collar and used as the “true location” of the collar. We acquired the true location using a Trimble Juno and Terrasync antennae (Trimble Navigation Limited, Westminster, Colorado). The collars transmitted for 6 days (from 1 to 7 December 2017); stored-on-board data included 1 extra day. Roughly 5 cm of snowfall occurred during that time interval.

During winter 2017–2018, 10 adult (>1.5 years) female deer were captured at each study site (Figure 1). A total of 19 deer were captured via net-gunning from helicopter (Hells Canyon Helicopters, Clarkston, Washington), and 1 deer at the ING site was ground-captured using a Clover trap (DelGiudice et al. 2001). Handling of animals consisted of blind-folding, hobbling, recording a rectal temperature (° C), measuring chest girth and hind leg length (cm), affixing an ear-tag to each ear, fitting a GPS collar, and administering a broad-spectrum antibiotic. Collars were programmed to obtain 1 location-fix every 2 hours during December–June and 1 location-fix every 4 hours during July–November. Location data were transmitted to a base station every 10 hours (maximum 6 locations per transmission). The collars’ GPS units were equipped with QFP programming, which will obtain a QFP location only when a GPS-fix is unsuccessful; they are stored-on-board along with activity data collected every 5 minutes using an accelerometer. These data are retrieved and downloaded once collars are recovered. Fourteen of the collars were designed to remain on the deer for >2 years. Six collars, 3 on each site, have a pre-programmed automatic collar-release mechanism (CR-5; Telonics, Inc., Mesa, Arizona) so they can be recovered in September 2018. We will assess the performance of these collars using the stored-on-board location and activity data.

We classified cover types on the ING study site using a mirror stereoscope (Model MS27, Sokkia Co., Ltd., Tokyo) and 9”x 9” color infrared aerial photographs (1:15,840 scale) taken during October 2012 to capture the color contrast of peak autumn foliage. We delineated forest stands according to a classification system developed to assign dominant and co-dominant tree species, height class, and canopy closure class for conifer stands (Table 1). Forage sites—defined as open areas with regeneration <2 m in height—swamps and lakes were also delineated. We interpreted forest stands to a minimum size of 0.5 ha (DelGiudice et al. 2013a). Habitat training sites were established at locations of fresh deer snow-urine (i.e., urine in snow) collection. The snow-urine samples were being collected and analyzed to assess the nutritional status of deer (DelGiudice et al. 1989, 2017), but these locations also allowed documentation of vegetation information relevant to the habitat classification system and aerial photointerpretation being conducted during winter 2017–2018.

We conducted a preliminary assessment of the feasibility of making fine-scale habitat measurements for better understanding individual use of cover types. We examined habitat use by selecting a sample of location-fixes within individual deer home ranges on the ING site, and characterized cover types by structure (forest stands only), area, and arrangement of conifer forest cover and forage openings. Specifically, we measured 20 sample locations (10 in dense conifer and 10 in forage openings) related to the following characteristics: cover type being used; dominant and co-dominant tree species; stand height and canopy closure classes; distance (m) from fix to center of stand being used; distance (m) to nearest conifer cover class, if not in use; distance (m) to nearest opening/foraging site, if not in use; and area of cover type in use. Measurements were made using the Near tool in ArcMap 10.4 (ESRI Redlands, California). A 95% kernel home range was calculated using *adehabitat* (Calenge 2006) in program R (R Core Team) and will facilitate comparison of habitat composition within home ranges and between the 2 study sites.

RESULTS AND DISCUSSION

During the pre-deployment collar-testing, 802 of an expected 860 GPS locations to be transmitted were successfully transmitted (93%). Collars placed in dense conifer stands had the lowest GPS transmission-success rate (79%) compared to $\geq 96\%$ for collars in the other cover types (Table 2). Overall, mean location error of the transmitted locations was 6.2 m (± 0.68 [standard error]). Mean location error of collars was different among cover types ($F_{3, 798} = 33.2$; $P < 0.001$), with collar errors in dense conifer being greater than in browse ($P < 0.001$), hardwood ($P < 0.001$), and open ($P < 0.001$). There was no difference in location error among hardwood, browse, and open cover types ($P > 0.05$). When all data were downloaded directly from the collars, including QFP locations, we received 100% overall fix-success for all collars (Table 3). Of the 1,008 locations (included the extra day of stored-on-board) downloaded from all collars, 89% (897) had a location error < 10 m and 98% (988) had a location error < 25 m. A total of 4 QFP locations ($< 1\%$) were acquired during the 7 days of testing, all in dense conifer cover with a mean location error of 6.8 m (± 2.6). Pre-deployment collar-testing provided valuable information about the expected performance of our collars and how that might influence the accuracy of our examination of habitat use once deployed on free-ranging deer.

We recovered collars from 6 wolf-killed deer (10 March–19 May 2018) and downloaded and analyzed the data as we had done during the pre-deployment collar-testing; 1 collar was not recovered in time to be included in this research summary (DelGiudice et al 2018). Of the 6 collars recovered, GPS transmission-success rate was 80% and fix-success of the 3,093 expected locations was 100%, with 12% being QFP locations (Table 4). Overall mean horizontal error estimated by Telonics was 15 m (± 0.22). Fix-success rates from recovered collars were consistent with rates of collars used in the pre-deployment testing. Higher horizontal error estimates and lower transmission rates may be due to the increased frequency of dense conifer use on winter ranges (Morrison et al 2003, DelGiudice et al 2013a,b). The mean location error estimates are far superior (smaller) to those reported from previous GPS collar studies (32–100 m; Rempel et al. 1995, Moen et al. 1996, Dussault et al. 1999). The addition of QFP locations is critical to our habitat study, providing 100% fix-success rates, compared to 37–40% reported elsewhere (Moen et al. 1996, Dussault et al. 1999).

A total of 465 cover type stands spanning 3,969 ha were classified for the ING site (Figure 2). Dense conifer stands (473 ha) and forage openings (488 ha) each accounted for about 12% of the study area. Assuming 100% fix-success rates from our collars based on collar-testing, we expected 10,973 winter (10 March–1 May 2018) deer locations from the 2 sites that will be used for winter habitat analyses. Of the 10 locations from sampled deer in dense conifer stands, the mean distance to forage was 162 m (± 44) and 107 m (± 26) to the center of the stand in use (Table 5). The mean area of dense conifer stands being used was 12 ha (± 3). Of the 10 locations from sampled deer in forage openings, the mean distance to dense conifer stands was 87 m (± 22) and 162 m (± 42) to the center of the forage opening in use (Table 6). The mean area of forage stands was 13 ha (± 4). These measurements are consistent with previous research suggesting dense conifer cover should be arranged within 355 m of forage openings (Morrison et al. 2003, Potvin et al. 2003, Beyer et al. 2010). The small sample size used in this example does not provide enough information to make management prescriptions, but provides insight to the feasibility of making these fine-scale habitat measurements using our combination of GPS collars, remote sensing, and GIS technologies. Analyses of the full data sets are in progress.

Future work will include classifying and inventorying cover types on the EL site using similar methods, as well as analysis of all 10,973 expected winter locations from the 2 study sites to assess winter habitat use and requirements. Along with the measurements made in this summary, we will measure distance (m) to the nearest edge of the stand being used, as well as,

perimeter (edge) and perimeter:area ratio of the cover types in use for all deer locations. Ultimately, the ability to make these fine-scale habitat measurements using GPS collars, remote sensing, and GIS as winters progress and vary annually will allow us to assess the area, shape, juxtaposition, and arrangement of dense conifer cover, forage openings, and other cover types to assist managers in formulating prescriptions that effectively integrate forest and habitat management strategies and practices. Based on the strong results from collar-testing and deployment during winter 2017–2018, 40 additional collars will be deployed (20 on each site) during winter 2018–2019.

ACKNOWLEDGEMENTS

We thank B. Matykiewicz and B. Wagner for all of their strong efforts applied to trapping and handling deer, collecting snow-urine samples, and gathering habitat information. We are grateful to P. Backman for her efforts with mortality investigations and for her knowledge of the Elephant Lake study site. We appreciate the time and skills of C. Humpal applied to laboratory analyses of our deer snow-urine and bone marrow samples. We also acknowledge the United States Forest Service LaCroix and Deer River Ranger Districts for providing housing during the winter field season. This project is supported by the Minnesota Department of Natural Resources Section of Wildlife and the Wildlife Restoration (Pittman-Robertson) Program. The Minnesota Deer Hunters Association provided supplemental funding for post-doctoral research assistance.

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Table 1. Habitat classification system used with interpretation of color infrared aerial photographs from October 2012 to inventory winter habitat of white-tailed deer on the Inguadona Lake study site in northcentral Minnesota, winter 2017–2018. This classification system will also be used to inventory winter habitat on the Elephant Lake site in northeastern Minnesota.

Cover type	Class	Description	Code
Conifer	Northern white cedar		WC
	Balsam fir		BF
	Black spruce		BS
	Tamarack		T
	Red pine		RP
	Jack pine		JP
	White pine		WP
	Mixture of 2 dominants		Symbol/Symbol
Hardwoods	Trembling aspen		TA
	Paper birch		PB
	Maple		RM
	Other		O
Height (m)	1	≥2 and <6	1
	2	≥6 and <11	2
	3	≥11	3
	Mixed 1	<6 and 6 to <11	4
	Mixed 2	<6 and ≥11	5
	Mixed 3	≥6 and <11 and ≥11	6
Canopy closure (%)	Open	<40%	a
	Moderately dense	≥40% and <70%	b
	Dense	≥70%	c
Openings	Forage/Opening		F
	Swamp/Bog		SW
Water	Pond	< 5 acres	P
	Lake	≥ 5 acres	L

Table 2. Summary statistics by habitat type for *transmitted* data from global positioning system (GPS) collars.^a Three collars were tested in each type at the Carlos Avery Wildlife Management Area, Minnesota, 1–7 December 2017.

Habitat type	Mean location error (m) ^b	Median location error (m)	Standard error	Minimum location error (m)	Maximum location error (m)	Number of GPS locations transmitted ^c	Percentage of GPS locations successfully transmitted
Browse	4.7	4.2	0.18	2.5	27.6	212	98%
Conifer	9.7	7.0	0.68	2.5	58.6	167	79% ^d
Hardwood	6.6	5.6	0.40	1.5	37.6	207	96%
Open	4.8	4.4	0.13	3.4	12.9	216	100%
Overall	6.2	4.7	0.20	1.5	58.6	802	93%

^aGlobalstar Recon GPS units (Model IGW-4660-4; Telonics, Inc., Mesa, Arizona).

^bLocation error was calculated by taking the Euclidean distance from GPS location and the true location for each GPS-fix. True locations were obtained using a Trimble Juno and Terrasync antennae at the location of each collar (see Methods).

^cTransmitted data include 6 days of transmissions (expected 216 locations per cover type, 860 total).

^dExpected number of GPS locations for conifer is 212 due to the 4 Quick Fix Pseudorange locations acquired that cannot be transmitted (see Methods).

Table 3. Summary statistics by habitat type for data *downloaded* directly from global positioning system (GPS) collars.^a Three collars were tested in each habitat type at the Carlos Avery Wildlife Management Area, Minnesota, 1–8 December 2017.

Habitat type	Mean location error (m) ^b	Median location error (m)	Standard error	Minimum location error (m)	Maximum location error (m)	Number of successful fixes ^c	Overall fix-success rate (%)
Browse	3.1	2.7	0.14	0.3	25	252	100%
Conifer	10.3	6.4	1.05	0.3	161	252	100%
Hardwood	5.9	4.6	0.35	0.4	42	252	100%
Open	3.3	2.9	0.14	0.2	17	252	100%
Overall	5.7	3.6	0.29	0.2	161	1008	100%

^aGlobalstar Recon GPS units (Model IGW-4660-4; Telonics, Inc., Mesa, Arizona).

^bCalculations are the same as described in Table 2.

^cDownloaded data include 7 days of locations (1 extra day of stored-on-board, expected 252 locations per cover type, 1,008 total).

Table 4. Summary statistics of location-fix data downloaded from global positioning system (GPS) collars^a recovered from 6 wolf-killed adult, female white-tailed deer during winter 2017–2018 and associated performance metrics.^b Horizontal error was calculated by Telonics and downloaded with the location data. Quick Fix Pseudoranging (QFP) locations were recorded only when a GPS-fix was unsuccessful. Collars were deployed on a winter range study site in northcentral (Inguadona Lake) or northeastern (Elephant Lake) Minnesota.

Collar ID	Study area ^c	Mean horizontal error (m)	Overall fix-success rate	Percent QFP locations	GPS-fix transmission-success rate ^d
697085A	ING	16	100%	13%	86%
697087A	EL	17	100%	12%	62%
697092A	ING	14	100%	14%	89%
697095A	ING	14	100%	0%	86%
697098A	ING	15	100%	17%	88%
699965A	EL	16	100%	8%	69%
Overall		15	100%	12%	80%

^aGlobalstar Recon GPS units (Model IGW-4660-4; Telonics, Inc., Mesa, Arizona).

^bExcludes 1 mortality in which the collar was not recovered in time for analysis.

^cING = Inguadona Lake and EL = Elephant Lake.

^d Transmission-success rate is calculated from the GPS locations only, does not include QFP locations.

Table 5. Fine-scale habitat measurements from a sample of 10 global positioning system (GPS) collar locations of adult, female white-tailed deer in dense conifer stands^a on the Inguadona Lake study site in northcentral Minnesota, 10 March–30 April 2018.

Dominant species	Height class ^b	Distance to nearest forage opening (m)	Distance to center of stand in use (m)	Area of stand in use (ha)
Red pine	2	363	61	9
Black spruce	6	293	108	27
Red pine	3	45	13	9
Red pine	3	22	30	2
Red pine	6	65	112	6
Red pine	3	228	235	7
Tamarack	4	402	125	11
Balsam fir	3	56	32	5
Northern white cedar	3	41	79	16
Red pine	3	100	272	24
Mean (\pm SE)		162 (\pm 44)	107 (\pm 26)	12 (\pm 3)

^aDense conifer stands have canopy closure of $\geq 70\%$.

^bHeight classes are measured in meters: 1 (≥ 2 and < 6), 2 (≥ 6 and < 11), 3 (≥ 11), 4 (< 6 and 6 to < 11), 5 (< 6 and ≥ 11), and 6 (≥ 6 and < 11 and ≥ 11).

Table 6. Fine-scale habitat measurements from a sample of 10 global positioning system (GPS) collar locations of adult, female white-tailed deer in forage openings on the Inguadona Lake study site in northcentral Minnesota, 10 March–30 April 2018.

Habitat type	Distance to dense conifer (m) ^a	Distance to center of opening in use (m)	Area of opening in use (ha)
Forage	22	124	4
Forage	99	37	7
Forage	42	63	3
Forage	48	287	18
Forage	231	173	4
Forage	203	477	38
Forage	56	155	24
Forage	81	11	3
Forage	2	211	15
Forage	83	81	12
Mean (±SE)	87 (±22)	162 (±42)	13 (±4)

^aDense conifer stands have canopy closure of ≥70%.

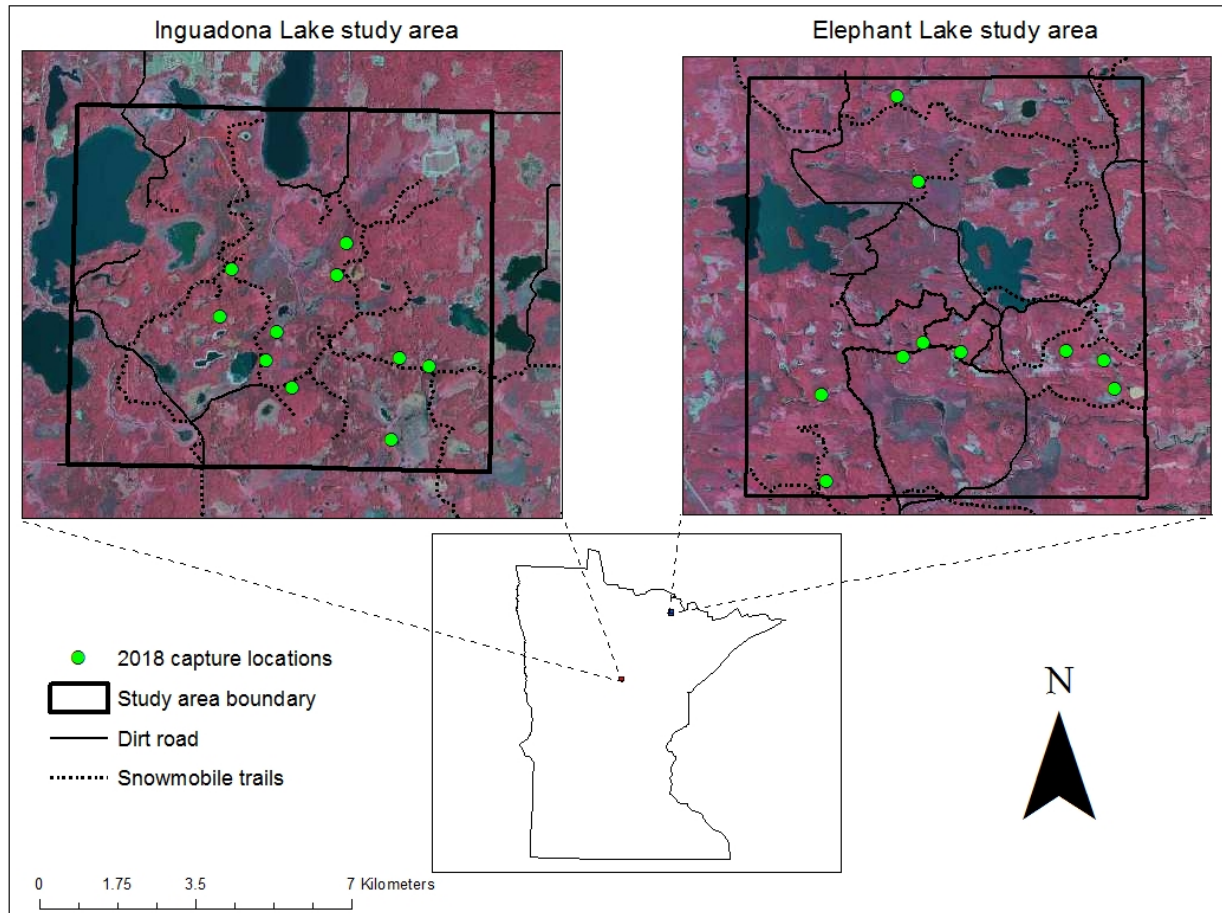


Figure 1. Color infrared aerial photographs (1:15,840) and map of winter range sites for study of habitat use by adult (>1.5 years), female white-tailed deer in northcentral (76 km²) and northeastern (120 km²) Minnesota, winters 2017–2018 and 2018–2019. Ten capture locations for 2017–2018 are depicted for each site.

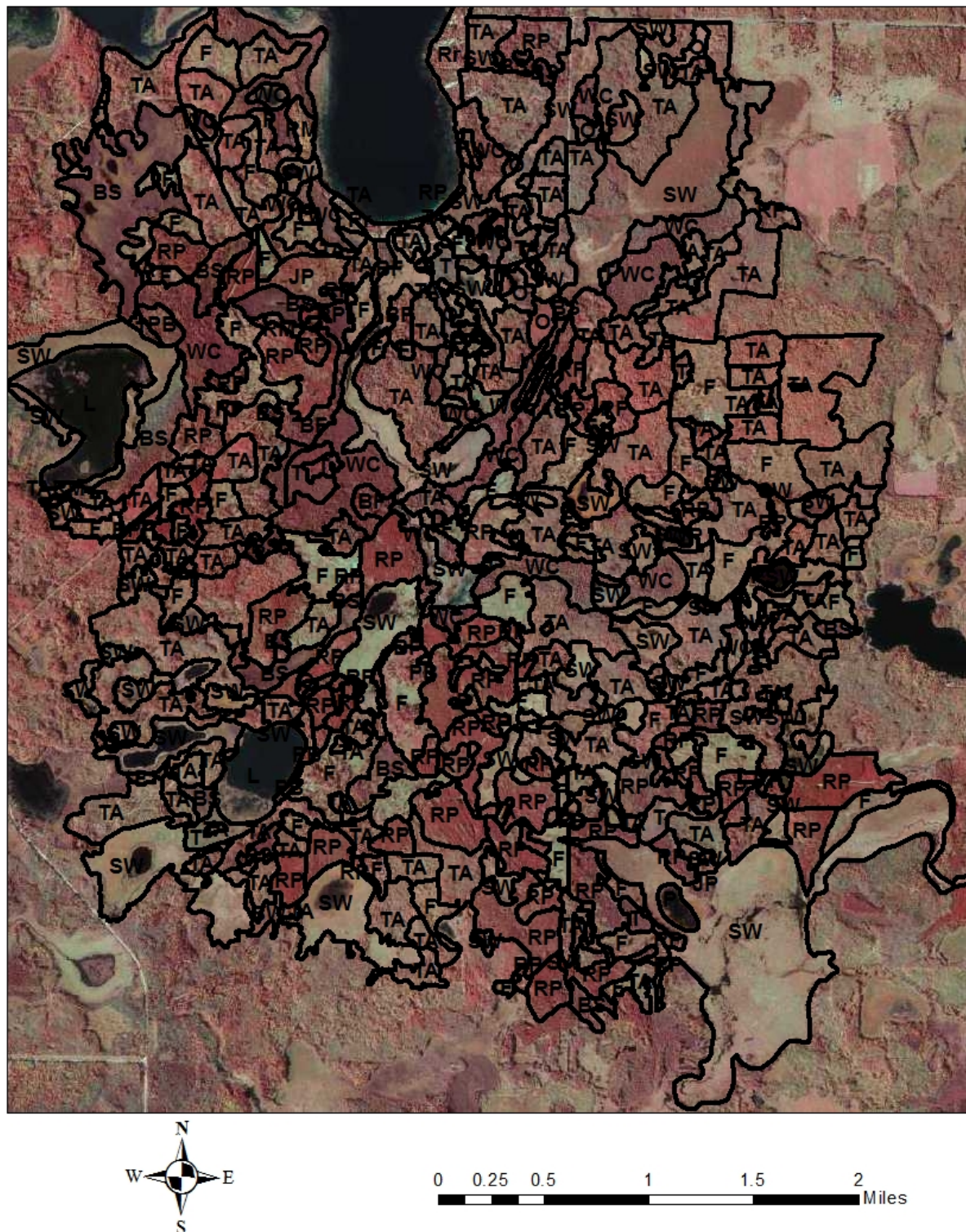


Figure 2. Habitat type boundaries delineated from interpretation of 2012 color infrared aerial photographs of the Inguadona Lake study site in northcentral Minnesota, according to a classification system developed for examination of winter habitat of white-tailed deer (Table 1). This interpretation will be used to make fine-scale measurements of habitat use by deer during winters 2017–2018 and 2018–2019.



WINTER SURVIVAL AND CAUSE-SPECIFIC MORTALITY OF WHITE-TAILED DEER IN NORTHERN MINNESOTA: UPDATING WITH GPS COLLARS

Glenn D. DelGiudice, Bradley D. Smith,¹ and William J. Severud¹

SUMMARY OF FINDINGS

Ongoing studies that examine the influences of environmental, intrinsic, and demographic factors on survival and cause-specific mortality rates of white-tailed deer (*Odocoileus virginianus*) have been critical to enhancing our understanding of population performance and to improving management. A recent evaluation report from the Office of the Legislative Auditor recommended that the "...DNR should conduct field research to collect and utilize more information about Minnesota's deer... and inform the department's vital rate estimates of deer births and deaths, and better reflect deer population dynamics" to improve our understanding of demographics and habitat requirements. Using cutting-edge global positioning system (GPS) collar, remote sensing, and geographic information system (GIS) technologies, we recently launched a study that will inform a level of understanding of habitat requirements and drivers of population performance (survival and reproduction) required by managers to prescribe forest manipulations that best support population goals. Herein, our objectives are to compare winter survival rates and cause-specific mortality (and influential factors) of adult female deer residing on study sites in northcentral (Inguadona Lake) and northeastern (Elephant Lake) Minnesota. Specifically, we predicted that survival, percent winter mortality, and the impact of wolf (*Canis lupus*) predation would be influenced by winter severity in a way that is consistent with our understanding of this relationship garnered from a previous long-term (1991–2005) study in northcentral Minnesota. Mortalities of 6 of 20 GPS collared adult, female deer occurred from 10 April to 28 May on the 2 sites, 3 at Elephant Lake and 3 at Inguadona Lake. Wolf predation was the proximate cause of all of these mortalities, and most of these deer were in poor condition when they were killed. Overall, the survival rate decreased to 0.68 (95% confidence interval 0.50–0.93) by 28 May. Wolves preyed on a fourth deer in poor condition at Inguadona Lake, but it occurred within 2 days of being captured, so we excluded it from analyses as a capture-related mortality. Typically, adult female deer enter winter in the best physical condition compared to fawns and adult males, and thus have the highest probability of surviving winter. This at least suggests that overall mortality rates across sex and age classes during this past winter could have been even higher than indicated by our preliminary adult female data. Ongoing federal protection of wolves in Minnesota limits the Minnesota Department of Natural Resources' (MNDNR) management options, and consequently, has at least contributed to the estimated wolf population almost doubling from winter 1988–1989 (1,521 wolves) to the present (~2,900 wolves). Caution is warranted in interpreting our preliminary findings, but they do

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highlight the need for multi-year follow-up study to better understand whether deer-habitat-wolf predation relationships have been changing since completion of the MNDNR's previous long-term study.

INTRODUCTION

Studies that have examined the influences of extrinsic (e.g., location, season, habitat, predation, and human activities), intrinsic (e.g., age, sex, condition), and demographic (e.g., density) factors on survival and cause-specific mortality rates have enhanced our understanding of the population performance and dynamics of white-tailed deer (*Odocoileus virginianus*) and other ungulates in northern Minnesota and elsewhere (Nelson and Mech 1986a,b; Fuller 1990; Bartmann et al. 1992; DelGiudice 1998; Gaillard et al. 2000; DelGiudice 2002, 2006). A long-term (1991–2005) study of female deer, the reproductive component of populations, reported that the relative risk of mortality was strongly related to the severity of winter conditions (i.e., indicated by a winter severity index [WSI]; DelGiudice et al. 2002, 2006) in northcentral Minnesota. Indeed, the risk of death increased as winters progressed, and by the end of winter was at least 10 times greater during the most severe winter (1995–1996) compared to the mildest winter (1990–1991). That study also documented that the relative risk of death by natural causes was consistently greater than by all other causes of mortality (e.g., hunting), and that wolf (*Canis lupus*) predation, directly related to snow depth, was the primary cause of mortality. Furthermore, the risk of adult deer mortality by wolves increases sharply after 6 years of age.

The Office of the Legislative Auditor (OLA) recently issued an evaluation report of the Minnesota Department of Natural Resources' (MNDNR) management of the state's deer population (OLA 2016). The evaluation report focused on improving population estimates and acknowledged throughout that improved habitat management would have to be the other key component of this plan to establish and meet population goals. More detailed information and a greater understanding of winter habitat requirements of deer in northern Minnesota has been an ongoing need of wildlife managers and foresters to enhance their ability to plan, integrate, and implement long-term forest and habitat management strategies and associated activities. Relative to population and habitat management, the OLA report recommends that the "...DNR should conduct field research to collect and utilize more information about Minnesota's deer... and inform the department's vital rate estimates of deer births and deaths, and better reflect deer population dynamics" to improve our understanding of demographics and habitat requirements. Using cutting-edge global positioning system (GPS) collars and remote sensing and geographic information system (GIS) technologies, this recently-launched study will increase understanding of habitat requirements and methods to evaluate demographics required by managers to prescribe forest manipulations that best support population goals (DelGiudice et al. 2017).

Fieldwork for this study was initiated during winter 2017–2018. A critical overall objective of this first year is to establish the technical feasibility of making fine-scale measurements of habitat use by white-tailed deer (see companion research summary by Smith et al. 2018) and to monitor winter survival and cause-specific mortality as a means of assessing habitat quality (DelGiudice et al. 1989a,b) and updating input for state modeling of northern deer populations. We predicted that the influence of winter severity on crude winter mortality and the wolf predation rate of adult female deer would be consistent with findings from our previous long-term study (DelGiudice et al. 2002, 2006).

OBJECTIVES

1. To compare winter survival rates of adult female deer residing on the Inguadona Lake and Elephant Lake sites, located in northcentral and northeastern Minnesota, respectively, where there are differences in winter severity, habitat composition, and deer density
2. To determine specific causes of mortality and contributing factors

STUDY AREA

As discussed above and in a companion research summary in this issue (Smith et al. 2018), the study included 2 deer winter range sites, Inguadona Lake (46 km²) in northcentral and Elephant Lake (76 km²) in northeastern Minnesota. These sites allow natural comparisons of potential influences of differences in winter severity, habitat composition, and deer density on habitat use and requirements and associated vital estimates. D'Angelo and Giudice (2015) reported pre-fawning deer densities of 7–9 and 3–5 deer/km² in the vicinity of the Inguadona Lake and Elephant Lake sites, respectively. The MNDNR calculates a WSI throughout the state by accumulating 1 point for each day with an ambient temperature $\leq -17.7^{\circ}\text{C}$ and an additional point for each day when snow depth is ≥ 38 cm during 1 November–31 May. Generally, winters with maximum WSI values (by 31 May) <100 , $100\text{--}180$, and >180 are assessed as mild, moderately severe, and severe, respectively, relative to impacts on deer survival; however, multiple factors may influence this interpretation annually and geographically (DeGiudice et al. 2006). Maximum WSI at Inguadona Lake and Elephant Lake at the end of winter 2017–2018 was 61 and 122, respectively. Additional details addressing site boundaries, location, topography, forest composition, long-term weather, and wolf and black bear (*Ursus americanus*) densities are provided in Smith et al. (2018).

METHODS

During 10–11 March 2018, 19 adult (>1.5 years old), female white-tailed deer were captured by net-gunning from helicopter (Hells Canyon Helicopters, Clarkston, Washington), 9 and 10 on the Inguadona Lake and Elephant Lake sites, respectively. A tenth deer was captured on 25 February by Clover trap at Inguadona (Clover 1956). Except for this deer (which was immobilized with xylazine and ketamine, and reversed with yohimbine), all deer were physically restrained for handling (Smith et al. 2018). All deer were fitted with a Globalstar Recon GPS collar (Model IGW-4660-4; Telonics, Inc., Mesa, Arizona). We programmed all collars to collect 1 location-fix every 2 hours during December–June and 1 location-fix every 4 hours during July–November. The collar's mortality sensor relies on a 3-axis accelerometer. The unit samples this accelerometer every second. "Active" is recorded when the reading of any of the 3 axes changes by more than 0.3 g (gravitational force) since the last active second. Less than 5 accumulated seconds of activity during the previous 8-hours causes the unit to detect mortality, which triggers the collar to send a mortality notification through the Globalstar satellites, followed by sending an email to our team. This launches our field investigation. This also increases the VHF pulse rate to notify researchers in the field. Our field investigations included a thorough search for site and carcass evidence to determine the specific cause of mortality. When available, we collected a mandible (to extract a fourth incisor) and femur (or other long bone as necessary) to age the deer to the year and assess body condition (Gilbert 1966, Mech and DeGiudice 1985). We conservatively assigned ultimate cause of death as "capture-related" when the mortality occurred within 14 days of capture, regardless of the proximate cause (e.g., wolf-kill; DeGiudice et al. 2002, 2006) or handling method (i.e., physically or chemically immobilized).

We calculated Kaplan-Meier survival estimates using the R package *KMsurv* (R Core Team 2017). We examined relationships between the WSI and percent winter mortality by simple linear regression analyses in Excel (Version 14.0.7153.5000, Microsoft Corporation 2010).

RESULTS AND DISCUSSION

Overall, survival of our GPS collared, adult female deer decreased markedly through the end of May (~80 days) to 0.68 (95% confidence interval 0.50–0.93) and was similar at Inguadona and Elephant Lakes (Figure 1). Natural mortality during late winter 2017–2018 was notably higher than expected. Overall, the late winter mortality rate was 35% (7 of 20 deer). The first mortality occurred at Inguadona Lake on 13 March within 2 days of capture; consequently, we assessed it as capture-related, but the proximate cause was wolf predation. Furthermore, ocular assessment of the collected long bone marrow (i.e., red) indicated near exhaustion of fat in this depot, thus this individual was in poor condition when it was killed (Mech and DelGiudice 1985). Laboratory analyses of marrow fat content of all collected bone specimens are pending. Importantly, because this captured animal was physically restrained for only 5 minutes, and not chemically immobilized, there was no risk of residual drug-effects and limited potential influence of the brief handling on its vulnerability to subsequent predation.

Six additional mortalities occurred from 10 April to 28 May on the 2 sites, 3 at Elephant Lake and 3 at Inguadona Lake. Wolf predation was the proximate cause of all of these mortalities as well. Four of these deer were in only fair to poor condition, whereas 2 could not be assessed due to insufficient evidence (e.g., no long bone recovered). Excluding the capture-related mortality from our calculations reduced the natural mortality rate to 33% at Inguadona; it was 30% at Elephant Lake and 32% overall. Given the low to moderate, end-of-the-season, maximum WSI values for Inguadona (66) and Elephant Lake (122), these female mortality rates, overall and due to wolf predation specifically, were extraordinarily high (Figure 2). Winter wolf predation on northern deer is directly related to snow depth, attributable to deepening snow increasingly impeding deer mobility and escape (deer have a heavier weight-load-on-track) and to its steady compromising effect on energy balance and endurance (Moen 1976, Nelson and Mech 1986b, DelGiudice 1998, DelGiudice et al. 2002). Consequently, most winter mortalities, both in our previous long-term and present studies, occurred during March–May, when snow cover is deepest or condition is poorest.

The preponderance of evidence in this study suggests that poor condition was a noteworthy contributing factor to the high mortality rates by wolf predation. Given that winter conditions were not particularly severe at either site, as indicated by maximum WSI values, this prompts consideration of the role of winter habitat deficiencies compromising the ability of deer to adequately fulfill their biological requirements as the season progressed. Indeed, it is additionally noteworthy that winter survival and wolf predation rates were similar on both sites, despite the maximum WSI at Inguadona Lake being half that at Elephant Lake. Increasing concerns about the limited quantity and quality of habitat (e.g., dense conifer cover) on deer winter range in northern Minnesota, in large part, prompted the need for the present study (T. Rusch, L. Petersen, and P. Backman, MNDNR, Section of Wildlife, personal communication), as did OLA's evaluation report strongly recommending deer research that enhances the MNDNR's understanding of vital estimates and associated population performance and dynamics for improved management be continued (OLA 2016). Typically, adult female deer enter winter in the best physical condition (i.e., most replete fat reserves), and thus have the lowest hazard risk or highest probability of surviving winter compared to fawns and adult males (Mautz 1984, Robbins 1993, DelGiudice et al. 2002). This at least suggests that overall winter mortality rates during this past winter could have been even higher than indicated by our preliminary adult female data, which also did not include winter mortality that occurred before collar deployment on 10 March. Ongoing federal protection of wolves in Minnesota limits the MNDNR's

management options, and consequently, has at least contributed to the estimated population almost doubling from winter 1988–1989 (1,521 wolves), just as the previous deer study was initiated (winter 1990–1991), to the present (~2,900 wolves, Erb and DonCarlos 2009, Erb et al. 2017). Caution is warranted in interpreting our preliminary findings, but they appear to critically highlight the need for multi-year follow-up study to better understand whether these complex deer-habitat-wolf predation relationships have been changing since completion of the previous long-term study (DelGiudice et al. 2002, 2006, 2007, 2013a,b).

ACKNOWLEDGMENTS

We appreciate the dedicated efforts of B. R. Matykiewicz and B. L. Wagner as field biology technicians whose duties included capturing and handling deer, collecting snow-urine samples for nutritional assessments, and gathering vegetation data for habitat classification and inventories. We thank P. Backman for her support of study site selection, with deer mortality investigations and overall logistics, and for her knowledge of the Elephant Lake study site. We also appreciate the effort and technical skills of C. A. Humpal in the laboratory analyzing snow-urine samples and deer remains and acknowledge the United States Forest Service LaCroix and Deer River Ranger Districts for providing housing during the winter. J. Erb loaned us field equipment as needed. This project is supported by the Minnesota Department of Natural Resources Section of Wildlife and the Wildlife Restoration (Pittman-Robertson) Program. The Minnesota Deer Hunters Association provided supplemental funding to support valuable post-doctoral research assistance.

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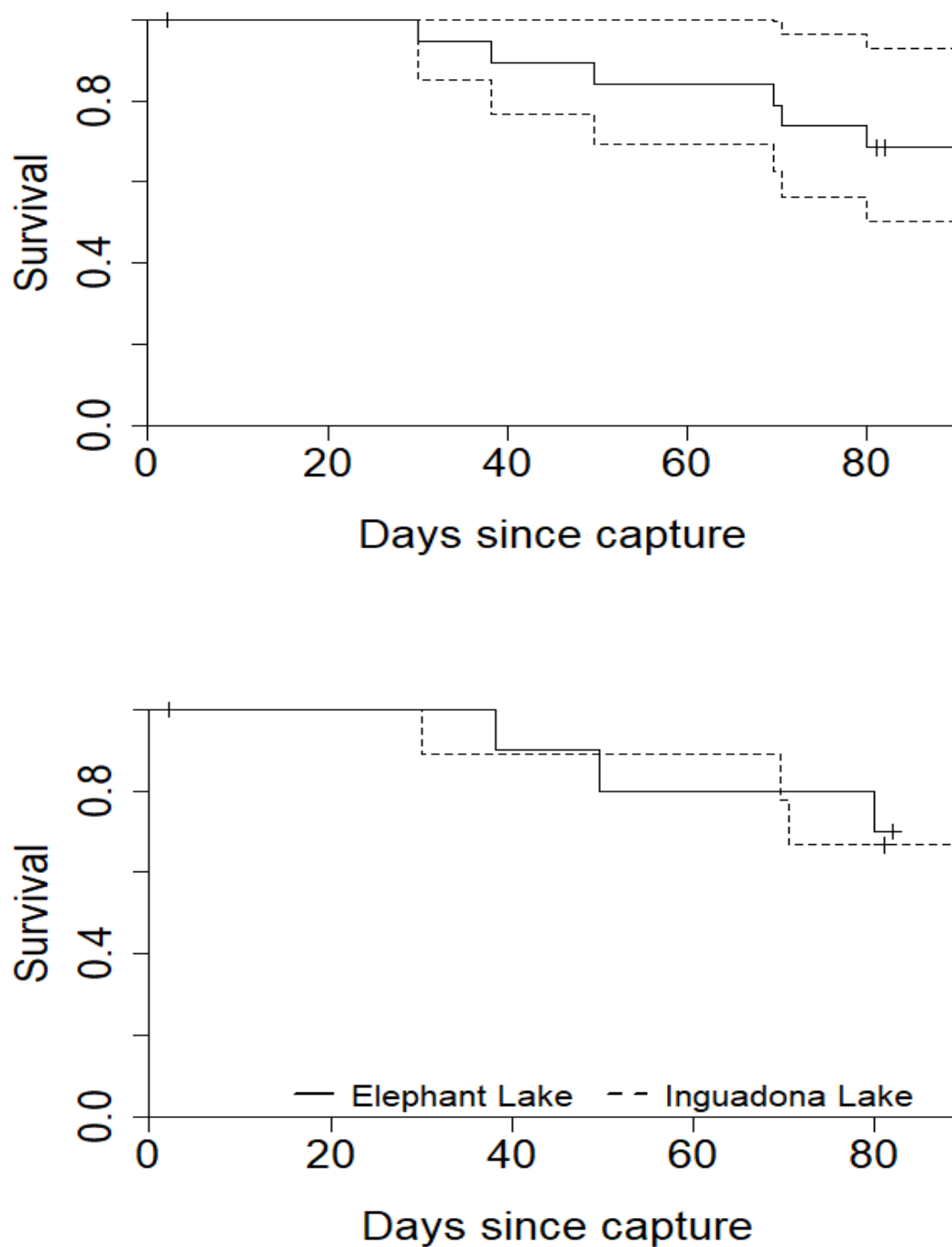


Figure 1. Kaplan-Meier survival for all 20 global positioning system-collared, adult (>1.5 years) female white-tailed deer (top, pooled) on the Inguadona Lake (northcentral) and Elephant Lake (northeastern) study sites and comparing survival for the 10 deer on each of the sites (bottom), Minnesota, 11 March–31 May 2018. The early single tick mark represents the deer censored due to capture-related mortality, and the double mark represents the last day post-capture included in the analysis. Dashed lines in the top graph represent 95% confidence intervals.

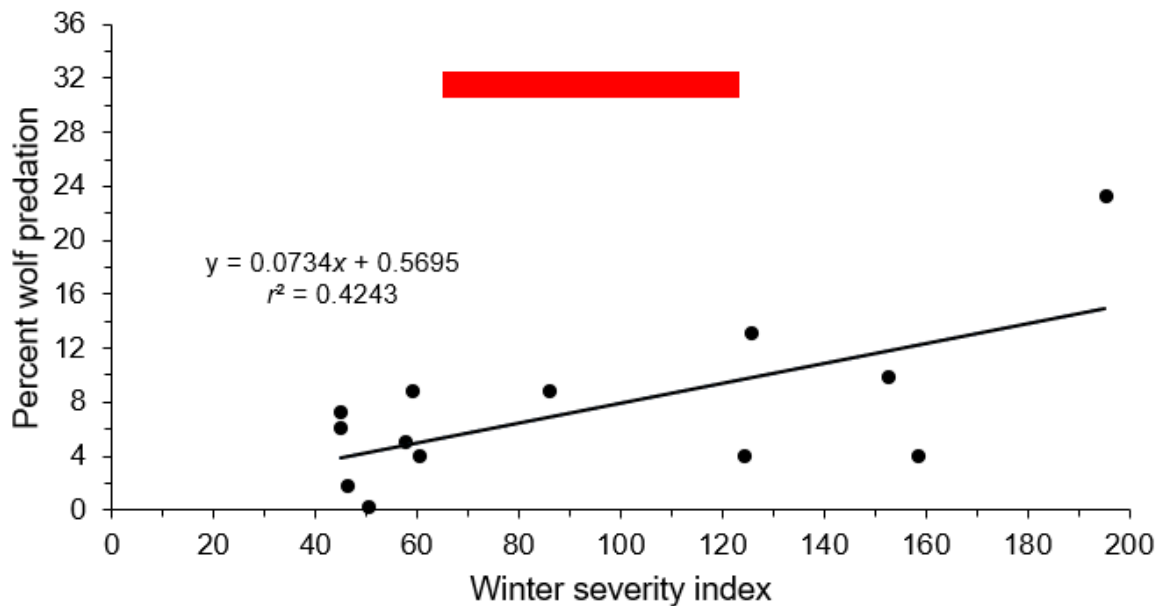
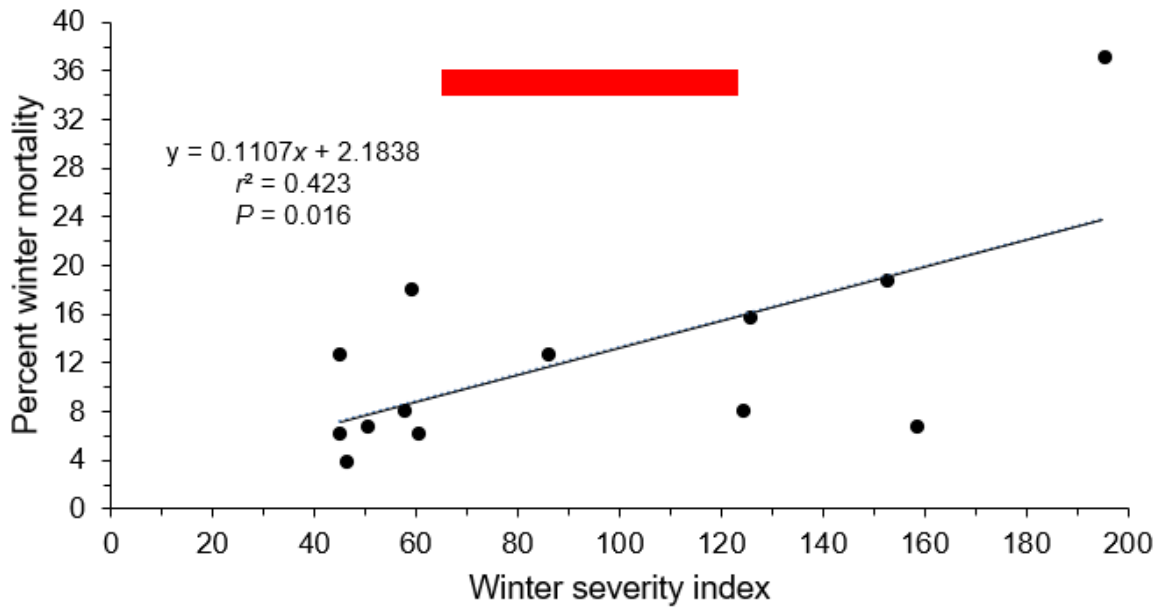


Figure 2. Comparison of pooled percent crude winter mortality (35%, top) and wolf predation rates (32%, bottom), depicted by the red bars, for 20 global positioning system-collared, adult (>1.5 years), female white-tailed deer from a study site in northcentral (Ingwadona Lake) and northeastern (Elephant Lake) Minnesota during winter 2017–2018 to the long-term relationship of these rates for radiocollared adult, female deer to maximum winter severity index (WSI) in northcentral Minnesota, during winters 1990–1991 to 2002–2003 (DeGiudice et al. 2006). The red bar spans from a maximum WSI of 61 (Ingwadona Lake) to 122 (Elephant Lake).



WINTER NUTRITIONAL RESTRICTION AND DECLINE OF MOOSE IN NORTHEASTERN MINNESOTA, WINTERS 2013–2018

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SUMMARY OF FINDINGS

The moose (*Alces alces*) population in northeastern Minnesota has declined an estimated 66% from 2006 to 2018. As was the case in northwestern Minnesota's moose decline during mid-1980–2007, a number of complex ecological relationships between undernutrition, pathogens, predation, and environmental factors (e.g., habitat, temperature) are likely exerting pressure on moose and contributing to this recent 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); urea nitrogen:creatinine (UN:C) ratios have shown the greatest potential as a metric of winter nutritional status with values <3.0, 3.0–3.4, and ≥ 3.5 mg:mg being indicative of moderate (normal), moderately severe, and severe nutritional restriction, respectively. During 4 January–28 March 2013–2018, we collected annual totals of 123, 307, 165, 189, 160, and 332 moose snow-urine samples, and mean seasonal UN:C ratios were 3.7, 2.9, 2.9, 3.5, 3.7, and 2.6 mg:mg for the 6 winters, respectively. The mean population UN:C ratios for winters 2013, 2016, and 2017 were above the threshold indicative of severe nutritional restriction (i.e., a starvation diet) and accelerated body protein catabolism. During 2014, 2015, and 2018 the corresponding values reflected moderate nutritional restriction. Most indicative of the unique severity of nutritional restriction in 2013, nearly one-third of all samples collected yielded UN:C ratios ≥ 3.5 mg:mg.

Perhaps the ultimate value to management of nutritional assessments of free-ranging animals is realized when the findings can be related to the performance and dynamics of the population and other ecological factors challenging that performance. Through 2017, our population-level nutritional assessments were closely tracking separate population estimates ($r^2 = 0.75$) of moose in northeastern Minnesota. However, this relationship weakened markedly with the inclusion of the 2018 population estimate and snow-urine data. This likely was attributable in part to the notable uncertainty associated with the annual population estimates and its continued statistical stability, but apparent decline. Biologically, the mean population-level UN:C ratio (2.6) and relatively low incidence of snow-urine samples with UN:C ratios indicative of severe nutritional restriction (14.8%) were consistent with the population's continued stability. Although nutritional restriction varied among the 6 winters, data suggested a level of deprivation not supportive of population growth. Climate change, reflected by the heat stress index for moose, and variation in winter conditions, as indexed by the Winter Severity Index (WSI), were not related to nutritional restriction of moose. For the first 5 winters (the only years for which survival estimates are available), we documented that the level of severe nutritional restriction

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was inversely related ($r = -0.86$) to variation of *natural* winter survival of global positioning system (GPS) collared adult moose. While these relationships do not substantiate cause-and-effect, presently it provides the best preliminary empirical evidence that inadequate winter nutrition at the population level is intricately related to the declining trajectory of moose numbers in northeastern Minnesota.

INTRODUCTION

Declines in regional populations of moose (*Alces alces*) along the southern periphery of their global range have been common in recent decades (Timmerman and Rodgers 2017). In northeastern Minnesota the estimated 2018 population (3,030 moose) is 66% less than in 2006 (8,840 moose, DelGiudice 2018), exhibiting a trajectory similar to that documented previously for moose in northwestern Minnesota, where the population decreased from ~4,000 in the mid-1980s to <100 moose by 2007 (Murray et al. 2006). Furthermore, mean annual mortality rates of collared adult moose associated with the declines were similarly high (21%) in the northwest and northeast (Murray et al. 2006; Lenarz et al. 2009; R. A. Moen, unpublished data). In northwestern Minnesota, malnutrition and pathogens were identified as important factors influencing the population's decreasing trajectory (Murray et al. 2006). In northeastern Minnesota a recent (2013–2017) aggressive study of global positioning system (GPS) collared, adult moose reported a mean annual mortality rate of 14.7%, with health-related factors (e.g., parasites, disease) accounting for about two-thirds of the deaths, wolf (*Canis lupus*) predation for one-third, and complex interactions between the 2 categories were well-documented (Carstensen et al. 2018). In the earlier studies, climate change (i.e., warming temperatures) was implicated in both population declines (Murray et al. 2006; Lenarz et al. 2009, 2010).

Temperature-survival relationships are complex, and indicate that climate change can directly and indirectly impact ungulate populations (Bastille-Rousseau et al. 2016, Davis et al. 2016, Street et al. 2016). Moose are particularly well-adapted to cold climates, but temperatures that exceed “heat stress” thresholds of 14° to 24° C during summer and –5° C during winter may increase metabolic rates, induce energy deficits, and hasten deterioration of body condition (Renecker and Hudson 1986, 1990; Broders et al. 2012; McCann et al. 2013). These thresholds may be influenced by exposure to solar radiation and wind (Renecker and Hudson 1990, McCann et al. 2013). Nutritional and health status (e.g., disease, parasites), behavioral responses (e.g., altering movement, foraging, and bedding patterns), and quality of available habitat have the potential to affect the animal's ability to mitigate negative impacts from heat stress (Van Beest et al. 2012, McCann et al. 2016, Street et al. 2016).

Energy balance is central to animal fitness, which is critical to survival and reproduction, the 2 drivers of population performance (Robbins 1993). The natural “nutritional bottleneck” of winter typically imposes the greatest challenge to the supply side of energy budgets of moose and other northern ungulates (Mautz 1978, Schwartz and Renecker 2007). Gestation at this time increases energetic and nutritional demands, particularly during late-winter and early-spring (Robbins 1993). Although moose are generally well-adapted to this seasonal nutritional deprivation, elevated ambient temperatures exceeding heat stress thresholds, coupled with the influence of other compromising extrinsic factors (e.g., pathogens, poor quality forage and low availability of thermal cover, densities of conspecifics or other nutritionally competing species) can exacerbate energy deficits and associated consequences relative to adult and juvenile survival, subsequent reproductive success, and population dynamics (Robbins 1993; DelGiudice et al. 1997, 2001).

Winter nutritional restriction of moose and other northern ungulates can be physiologically assessed at the population level 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), the end-product of protein metabolism, is one of many chemistries investigated for its value as a physiological metric of the severity of nutritional restriction (DelGiudice et al. 1991a,b, 1994). In healthy moose, urinary UN:C values decrease (N conservation) in response to diminishing intake of crude protein and digestible energy, but as dietary restriction and negative energy balance become more severe and fat reserves are depleted, ratios increase to notably elevated values in response to accelerated net catabolism of endogenous (body) protein. Snow-urine UN:C ratios exhibited differential effects of a winter tick (*Dermacentor albipictus*) epizootic and habitat differences on the severity of nutritional restriction of moose on Isle Royale, Michigan, and were strongly related to dynamics of the population, including a pronounced decline and recovery to historically high numbers (DelGiudice et al. 1997).

OBJECTIVES

1. To determine how nutritional restriction varies annually and as winter progresses
2. To examine potential relationships between the severity of nutritional restriction and the winter heat stress index (HSI) for moose, seasonal survival rates of GPS collared adult moose, and annual population estimates

We hypothesized that increasing winter ambient temperatures, exceeding the HSI threshold, are contributing to the severity of nutritional restriction and energy deficit of moose. We also predicted that the severity of nutritional restriction would be inversely related to the performance of the population in northeastern Minnesota, primarily through its effect on adult survival and possibly calf production. Findings will set the stage for additional work assessing nutritional relationships of moose to variations in habitat and other factors.

STUDY AREA

We assessed winter nutritional restriction of moose within a 6,068-km² study area located between 47°06'N and 47°58'N latitude and 90°04'W and 92°17'W longitude in northeastern Minnesota (Figure 1). Including bogs, swamps, lakes, and streams; lowland stands of northern white cedar (*Thuja occidentalis*), black spruce (*Picea mariana*), and tamarack (*Larix laricina*); and upland balsam fir (*Abies balsamea*), jack pine (*Pinus banksiana*), white pine (*P. strobus*), and red pine (*P. resinosa*), this region has been classified as Northern Superior Upland (Minnesota Department of Natural Resources [MNDNR] 2015). Trembling aspen (*Populus tremuloides*), white birch (*Betula papyrifera*), and conifers are frequently intermixed.

Wolves (*Canis lupus*) and American black bears (*Ursus americanus*) are predators of moose (Fritts and Mech 1981, Severud et al. 2015) with recent densities estimated at 4.0 wolves and 23 bears/100 km² (Garshelis and Noyce 2015, Erb et al. 2017). White-tailed deer (*Odocoileus virginianus*) are managed at pre-fawning densities of <4 deer/km², and are the primary prey of wolves in most of northern Minnesota (Nelson and Mech 1986, DelGiudice et al. 2002). The MNDNR assesses winter severity (1 November–31 May) by a Winter Severity Index (WSI), calculated by accumulating 1 point for each day with a temperature ≤−17.7° C (0° F, temperature-day) and 1 point for each day with snow depth ≥38 cm (15 inches, snow-day), for a potential total of 2 points per day. Maximum WSI values varied markedly across moose range, 35–160, 184–245, 54–152, 31–142, 50–159, and 50–179 for winters 2012–13 to 2017–18, respectively (Minnesota State Climatology Office 2018). Mean daily minimum and maximum temperatures varied markedly during November–April from 2012–13 to 2017–18 at Ely, Minnesota (Midwestern Regional Climate Center 2018; Figure 2). The heat stress index (HSI_{Min} and HSI_{Max}, see Figure 3) for moose during the “cold season” (November–March) was calculated by daily accumulation of degrees Celsius exceeding −5° C for the maximum and minimum ambient temperatures, respectively (Renecker and Hudson 1986).

METHODS

We collected fresh snow-urine specimens of moose during 4 January–28 March 2013– 2018. Our field team drove (by truck or snowmobile) a route of approximately 201 km to distribute the sampling throughout the study area (Figure 1). Field technicians were not restricted to this route, rather they 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 @ MNDNR, Section of Wildlife) and a Superior National Forest map (U. S. Forest Service) to navigate in the field.

Generally, sampling was conducted within 7 days of a fresh snowfall, most often within 2–4 days, so that we could associate urine chemistry data and nutritional assessments with specific narrow temporal intervals. Upon observing fresh moose sign, technicians tracked the individual(s) on foot as necessary until they found a fresh snow-urine specimen. The objective for the 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 year calves comprised only 13–17% of the population (DelGiudice 2018). We focused primarily on the adult age class to facilitate optimum comparability of physiological assessment data.

Specimens were collected and handled as described by DelGiudice et al. (1991a, 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 or other sign in the area also were recorded.

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

Winter (January–March) was divided into 6, 2-week sampling intervals (~1–15 January, 16–31 January, 1–14 February, 15–28 February, 1–15 March, and 16–31 March). Sample sizes for the 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 standard error). 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. We examined relationships between proportions of snow-urine specimens with UN:C values indicative of severe nutritional restriction (≥ 3.5 mg:mg) and populations estimates, seasonal survival, and HSI by simple linear regression analyses in Excel (Version 14.0.7153.5000, Microsoft Corporation 2010).

RESULTS AND DISCUSSION

During January–March 2013–2018, 1,289 urine specimens from moose were collected and analyzed to assess nutritional status at the population level. Specifically, annual totals of 123, 307, 165, 189, 160, and 332 moose snow-urine samples, respectively, were collected during 5–6, 2-week sampling intervals using our designated routes and were adequately concentrated for chemical analysis. The greater number of samples collected during 2014 was largely due to the

early and prolonged deep snow cover, whereas during 2018, the greatest number of samples was attributable to more intense sampling during the middle of the winter sampling period.

Overall, mean UN:C ratios were 3.7, 2.9, 2.9, 3.5, 3.7, and 2.6 mg:mg for winters 2013 to 2018, respectively (Figure 4). The mean population UN:C ratio for entire winters 2013, 2016, and 2017 were above the threshold indicative of severe nutritional restriction or a starvation diet (≥ 3.5 mg:mg) and accelerated body protein catabolism. But the elevated mean UN:C of 2016 and 2017 were influenced largely by a small number of collected samples that exhibited very high UN:C ratios indicative of a moribund condition (≥ 22.0 mg:mg), whereas during 2013, nearly one-third of all samples collected yielded UN:C ratios indicative of severe nutritional restriction (≥ 3.5 mg:mg, Figure 5). According to Figure 5 and the summed proportions of samples with UN:C ratios indicative of moderately severe and severe restriction, it appears that winters 2013 to 2015 were the most nutritionally challenging to moose, whereas during winters 2016 to 2018, UN:C ratios more consistently indicated moderate restriction to be most dominant.

Mean urinary UN:C ratios by 2-week interval of winter 2013 indicated that nutritional restriction was normal or moderate during late-January, but became severe throughout February and early-March, and was still assessed as moderately severe in late-March (Figure 6). As severe nutritional restriction of moose progresses with winter, those animals may be under-sampled as some eventually die directly from undernutrition or because they've become predisposed to another proximate cause of mortality (e.g., wolf predation, Carstensen et al. 2018), and those still alive urinate less, which is a physiological mechanism to conserve water and electrolytes. However, the 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%) during 2013 (Figure 7). Such elevated values have been associated with long-term fasting in controlled nutrition studies of captive white-tailed deer and starvation of free-ranging elk (*Cervus elaphus*), bison (*Bison bison*), and moose (DelGiudice et al. 1991a, 1994, 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 5).

During 2014, mean urinary UN:C ratios in all 2-week intervals, except early February, remained just below the moderately severe category (Figure 6), and the percentage of samples with ratios indicative of severe nutritional restriction gradually decreased as this winter progressed (Figure 7), either due to an easing of conditions restricting access to forage or because severely stressed individuals were being under-sampled, which may be most plausible as previously explained. Adverse effects of the late, but prolonged conditions of winter 2013, including warm temperatures, may have contributed to the high spring-summer calf loss and absence of the need for dams to lactate (Severud et al. 2015). This also may have allowed 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 from moose classified nutritional restriction as moderate (normal), whereas 36% reflected moderately severe to severe restriction, which was less than in 2013 (Figure 5). 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 5). However, a higher percentage of moose appeared to be experiencing moderate or normal restriction and a smaller percentage moderately severe restriction than in 2013 and 2014 (Figure 5). Rapidly diminishing snow cover prevented 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. In an attempt to better understand within-winter temporal patterns of assessed nutritional restriction across years, we

will be conducting more detailed analyses of UN:C data relative to the temporal and spatial distributions of sampling, progressive winter conditions, and sample size.

According to maximum WSI values, winter 2014 was the most severe of the 6 in northeastern Minnesota's moose range, followed by 2018, 2013, 2017, 2015, and 2016. 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, which are not hindered as much by deep snow (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 for moose and deer (Telfer and Kelsall 1984, DelGiudice 1998, DelGiudice et al. 2002, Schwartz and Renecker 2007). During 2013, conditions became severe during mid- to late-winter; consequently, a high number of snow-days did not accumulate, but the season was prolonged. Severe nutritional restriction of moose in 2013 was most similar to that which occurred in moose during several winters (1988–1990) on Isle Royale, also associated with severe winter tick infestations and a 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 severe during winter 2013 than in any of those that followed through 2018 (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). During the first 5 years, our population-level nutritional assessments closely tracked ($r^2 = 0.75$) population estimates of moose from the annual aerial survey (DelGiudice et al. 2018); however, with the addition of the 2018 survey results and nutritional assessment data the relationship weakened markedly (Figure 8). This is likely due to several factors. First, there is a great deal of uncertainty (wide 90% confidence intervals) associated with the annual aerial estimates of moose numbers (DelGiudice 2018). Second, there are spatial and temporal incongruences between data collections for the population estimates versus for the nutritional assessments. Relatively-speaking, the ~9-day aerial survey provides an estimate that is a winter "snapshot," whereas sample collections for the nutritional assessments span early to late winter (90 days). Finally, we do not yet understand the timeframe associated with potential biological effects on these moose of variation in nutritional restriction within a season or the specific mechanisms involved. It is noteworthy that our population estimates indicate that moose numbers have been relatively stable since 2012, with the exception of 2013. During this winter the population appeared to decrease abruptly; however, general survey conditions were poor, and we could not quantify their potential influence as an artifact on the point estimate. Of the 6 winters, 2013 was the only one in which a severe winter tick infestation occurred and had uniquely strong nutritional consequences for moose at the population level, reflected by urinary UN:C ratios (Figure 5). As described earlier, this has been similarly documented on Isle Royale (DelGiudice et al. 1997). The incidence of samples with UN:C indicative of moderately severe to severe restriction was greatest during winters 2013–2015, whereas during 2016–2018, nutritional restriction has remained remarkably moderate and stable. Six points is the minimum number required for valid statistical assessments of these relationships (F. Martin, Department of Applied Statistics, University of Minnesota, personal communication). Presently, what appears most clear across years is that elevated UN:C values suggest a level of nutritional deprivation not supportive of positive population performance or growth. Continued monitoring of population performance and dynamics and winter nutritional status, and primary factors influencing them, should continue to improve our understanding of the mechanisms involved.

During 2013 to 2015, warming winter temperatures were strongly associated with variation in the nutritional status of moose. As the January and winter HSI_{Max} values increased, the incidence of severe nutritional restriction of moose increased ($r^2 \geq 0.93$, DelGiudice and Severud 2017), which we believed may have led to many of these animals becoming more vulnerable to various health-related causes of mortality and predation (Carstensen et al. 2015, DelGiudice et al. 1997). However, unexpectedly, in 2016 and 2017, despite having the highest winter HSI values calculated with daily maximum (958 and 833) or minimum (220 and 194) ambient temperatures, the smallest percentage of samples with UN:C ratios reflecting severe nutritional restriction and greatest percentage indicative of moderate restriction occurred (Figure 5). Overall, the relationship between winter HSI_{Max} or HSI_{Min} and the percentage of samples with UN:C indicative of severe nutritional restriction collapsed. Absence of apparent relationships continued through winter 2018. However, the incidence of severe nutritional restriction at the population level remained inversely related to variation of *natural* winter survival ($r = -0.86$, $P = 0.061$), but not significantly so to winter-summer survival ($r = -0.65$, $P = 0.231$) of GPS collared adult moose (Figure 9). Survival data collection was temporally more consistent with data collection for the nutritional assessments, and both data sets have a high level of certainty. Unfortunately, completion of the 5-year study of adult moose did not permit a winter survival estimate for the sixth year. However, importantly, the 5-year relationship of winter nutritional restriction to winter survival supports a reasonably strong biological explanation of the winter nutritional influence on the population trajectory, and it suggests that the study cohort of GPS collared moose was indeed representative of the free-ranging population in northeastern Minnesota. Clearly, there is still much to understand about these relationships.

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 specimens with UN:C ratios indicative of severe nutritional deprivation (Figure 10). The wide temporal and spatial distributions of severe nutritional restriction suggest that habitat deficiencies at the landscape scale may constitute a primary contributing factor. We continue to apply significant efforts into investigating the habitat-nutrition relationships, but habitat deficiencies related to forage availability and quality, vegetative species composition, or less-than-optimum arrangements of forage openings and forest stands affording seasonal thermal cover remain unclear. Data from future 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 provide a basis for formulating management recommendations that may be implemented and evaluated in the near future.

ACKNOWLEDGMENTS

We thank S. Hurd, M. Pike, N. Martorelli, B. Matykiewicz, J. Ostroski, L. Kruse, M. Bowman, D. Dewey, R. Willaert, R. Ryan, R. Peterson, C. Olson, and K. Foshay for their dedication and strong efforts necessary to sampling these free-ranging moose, and R. Wright for contributing his GIS skills. We also are grateful to T. Rusch, D. Plattner, M. Meskill, L. Cassioppi, M. Magnuson, and N. Thom for their assistance and cooperation in setting up office space and securing key equipment. We appreciate and acknowledge the laboratory support and skills of C. Humpal. This study has been supported largely by the Minnesota Department of Natural Resources Section of Wildlife, Minnesota Environmental and Natural Resources Trust Fund (ENRTF), and Wildlife Restoration (Pittman-Robertson) Program. The Minnesota Deer Hunters Association has provided supplemental funding for stipends for full-time seasonal field biology volunteers.

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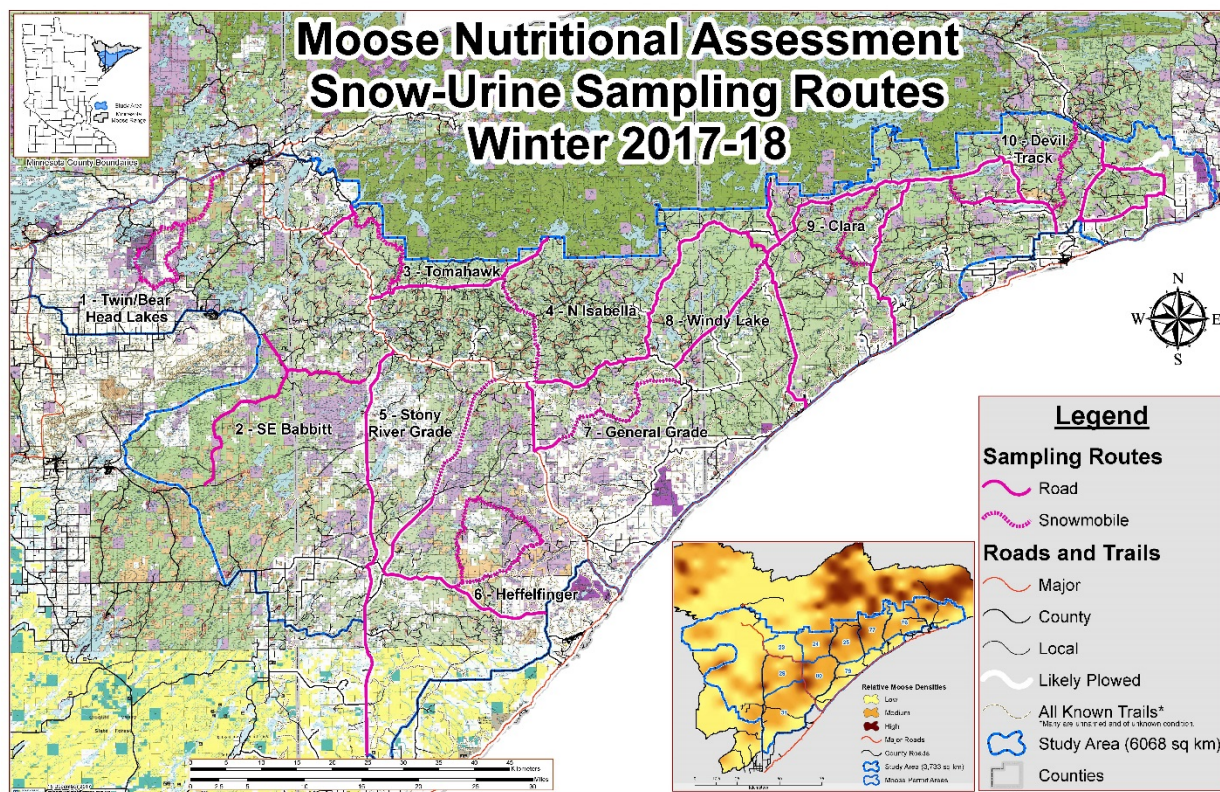


Figure 1. Map depicting the moose study area in northeastern Minnesota and the routes (i.e., roads and snowmobile trails in purple) used to distribute the sampling of fresh moose urine in snow (snow-urine) for nutritional assessments throughout the area, January–March 2013–2018.

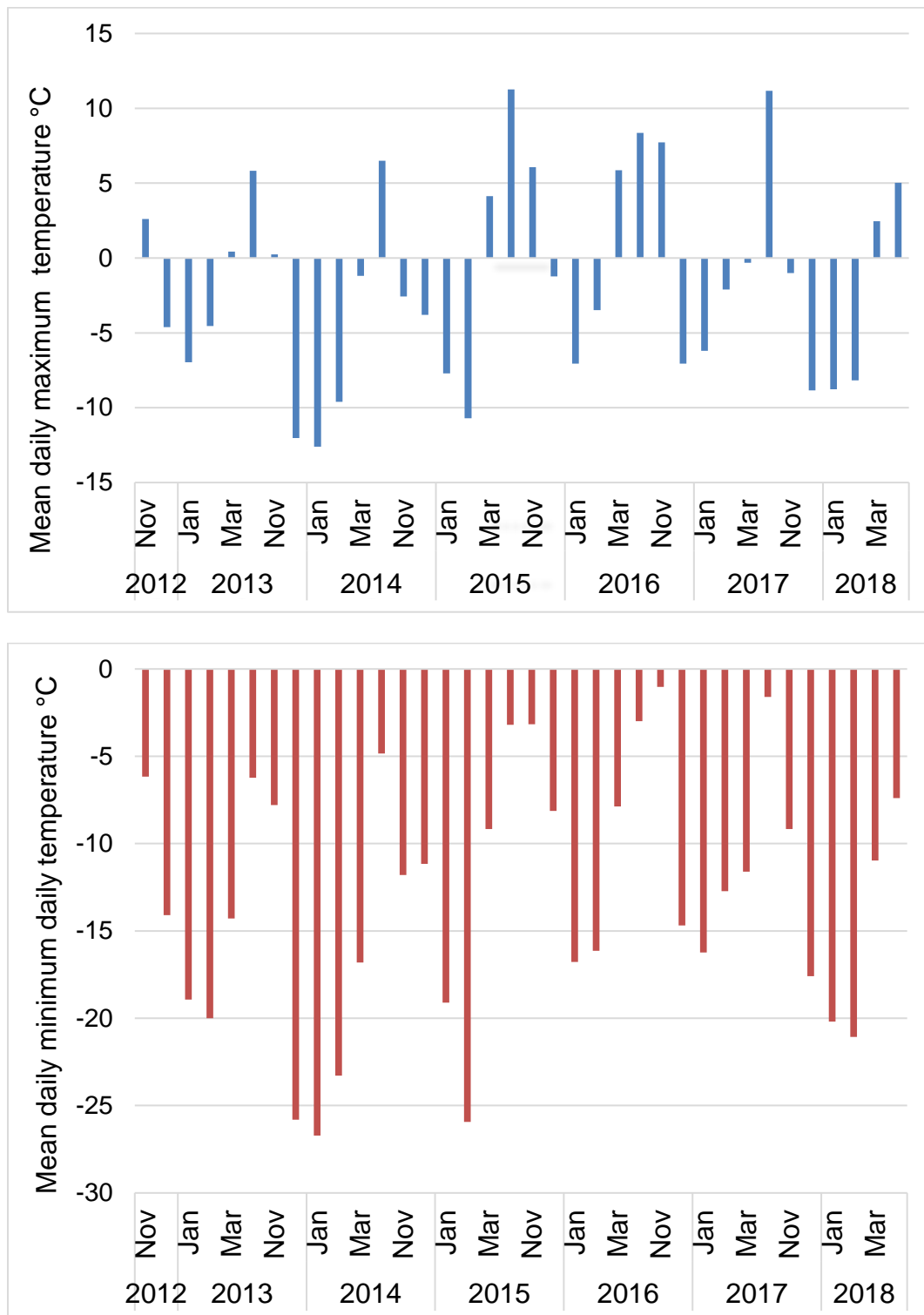


Figure 2. Mean daily maximum (top) and minimum (bottom) ambient temperatures, Ely, Minnesota, November–April 2012–2018 (Midwestern Regional Climate Center 2018).

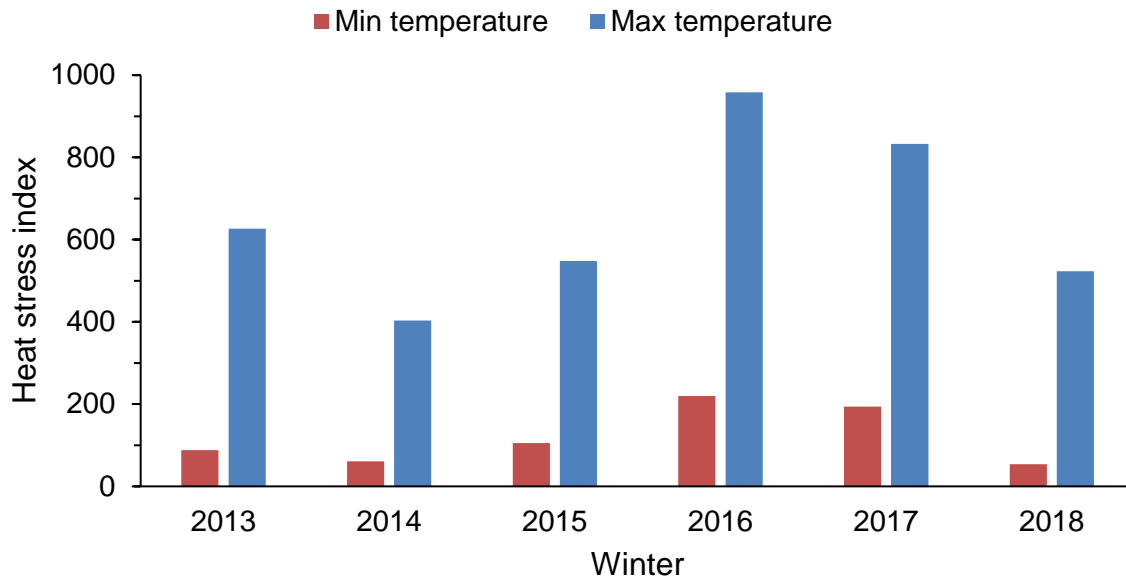


Figure 3. Winter heat stress index (HSI) for moose, calculated by accumulating daily °C exceeding a -5° C threshold for the minimum and maximum ambient temperatures (1 November–31 March, Renecker and Hudson 1986, 1990) in northeastern Minnesota, 1 November–31 March 2012–2018.

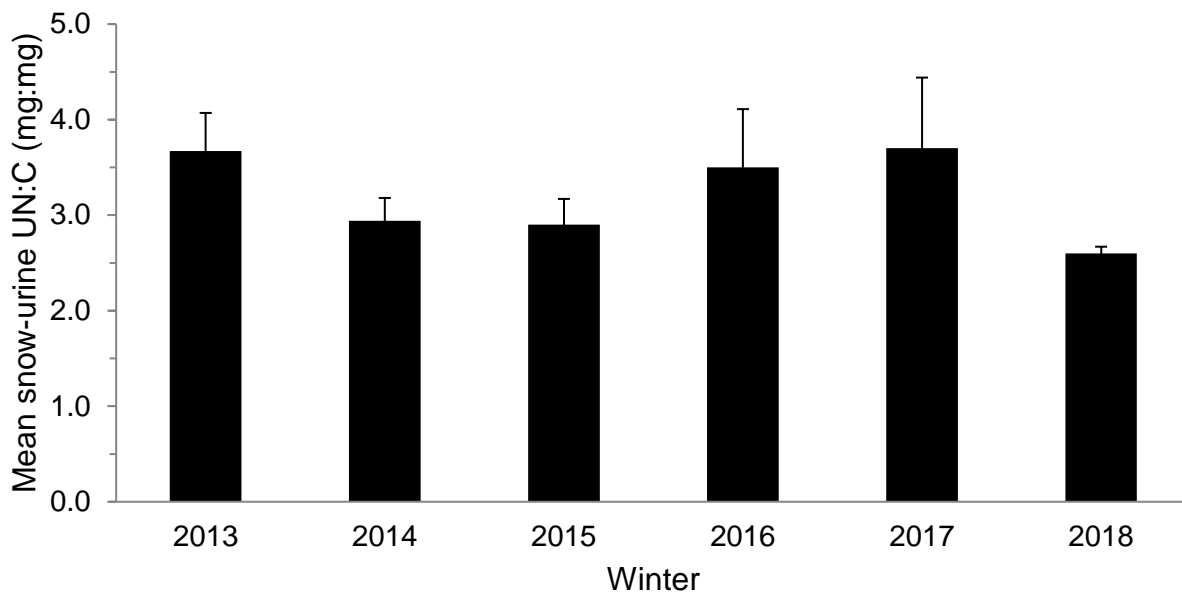


Figure 4. Overall mean (+SE) urea nitrogen:creatinine (UN:C) ratios of samples of fresh urine voided in snow (snow-urine) by moose and serially collected for assessments of nutritional restriction throughout northeastern Minnesota, January–March 2013–2018.

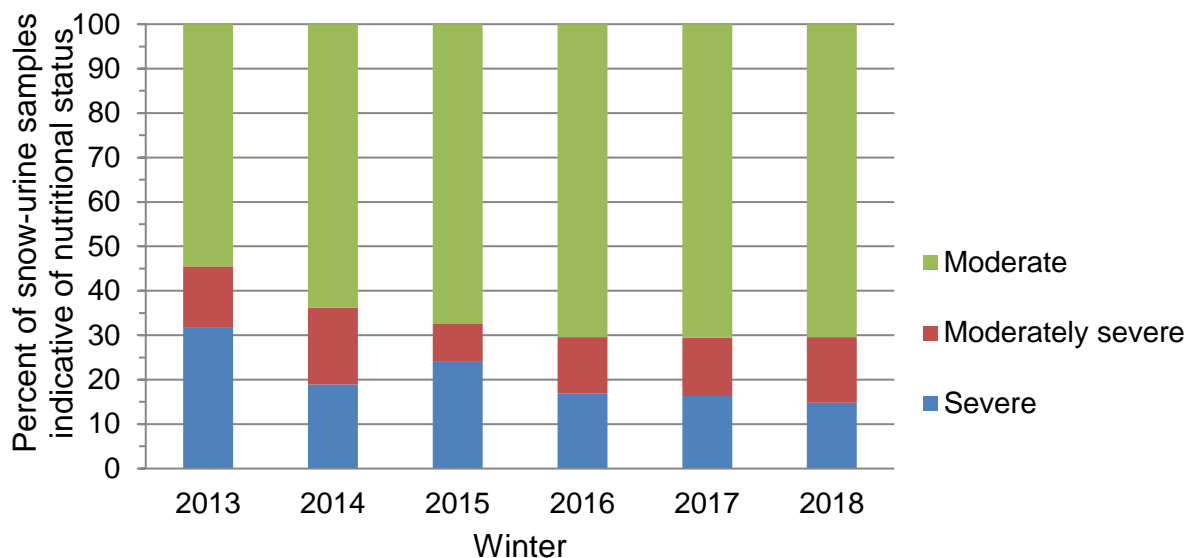


Figure 5. Overall percent of serially collected moose urine samples voided in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios indicative of moderate/normal (UN:C <3.0 mg:mg), moderately severe (UN:C = 3.0–3.4 mg:mg), and severe nutritional restriction (UN:C ≥3.5 mg:mg) throughout northeastern Minnesota, January–March 2013–2018.

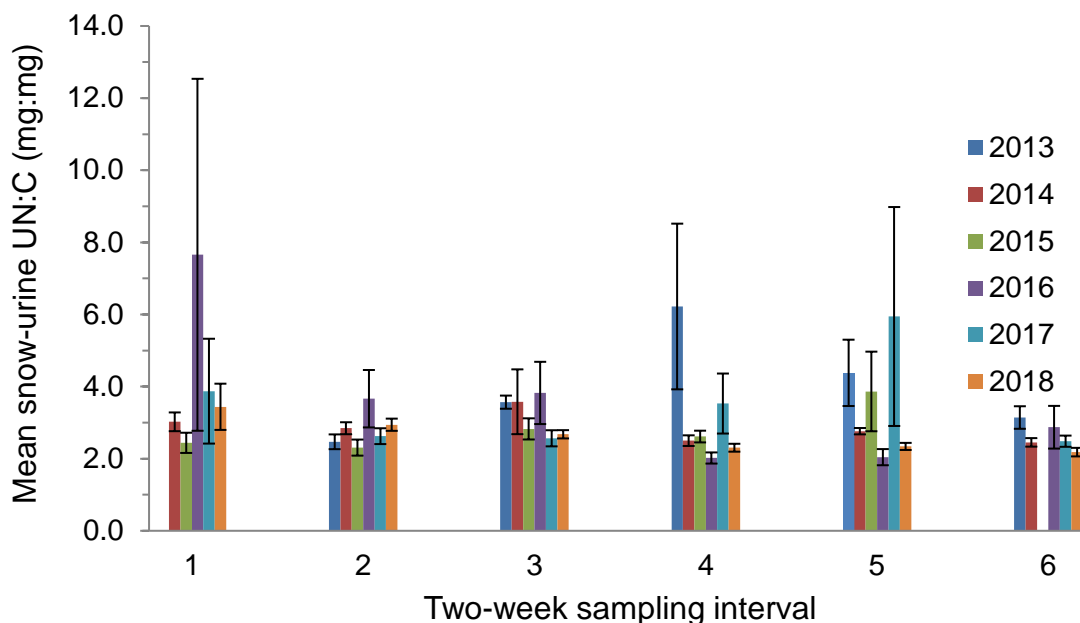


Figure 6. Mean (\pm SE) urea nitrogen:creatinine (UN:C) ratios of samples of fresh urine voided in snow (snow-urine) by moose and collected during 2-week sampling intervals for assessments of nutritional restriction throughout northeastern Minnesota, January–March 2013–2018.

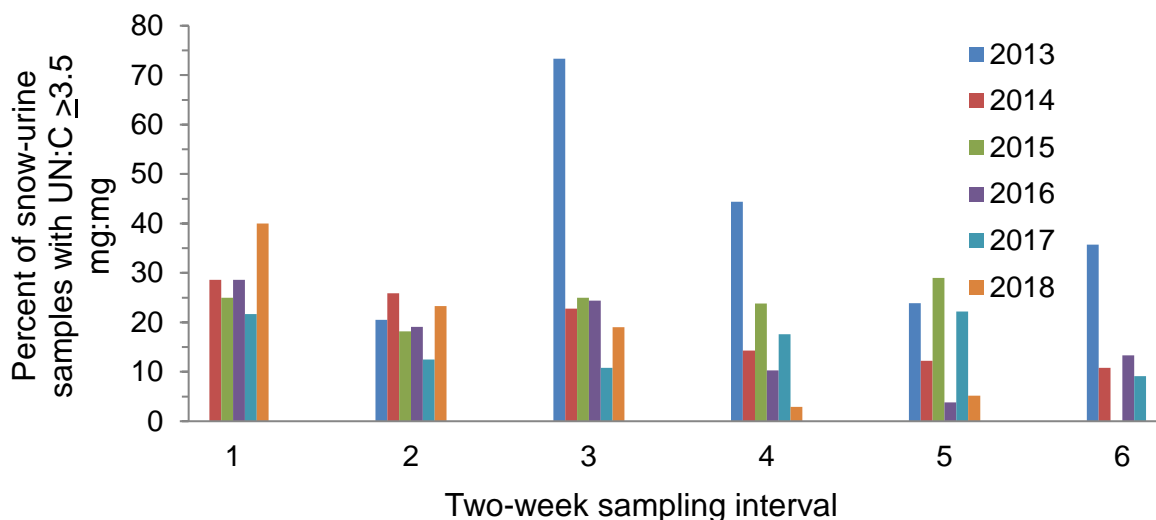


Figure 7. Percent of fresh urine samples voided in snow (snow-urine) by moose and collected during 2-week intervals with urea nitrogen:creatinine (UN:C) ratios indicative of severe nutritional restriction (UN:C ≥ 3.5 mg:mg) throughout northeastern Minnesota, January–March 2013–2018.

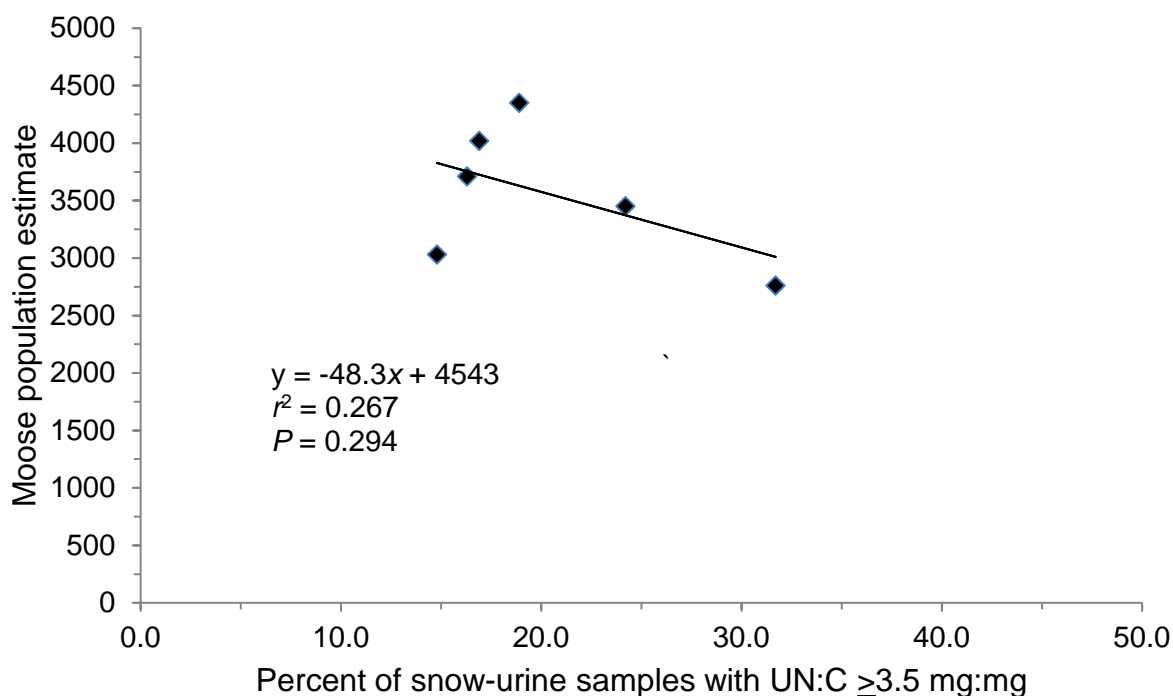


Figure 8. Relationship of the incidence of severe winter nutritional restriction of moose, indicated by the percentage of collected samples of urine in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios ≥ 3.5 mg:mg, to annual population estimates of moose in northeastern Minnesota (estimates from DelGiudice 2018), January–March 2013–2018.

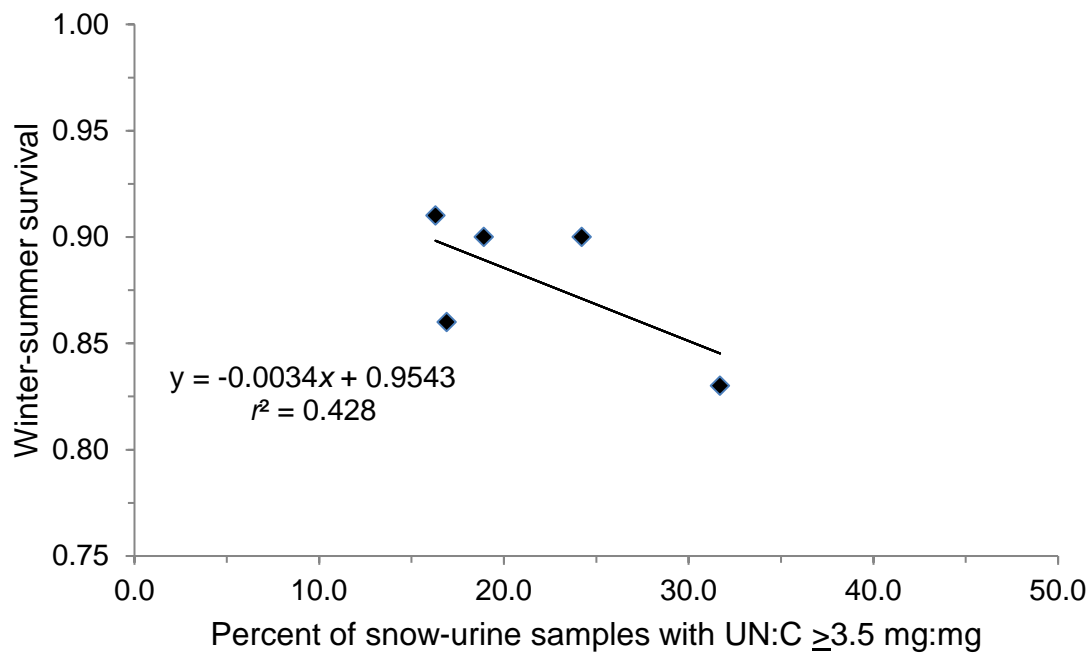
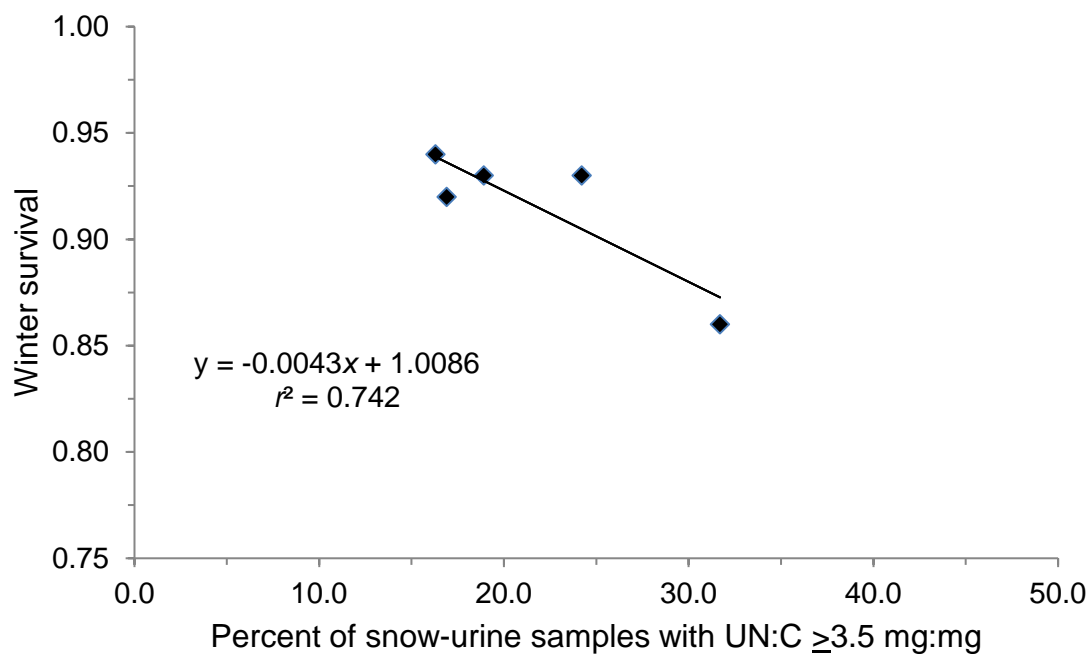


Figure 9. Relationships of the incidence of severe winter nutritional restriction of moose at the population level, indicated by the percentage of collected samples of urine in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios ≥ 3.5 mg:mg, to winter (top, 1 November–31 May

2013–2017) and winter-to-summer (bottom, 1 November–31 August 2013–2017) survival of GPS-collared adult moose in northeastern Minnesota.

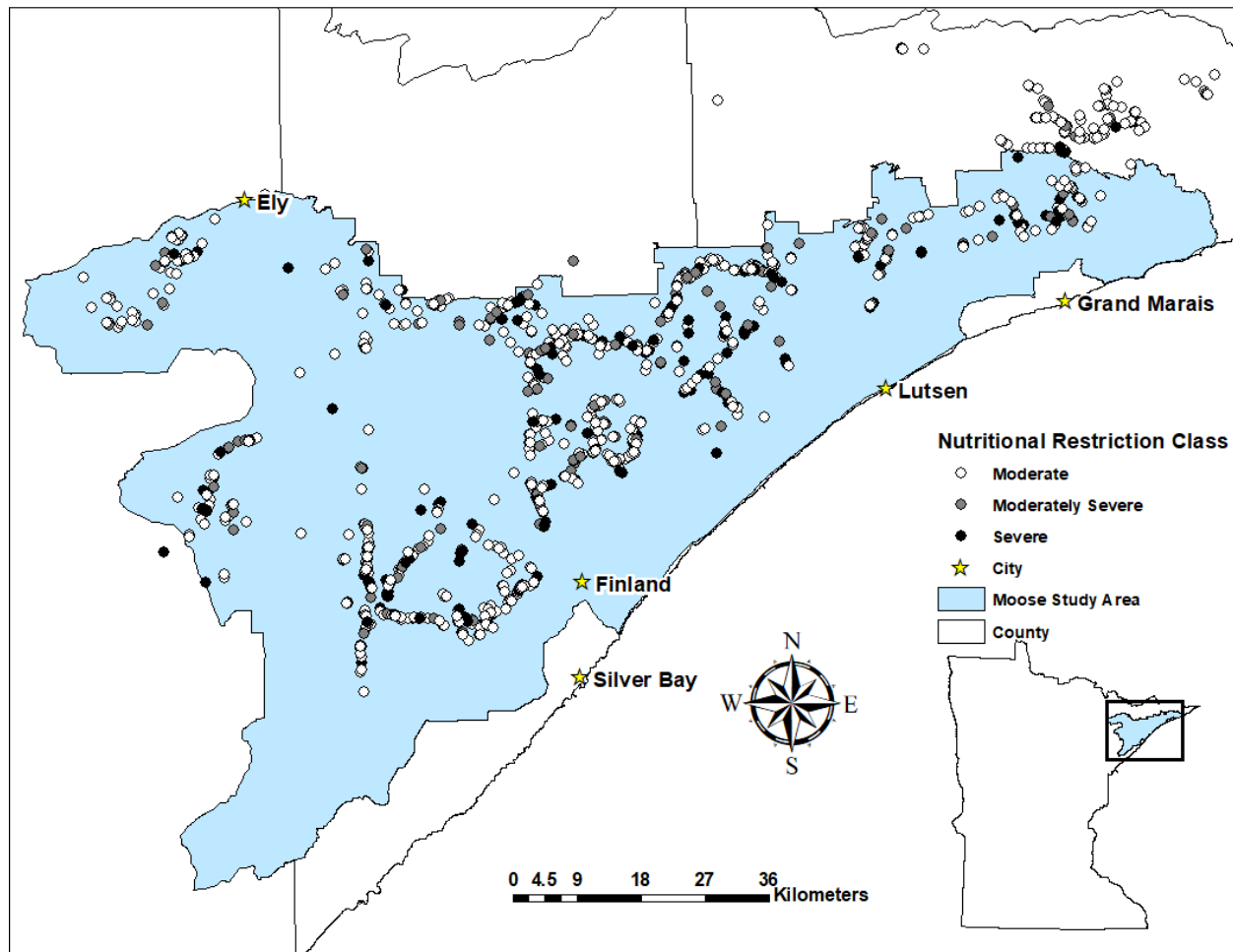


Figure 10. Spatial distribution of 1,289 samples of fresh urine in snow (snow-urine) from moose, serially collected for chemical analysis to assess the severity of winter nutritional restriction. Urinary urea nitrogen:creatinine (UN:C) ratios of <3.0 , $3.0\text{--}3.4$, and ≥ 3.5 mg:mg are indicative of moderate/normal (white circles), moderately severe (gray circles), and severe (black circles) nutritional restriction in northeastern Minnesota, January–March 2013–2018.



SHARP-TAILED GROUSE RESPONSE TO FALL PRESCRIBED FIRE AND MOWING

Charlotte Roy and Lindsey Shartell

SUMMARY OF FINDINGS

We examined sharp-tailed grouse (i.e., sharptail, *Tympanuchus phasianellus*) responses to prescribed fire and mechanical treatment (i.e., mowing) in the fall (mid-August through November) during 2015-2017 in northwest Minnesota. We surveyed sharp-tailed grouse use at sites and measured vegetation before and after management at 9 mowing treatments and 12 prescribed burns, ranging in size from 12 to 664 ac (5–269 ha) and totaling 2,495 ac (1,010 ha). We also surveyed 17 control sites ranging in size from 19 to 460 ac (8–186 ha) and totaling 1,455 ac (589 ha) using a similar survey schedule. We surveyed sharp-tailed grouse use 0–28 (mean 8.7) days before (PRE), 1 week after (1WK), 1 month after (1MO), and 1 year after (1YR) management by conducting fecal pellet transects and documenting sharptails observed at the site. We detected sharp-tailed grouse pellets at 4 of the 21 treatment sites and 4 of the 17 control sites prior to treatment. Following treatment, sharp-tailed grouse pellets were detected in ≥ 1 fall survey (1WK or 1MO) at 13 treatment sites and 4 control sites. Sharptails were observed at only 1 treatment site and at no control sites in PRE surveys, but in later fall surveys (1WK or 1MO), sharptails were observed at 4 treatment and 2 control sites. In 1YR surveys, a naïve estimate of occupancy of sites treated in fall 2015 and 2016 was higher than before management, whereas control sites remained unchanged from pre-treatment values. Our results thus far indicate that sharptail pellets provide a more useful indicator of site use than observations of grouse, and that 1 year after management sharp-tailed grouse use is greater at treated sites than control sites. Another field season is planned to increase sample sizes and improve statistical estimation of differences in occupancy, detection, and vegetation metrics.

INTRODUCTION

Sharp-tailed grouse rely on early successional habitats of open grass and brushland. Historically, these habitats were created and maintained through periodic wildfire. More recently, fire suppression has played a role in reducing habitat for sharp-tailed grouse (Berg 1997). Prescribed fire has become an important management tool for maintaining open grass and brushland habitats, but it can be difficult to implement effectively or safely under many conditions (e.g., too wet, windy, humid, dry) and can require considerable staff and resources to execute. Thus, wildlife managers supplement prescribed burning with mechanical habitat management tools (e.g., shearing, mowing) to maintain early successional habitats. Although mechanical treatments set succession back, they may not produce the same wildlife response as fire does. Wildlife managers have expressed concern that sharp-tailed grouse are not responding to management in the way they would expect if habitat were limiting.

Fall may be a particularly important season for management because juvenile sharptails disperse to surrounding habitat in the fall. Currently, most prescribed burns on state and other lands in the sharp-tailed grouse range occur in the spring (Roy and Shartell, unpubl. data from DNR Wildlife Managers). Region 1 (R1) regularly conducts fall burning, however Regions 2 and

3 (R2/3) have not been burning in the fall because of concerns about peat fires during drier conditions and challenges mobilizing a large number of fire-qualified staff on short notice during the fall (R1 has a Roving Crew to assist with prescribed fire treatments and R2 does not). This study aims to measure the response of sharptails to prescribed burning and mechanical treatments in the fall, as compared to untreated controls.

Historically, fires occurred throughout the year and maintained early successional habitats, such as open grass and brushland, on the landscape. Grassland fires were started by lightning during the growing season, and Native Americans set fires during both the spring and fall dormant seasons in both grasslands and forests to aid hunting (see review in Knapp et al. 2009). Stand replacing fires occurred at 0-10 year intervals in grass and shrub vegetation types, and in forest and woodland types, understory fires occurred at 0-10 year intervals, with more severe, stand-replacement fires occurring at less frequent intervals in Minnesota (Brown and Smith 2000).

Native Americans referred to the sharp-tailed grouse as the “fire grouse” or “fire bird” because of their association with habitats frequently burned, and kept open, by fire. Sharptails have been shown to respond to prescribed fire treatments. Kirsch and Kruse (1973) found that the numbers of broods hatched per 100 acres was higher in 2 burned areas compared to an unburned control 1 year after spring prescribed fires. Sexton and Gillespie (1979) reported that sharptails switched leks just 2 days after a spring burn, abandoning the former dancing ground in favor of the recently burned site 480 m to the north. Sharptails have also been observed returning to leks to dance the day after a burn (J. Provost, pers. comm.).

Burn season may have an effect on the response of sharptails to prescribed fire treatments. Burns conducted in the fall might attract dispersing juveniles searching for habitat. Numerous bird species are known to be attracted to fire, smoke, and recently burned areas (Smith 2000); smoke, flames, and dark burned ground could provide strong visual cues about habitat creation and its direction from a large distance. Young sharptails disperse during September and October (Gratson 1988), typically <6 km from brood rearing areas near nest sites. Sites burned in the fall are not followed by regrowth of vegetation during winter (Kruse and Higgins 1990) and could serve as lek sites the following spring. Sharp-tailed grouse also resume dancing at leks in the fall; Hamerstrom and Hamerstrom (1951) suggested that these fall dances, which include young males, might establish leks for the following spring.

Similar long-distance cues to habitat creation and maintenance are not provided by mechanical treatments. Thus, we might expect wildlife responses to management lacking these cues to be delayed or muted. In Florida shrub-grassland, burned plots were colonized by birds sooner than the mechanically treated plots, in which shrubs were chopped (Fitzgerald and Tanner 1992); birds were observed in burned plots the next day but not for months in chopped plots. Species richness and abundance remained lower in winter chop plots than in burned and control plots throughout this study. Fitzgerald and Tanner (1992) suggested that this was because burned plots provided more complex structure than mechanically treated plots.

Sharp-tailed grouse densities and responses to management treatments have been measured with numerous methods, but pellet counts are the simplest to execute. Pellet counts along transects have been shown to be indicative of the relative abundance of sage grouse (*Centrocercus urophasianus*, Hanser et al. 2011), density of red grouse (*Lagopus lagopus scoticus*, Evans et al. 2007), and habitat use of red grouse (Savory 1978). Pellet counts along transects in plots have been used to compare sage-grouse responses to mechanical and chemical treatments (Dahlgren et al. 2006). Schroeder and Vander Haegen (2014) used pellet counts along circular transects to examine the effects of wind farms on sage-grouse.

OBJECTIVES

- To compare sharp-tailed grouse use prior to and following fall management within burn, mow, and control treatments.
- To relate vegetation metrics to differences in sharp-tailed grouse use of burn, mow, and control treatments.

Hypotheses

- Sharp-tailed grouse use will increase following burning or mowing, with burned sites showing a greater increase in sharptail use than mowed sites, and both treatments having greater sharptail use than controls.
- Vegetation composition and structure will influence the use of treatment and control sites by sharp-tailed grouse, with increased use in early successional habitats.

METHODS

Study Areas

Our study was focused in the northwest sharp-tailed grouse region of Minnesota. Treated study sites were mainly on state lands, however 1 site owned and managed by The Nature Conservancy (TNC) and 3 private land sites were included. In 2015, we conducted pre-treatment surveys at 16 sites that were planned to be managed and 15 control sites. Of these, 10 sites (6 mows and 4 prescribed burns) were treated (Table 1). In 2016 we conducted pre-treatment surveys at 9 sites that were planned for management and 6 control sites. Of these, 4 sites (1 mow and 3 prescribed burns) were treated (2016 was an unusually wet year which restricted management opportunities). In 2017, we conducted pre-treatment surveys at 13 sites that were planned for management and 8 control sites. Seven sites were managed (2 mows and 5 prescribed burns).

Data Collection & Experimental Design

Treatment sites varied in size, date of management, vegetative composition, surrounding landscape, and local sharp-tailed grouse density. We attempted to match treatments in each DNR work area or sub-work area (some work areas are very large) with a control site of similar size and successional stage (e.g., crude habitat classification, visual assessment of percent cover shrubs and herbaceous vegetation, and average shrub height) *a priori* as determined by inspection of aerial imagery, conversations with managers, and site visits. Control sites were identified ≤ 6 km from treatment sites when possible (based on dispersal distances of young males in the fall; Gratson 1988). Control sites helped account for changes related to seasonal progression (i.e., changes in habitat use, social behavior, and vegetation) not related to management. Dahlgren et al. (2006) implemented a similar design to account for temporal differences in the application of management treatments for sage grouse. However we decided that a paired analysis was inappropriate due to the difficulty to closely match treatment and control sites. Thus, beginning in 2017 we selected one control for nearby sites treated on the same day. This also provide for a more balanced sample size among the 3 treatments (control, mow, and burn).

We surveyed treatment and control sites as close as possible in time, both before and after treatment (Smith 2002, also see Morrison et al. 2001:118-130). We walked systematically spaced parallel transects with a starting point placed on the site boundary and the transect traversing the treatment capturing both edge and interior portions. The sampling rate was standardized to 10 m of transect/ac (25 m/ha), with transects at least 150 m apart, based on placement of pellet transects in other studies (Evans et al. 2007, but half as dense as Dahlgren

et al. 2006, Hanser et al. 2011). We counted sharp-tailed grouse pellet piles ≤ 0.5 m from the transect, removing all pellets encountered (Evans et al. 2007, Schroeder and Vander Haegen 2014). At each pellet pile we recorded pellet freshness and vegetation category (i.e., grass, shrub, forb, grass-shrub mix, grass-forb mix, etc.). We also recorded all sharp-tailed grouse observed (heard, flushed, tracks seen) at the site while walking transects.

We sampled transects 4 times at each site—once before treatment, targeting measurements within 2 weeks of treatment (PRE), and 3 times after treatment; 1 week after treatment (1WK), 1 month after treatment (1MO), and 1 year after treatment (1YR). Treatment and control sites were sampled within 21 days of each other.

To adjust naïve occupancy rates for detection differences among treatment groups, vegetation categories, and other sources, we conducted pellet detection assessments. We accomplished this by surveying transects with pellets placed in known locations (but unknown to observers) and estimated detection probabilities for each vegetation and management category. Dahlgren et al. (2006) reported detectability of pellets along transects to be very high and similar in different types of vegetative cover. However, their study was conducted on sage grouse in sage brush, and sharp-tailed grouse habitats in Minnesota differ considerably in vegetative composition and structure.

We sampled vegetation within treatments using point-intercept sampling (Levy and Madden 1933, Dahlgren et al. 2006) to determine percent cover and average height of broad vegetation classes (i.e., tree, shrub, forb, and graminoid) before and after treatment. We sampled vegetation along 20-m transects placed perpendicular to the pellet transect, with the number of transects based on the size of the site. We marked the start of each vegetation transect using ground staples with numbered aluminum tags and flagging, and we used Global Positioning System (GPS) coordinates to allow re-measurement following treatment. During 2015-2016, we recorded maximum height for each vegetation class every 0.5 m for a total of 40 points per transect. After exploratory analysis of data and considering logistical tradeoffs, we reduced the amount of vegetation data collected in 2017, recording height and class every 1.0 m for a total of 20 points per transect. We used a pole with graduated measurements every dm to determine the type of vegetation intercepted (touching the pole) and the highest point at which each vegetation class touched the pole. We also recorded whether the vegetation was dead/dormant, combining those categories because it was unclear due to natural plant senescence whether vegetation was dormant or dead in late-fall surveys. Following treatment, we classified cut vegetation as dead/dormant, recorded height, and noted that the vegetation was cut. If no vegetation was present, the substrate type was recorded. For the purpose of this study, moss and lichen were considered a substrate type rather than vegetation.

Vegetation metrics were calculated for each study site. Proportion of cover in each class and mean maximum height were compared among treatment types and between sites with and without sharptail use. In our preliminary analysis, we included both live and dead vegetation, using the maximum height of either type at each point. Significant differences among survey periods were tested for using Tukey's Honest Significant Difference, and significant differences between sites occupied and unoccupied by sharptails was tested for using T-tests. For both a significance level of $P < 0.05$ was used.

RESULTS AND DISCUSSION

Sharp-tailed grouse pellets were detected on transects at 4 (19%) of the 21 treatment sites and 4 (24%) of the 17 control sites prior to treatment (Table 2). Following treatment, sharp-tailed grouse pellets were detected in ≥ 1 fall survey (1WK or 1MO) at 13 treatment sites (62%) and 4 control sites (24%). Sharptail observations on transects prior to treatment exhibited similar patterns, with detections at only 1 treatment site (0.05%) and no control sites (0%) in initial

surveys. In later fall surveys (1WK, 1MO), however, sharptails were observed at 4 treatment sites (19%) and 2 control sites (12%, Table 3). In 1YR surveys (completed for 2015 and 2016 sites to date), we detected pellets on transects at 6 (43%) of 14 treatment sites and 3 (23%) of 13 control sites, and sharptails were observed on transects at 2 treatment sites (14%) and 1 control site (8%). Naïve occupancy of treated sites was higher 1YR later, but occupancy of control sites remained unchanged (Figure 1).

Our pellet survey results thus far suggest that our methods are capturing sharptail use of treatment and control sites. Naïve occupancy rates (i.e., site use) from data collected thus far suggest increases in sharptail use of sites following management (Figure 1). Although occupancy and detection are confounded in naïve estimates for the 1WK and 1MO surveys (due to treatment effects on screening cover), surveys conducted 1 year (1YR) following treatments should have similar detection rates to pre-treatment measurements due to regrowth of vegetation the next growing season, especially in burn sites. Thus, the PRE vs. 1YR comparison should be reasonably straightforward and informative (e.g., Figure 2), whereas results from other time comparisons are more tenuous to interpret from naïve occupancy rates. Nevertheless, demonstrating that managed sites are used after management directly addresses manager concerns.

General field observations of vegetation prior to treatment indicated that mowing might be applied to sites at a later successional stage than prescribed fire. Prior to treatment, mow sites had a lower mean proportion of grass cover, greater mean proportions of forb and shrub cover, and taller shrubs than burn sites, however these differences were not significant (Table 4). The lack of significance could be due to the low sample size and high variability among sites.

Control sites had significantly lower graminoid height in 1MO surveys than in PRE surveys, which was likely the result of vegetation senescence (Table 5). One year later, we did not detect differences in vegetation cover or height at control sites compared to pre-treatment measurements (Table 5). At sites that were mowed, graminoid, forb, and shrub cover and height were all significantly lower in 1MO surveys, but in 1YR measurements only shrub height significantly differed from PRE survey measurements. At sites that were burned, graminoid cover, forb cover, and graminoid height were significantly lower in 1MO surveys, but in 1YR surveys no differences were detected (Table 5). Sites occupied by sharp-tailed grouse did not differ in mean vegetation cover or height from unoccupied sites during PRE or 1YR surveys (Table 6).

This report includes the fall surveys for the third year of data collection (PRE, 1WK, 1MO) but not the 1YR surveys that will be conducted in fall 2018. Results presented in this report are preliminary and subject to revision. We anticipate that 5 years of data collection may be necessary to understand the complex responses of sharp-tailed grouse to fall management treatments and associated vegetation changes. Managers throughout sharptail range in Minnesota have expressed a need for this type of information to more effectively manage for sharptails. Given the current sharptail population concerns in the east-central region, information on the effectiveness of various management options would be helpful for decision-making with finite resources for management. Managers in the northwest region are also interested in this information to ensure that their management actions are as effective as possible.

ACKNOWLEDGMENTS

We are thankful for the cooperation and support of Area Wildlife Managers, Assistant Managers, and staff at Aitkin, Baudette, Cloquet, Karlstad, Red Lake WMA, Roseau River WMA, Thief River Falls, Thief Lake WMA, and Tower. J. Eerkes at The Nature Conservancy in Karlstad was also very helpful in identifying sites and applying management. Special thanks to M. Schleif, V. Johnson, J. Colbaugh, A. Mosloff, A. Erickson, J. Brunner, A. Del Valle, and P. Coy for assistance with field data collection and data management. C. Scharenbroich loaded imagery into GPS units for field use. M. Larson provided comments that improved this report. J. Giudice provided statistical guidance and produced figures for this report. This study has been funded in part through the Wildlife Restoration (Pittman-Robertson) Program.

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Table 1. Management activities completed for sharp-tailed grouse habitat in northwest Minnesota during fall in 2015, 2016, and 2017 and associated control sites, in order of treatment date.

Site	Work area	Treatment	Treatment date	Treatment ac (ha)	Control ac (ha)
Roseau River	Roseau River	Mow	28 Aug–16 Sep 15	31 (12.5)	28 (11.3)
Skull Lake	Karlstad	Burn	1 Sep 2015	90 (36.4)	70 (28.3)
Halma	Karlstad	Mow	16–23 Sep 2015	41 (16.6)	39 (15.8)
Red Lake Mow	Red Lake	Mow	22 Sep 2015	12 (4.9)	22 (8.9)
Spooner	Baudette	Mow	28 Sep 2015	22 (8.9)	26 (10.5)
Caribou	Karlstad	Burn	28 Sep 2015	664 (268.7)	No control
TL 2015 Burn	Thief Lake	Burn	28 Sep 2015	58 (23.5)	31 (12.5)
Red Lake Burn	Red Lake	Burn	19 Oct 2015	152 (61.5)	176 (71.2)
Prosper	Baudette	Mow	19–30 Oct 2015	63 (25.5)	201 (81.3)
TL Mow	Thief Lake	Mow	30 Oct 2015	20 (8.1)	19 (7.7)
TL 2016 burn	Thief Lake	Burn	1 Sep 2016	31 (12.5)	37 (15.0)
Noracre ^a	Roseau	Burn	14 Sep 2016	71 (28.7)	22 (8.9)
Roseau brush	Roseau	Mow	27 Sep–7 Oct 16	23 (9.3)	29 (11.7)
Espelie	Thief River Falls	Burn	3 Oct 2016	443 (179.3)	460 (186.2)
Halma 2017	Karlstad	Mow	28 Aug–8 Sep 2017	62 (25)	61 (25)
Gates	Red Lake	Burn	8 Sep 2017	388 (157)	No control
K burn	Roseau	Burn	13 Sep 2017	90 (36)	93 (38)
F burn	Roseau	Burn	13 Sep 2017	99 (40)	Same as K
Prosper 2017	Baudette	Mow	27 Sep–26 Oct 2017	70 (28)	41 (17)
O burn	Roseau	Burn	9 Oct 2017	17 (7)	100 (40)
I burn	Roseau	Burn	9 Oct 2017	48 (19)	Same as O

^aNoracre burn was treated again (burned in spring 2017 and sprayed with herbicide in spring and summer 2017) before the 1YR survey, so it is not clear whether use in the 1YR survey was due to the burn or another treatment.

Table 2. Sharp-tailed grouse pellet detections at treatment and control sites in northwest Minnesota. Surveys were conducted before (PRE), 1 week (1WK), 1 month (1MO), and 1 year (1YR) after treatment. The number of pellet detections on transect are indicated numerically, and pellets detected off-transect are indicated with an OT, indicative of site use not captured in sampling. An asterisk indicates that snow impeded detection of pellets, and T indicates that tracks were detected in snow. Surveys with confirmed sharptail use through any source of sign are highlighted in gray. NS indicates that the 1YR survey has not yet been completed for sites managed during fall 2017.

Fecal pellets Site	Treatment				Control			
	PRE	1WK	1MO	1YR	PRE	1WK	1MO	1YR
Red Lake mow	0	0	0	2	0	0	0	1
Thief Lake mow	0	0	0*	0	0	0	0*	0
Spooner mow	0	0	2	0	0	0	0	0
Roseau 2015 mow	2 OT	1 OT	1	0	0	0	0	0
Halma mow	0	0	0	1 OT	1	1	2	0
TL 2015 burn	1 OT	0	1	1 OT	0	0	0	0
Skull Lake burn	0	1	0	1	0	0	0	0
Red Lake burn	0	0	0	0	0	0	0*	0
Prosper mow	0	1	0*	2	1	11	1T*	5 4 OT
Caribou burn	1	2	1 OT	0	-	-	-	-
TL 2016 burn	0	1	4 7 OT	7 1 OT	0	0	0	0
Noracre burn ^a	0	9 3 OT	0	3T*	0	0	0	0
Espelie burn	1	6	18 31 OT	1 3 OT	1 1 OT	1 3 OT	4 5 OT	3T* 2 OT
Roseau 2016 mow	1 OT	0	0	0	0	0	0	0
Halma 2017 mow	0	1 OT	1	NS	0	0	0	NS
Gates burn	0	3	0	NS	-	-	-	-
K burn	1 1 OT	0	7 11 OT	NS	-	-	-	-
F burn	4 1 OT	1	5 5 OT	NS	0	0	0	NS
Prosper 2017 mow	0	3T*	0	NS	0	0	0	NS
O burn	0	0	0	NS	-	-	-	-
I burn	0	0	0	NS	3 OT	1 OT	0	NS

^aNoracre burn was treated again (burned in spring 2017 and sprayed with herbicide in spring and summer 2017) before the 1YR survey, so it is not clear whether use in the 1YR survey was due to the burn or another treatment.

Table 3. The number of sharp-tailed grouse observed at treatment and control sites in northwest Minnesota. Surveys were conducted before (PRE), 1 week (1WK), 1 month (1MO), and 1 year (1YR) after treatment. Sharp-tailed grouse observed while off-transect are indicated with OT, indicative of site use not captured in sampling. Surveys with confirmed sharptail use through observations of any birds at the site are highlighted in gray. NS indicates that the 1YR survey has not been completed for sites managed in fall 2017.

Grouse observations	Treatment				Control			
Site	PRE	1WK	1MO	1YR	PRE	1WK	1MO	1YR
Red Lake mow	0	0	0	0	0	0	0	0
Thief Lake mow	0	0	0	0	0	0	0	0
Spooner mow	0	0	11	3 OT	0	0	0	0
Roseau 2015 mow	2 OT	5 OT	2 OT	0	0	0	0	0
Halma mow	0	0	1	0	0	2	0	0
TL 2015 burn	4	0	0	0	0	0	0	0
Skull Lake burn	0	0	0	0	0	0	0	0
Red Lake burn	0	0	0	0	0	0	0	0
Prosper mow	0	0	0	1	0	0	0	12-20
Caribou burn	0	5	13	2 2 OT	-	-	-	-
TL 2016 burn	0	0	0	0	0	0	0	0
Noracre burn ^a	0	0	0	0	0	0	0	0
Espelie burn	0	1	2 OT	0	5 OT	1	7 OT	0
Roseau 2016 mow	6 OT	0	0	0	0	0	0	0
Halma 2017 mow	0	0	0	NS	0	0	0	NS
Gates burn	0	0	0	NS	-	-	-	-
K burn	0	0	0	NS	-	-	-	-
F burn	1 OT	0	0	NS	0	0	0	NS
Prosper 2017 mow	0	0	0	NS	0	0	0	NS
O burn	0	0	0	NS	-	-	-	-
I burn	0	0	0	NS	0	0	0	NS

^aNoracre burn was treated again (burned in spring 2017 and sprayed with herbicide in spring and summer 2017) before the 1YR survey, so it is not clear whether use in the 1YR survey was due to the burn or another treatment.

Table 4. Mean pre-treatment vegetation cover and height for 4 vegetation classes at control ($n = 17$), mow ($n = 9$), and burn ($n = 12$) sites sampled for sharp-tailed grouse use in northwestern Minnesota from 2015-2017. No significant differences ($P < 0.05$) were observed.

	Control	Mow	Burn
Cover (proportion)			
Graminoid	0.93	0.90	0.98
Forb	0.30	0.44	0.22
Shrub	0.34	0.43	0.26
Tree	0.06	0.06	0.05
Height (m)			
Graminoid	0.52	0.53	0.54
Forb	0.33	0.37	0.28
Shrub	1.22	1.43	0.74
Tree	2.79	1.89	1.76

Table 5. Change in mean vegetation cover and height from pre-treatment to 1 month after (1MO, control $n = 17$, mow $n = 9$, and burn $n = 12$) and 1 year after (1YR, control $n = 13$, mow $n = 7$, and burn $n = 7$) at sites sampled for sharp-tailed grouse use in northwestern Minnesota from 2015-2017. Comparisons to 1YR surveys exclude sites managed in 2017. Significant differences ($P < 0.05$) between pre and post measurements are indicated with an asterisk.

	Control 1MO	Control 1YR	Mow 1MO	Mow 1YR	Burn 1MO	Burn 1YR
Cover (proportion)						
Graminoid	-0.03	0.00	-0.36*	-0.04	-0.43*	-0.08
Forb	-0.14	0.00	-0.36*	0.03	-0.18*	0.11
Shrub	-0.06	0.03	-0.29*	-0.12	-0.10	-0.06
Tree	-0.02	-0.01	-0.06	-0.05	-0.02	0.00
Height (m)						
Graminoid	-0.13*	-0.02	-0.39*	-0.09	-0.25*	-0.02
Forb	-0.06	0.00	-0.19*	-0.09	0.07	-0.02
Shrub	0.08	0.00	-1.20*	-0.85*	-0.03	0.03
Tree	-0.25	-0.01	-0.51	-1.29	0.19	-0.07

Table 6. Mean cover and height at sites occupied and unoccupied by sharp-tailed grouse pre-treatment (PRE, occupied $n = 11$, unoccupied $n = 27$) and one year after (1YR, occupied $n = 13$, unoccupied $n = 14$) at sites sampled for sharp-tailed grouse use in northwestern Minnesota during 2015-2017. 1YR surveys exclude sites managed in 2017. No significant differences ($P < 0.05$) between occupied and unoccupied sites were observed.

	PRE	PRE	1YR	1YR
Sharptail occupancy	unoccupied	occupied	unoccupied	occupied
Cover (proportion)				
Graminoid	0.94	0.94	0.92	0.87
Forb	0.33	0.26	0.35	0.35
Shrub	0.36	0.28	0.34	0.29
Tree	0.06	0.07	0.03	0.05
Height (m)				
Graminoid	0.52	0.55	0.51	0.47
Forb	0.34	0.29	0.33	0.27
Shrub	1.15	1.03	0.99	0.87
Tree	2.03	3.32	2.27	2.27

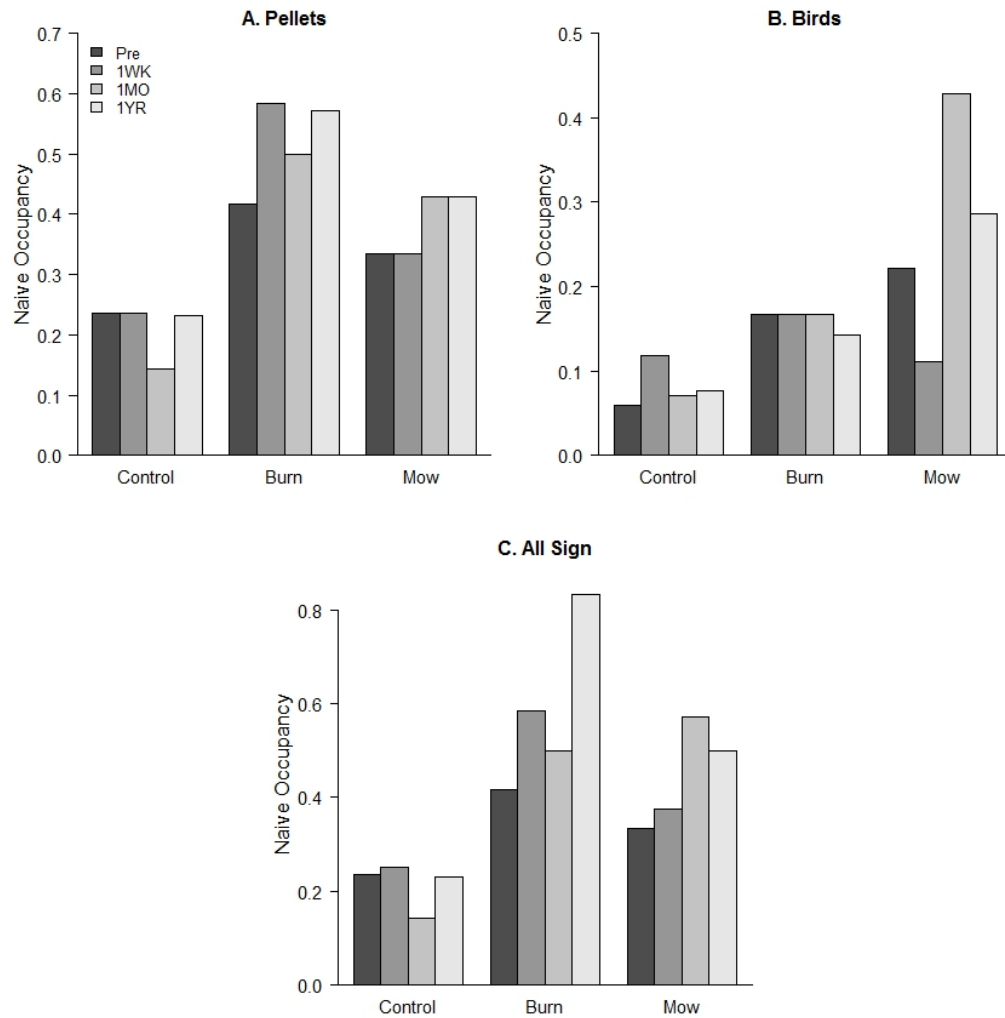


Figure 1. Naïve occupancy for sharptail pellets (A), sharptail observations (B), and all sign (includes off-transect detections, (C) during surveys conducted before (PRE), 1 week after (1WK), 1 month after (1MO), and 1 year after (1YR) treatment during 2015 and 2016 at study sites in northwest Minnesota to assess the effects of prescribed burning and mowing.

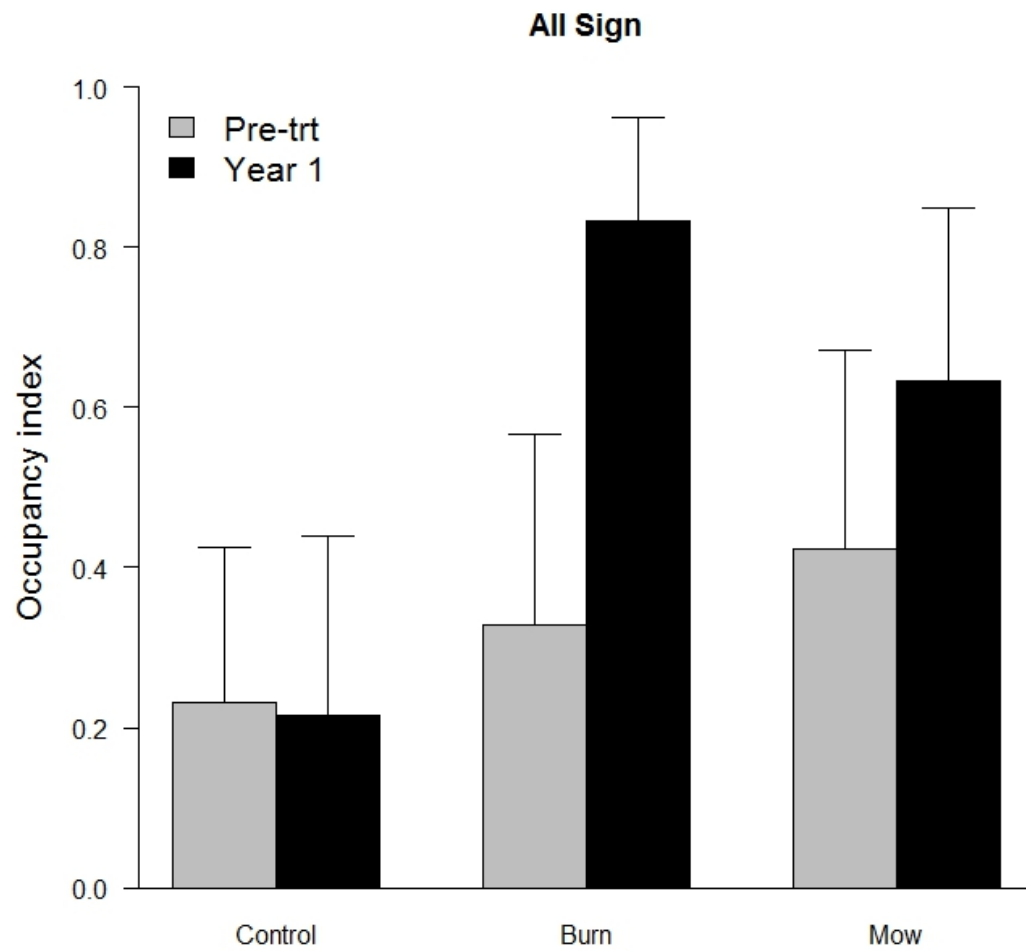


Figure 2. Mean naïve occupancy index with 85% confidence intervals at sites managed in northwestern Minnesota for sharp-tailed grouse during 2015 and 2016 based on a logistic regression model with an offset for transect length.

SURVIVAL, NEST SUCCESS, AND HABITAT SELECTION OF SHARP-TAILED GROUSE IN EAST-CENTRAL MINNESOTA – FINAL PROJECT REPORT

Lindsey M. Shartell

SUMMARY OF FINDINGS

Across 3 years of study, 39 female and 20 male sharp-tailed grouse (*Tympanuchus phasianellus*) were radiocollared at 7 leks in East-Central Minnesota. Lek attendance (average number of grouse observed per lek) was affected by wind speed, and female attendance occurred later than expected, peaking in early May, which was thought to be driven by late snow cover. Annual survival of radiocollared grouse was 0.41 ± 0.07 for females and 0.24 ± 0.10 for males. Monthly survival rates indicated that female grouse mortality was greatest during the breeding season with the highest mortality rate occurring in June. A total of 44 nests were located and monitored during the study, including 4 known re-nests. Nest sites included a wide variety of cover types and vegetation structure, but had greater overhead cover, shrubs present at the nest, lower vegetation density at 0.5-1.0 m from 15 m, lower soil moisture, greater vegetation density at 0-0.5 m from 2m, and fewer perches than non-nest sites. Nest survival rate (≥ 1 egg hatched) was 0.42 ± 0.09 . Vegetation metrics were not significantly related to nest success, however successful nests tended to have more overhead cover and higher vegetation density than unsuccessful nests. Broods were followed for 26 hatched nests over 3 years of study. Brood survival (≥ 1 chick at 50 days post hatch) for hatched nests was 42%. The average number of surviving chicks per nesting hen was 1.15. Hens used brushland, open upland, agriculture, and hayfield in greater proportion than their availability. Brooding hens showed similar use of cover types, with a notable increase in the use of hayfields. Lek complexes ranged in size from 40 – 1,460 ha and grouse locations were within 4.1 km of lek sites. Habitat management for sharp-tailed grouse should focus within 4 km of leks and provide nesting and brood rearing habitat in close association on both public and private lands.

INTRODUCTION

Sharp-tailed grouse ranged across much of the state of Minnesota prior to European settlement (Berg 1997). The open and brushland cover they inhabited was maintained by natural disturbances, particularly wildfire. Since that time, sharp-tailed grouse have been experiencing a long-term decline throughout their range (Gregg and Niemuth 2000; Silvy and Hagen 2004), and in recent decades have suffered marked population declines (Connelly et al. 1998) due to poor management and habitat loss (Riley 2004). In Minnesota, sharp-tailed grouse populations have declined sharply because the open brushland habitat they inhabit is vulnerable to destruction, senescence, conversion to incompatible cover, and succession to forest (Berg 1997). Estimated annual harvest rates for sharp-tailed grouse have fluctuated over time, with a high of 154,000 in 1949 (Berg 1997). Today, harvest rates have decreased to a 10-year average (2006-2015) of approximately 11,100 (Dexter 2015, Figure 1). Annual spring surveys of dancing grounds in Minnesota indicate that the mean number of sharp-tailed grouse per dancing ground has fluctuated since 1980 and remains within the observed range, however the number of leks surveyed in East-Central Minnesota had declined markedly (Roy 2016). Though

it is unclear how this decline is affected by survey effort, observations of historical leks becoming inactive are common in the East-Central (MNDNR, unpublished data).

Because of population decline, and subsequent lower harvest, MNDNR set a long-term goal to raise the annual sharp-tailed grouse harvest to 40,000 (MNDNR 2007). To attain this goal, forest planning efforts have identified priority open landscapes for brushland management (MNDNR 2007) with an objective of increasing the quantity and quality of habitat throughout the sharp-tailed grouse range in Minnesota. Brushland management, however, is expensive and comprises a considerable portion of the MNDNR Wildlife Operations budget. Open-brushland management expenditures on public lands exceeded \$700,000 annually from FY2012-FY2014 (MNDNR, unpublished data). Despite the amount of resources allocated to open-brushland management, significant information gaps exist in relation to sharp-tailed grouse habitat use in Minnesota.

Research on sharp-tailed grouse habitat specific to Minnesota has been limited, though some studies have been conducted in the northwestern (Artmann 1971; Schiller 1973; Wells 1981) and east-central (Hanowski et al. 2000) sharp-tailed grouse ranges. In addition, Berg (1997) summarized results from unpublished investigations to determine specific recommendations for habitat, such as size, composition, height, and density. The MNDNR and Minnesota Natural Resources Conservation Service (MN NRCS) provide detailed management guidance for sharp-tailed grouse habitat based on these studies, however it is unclear whether areas currently used by sharp-tailed grouse reflect the recommended characteristics or if managing for these features benefit sharp-tailed grouse populations. Annual dancing ground surveys and harvest estimates may provide some insight into the population level response to management (Connelly et al. 1998), however linking survival and fitness of sharp-tailed grouse to vegetation characteristics and associated management would better direct management resources (Martin 1992).

OBJECTIVES

1. Quantify adult and brood survival and identify causes of mortality.
2. Identify vegetation characteristics that influence nest site selection and nest success.
3. Evaluate breeding season habitat use by sharp-tailed grouse hens and broods.

METHODS

Study Area

Study sites were within the east-central Minnesota sharp-tailed grouse range. The east-central sharp-tailed grouse range is within the forested region of Minnesota. Sharp-tailed grouse habitat in this region consists mainly of open and brush lowlands and hayfields on both public and private lands. Grouse were captured at 7 leks in 2 concentrated areas in Aitkin County and St. Louis County. Leks selected for trapping occurred on public and private lands with known habitat management histories.

Field Methods

Sharp-tailed grouse were captured and radiocollared at active leks (dancing grounds) during the lekking period (from late March through early May) in 3 years (2013-2015). Grouse were captured using walk-in funnel traps and drift fences placed across the center of the lek (Toepfer et al. 1987). Staff and volunteers monitored trap sets daily from nearby ground blinds and recorded weather conditions and daily attendance of male and female grouse. Captured grouse were fitted with a necklace style radio transmitter (ATS Model A3960) equipped with a 12-hour mortality sensor. Date of capture, location, transmitter frequency, sex, age class (juvenile or adult), and weight (only in 2015) were recorded for each grouse.

Radiocollared grouse were located using truck mounted omni-directional antennas and hand-held 2-element antennas (Telonics RA-23K) in combination with portable receivers (ATS R2000). Location was determined either by searching on foot using homing techniques and circling or visually confirming location or from a distance using triangulation with a minimum of 3 directional azimuths. Bird ID (frequency), date, time, coordinates at the site, and azimuth to source of greatest signal (if using triangulation) was recorded. All GPS locations were recorded using the UTM Zone 15 North (NAD 1983) coordinate system.

Grouse were located every 2-3 days (minimum of 2 observations per week) to determine breeding status, habitat selection, and survival over the course of the breeding season (May-August). All observations were made during daylight hours, and the order of observations was interchanged to obtain a sample that accounts for differing time of day and associated activities (e.g. feeding, loafing, and roosting). For hens, once sedentary behavior was initiated (found repeatedly at the same location), the nest was confirmed by flushing the hen. Actual location, clutch size, and overhead cover (see methods below) were recorded. The average incubation time for sharp-tailed grouse is 23 days, during which hens usually remain on or within close distance of the nest. If nesting hens were found to be off of the nest, the nest was located to determine fate. In cases of depredation, the nest site was searched and any predator signs noted. After hatch, nests were observed to confirm clutch size and determine the number of successfully hatched eggs. Egg shells were collected, air-dried and stored for future projects using genetic analysis. In cases of mortality, location, condition, and cause of mortality (e.g., mammalian predation, avian predation, exposure/weather, hunting) were recorded and any predator or scavenger signs noted.

Following hatch, hens and broods were located every 2-3 days (minimum of 2 observations per week) to monitor habitat use and survival. Chicks are generally able to fly 10 days after hatch, though they may not flush when approached. Hen location was recorded at or near the site where the hen and/or brood was flushed or by circling within 5 m of the hen without flushing. To determine brood survival, hen behavior and visual confirmation of chicks was used. When possible, the number of chicks present was determined, however young broods were not purposefully flushed. After 30 days from hatch, hens were flushed and the immediate area searched to flush any chicks to determine brood survival and size. Brood counts continued through August or until approximately 50 days post-hatch, as the likelihood of chick dispersal increases at this point. During the fall and winter season, grouse were located monthly to check for mortality and to determine over-winter survival rates. In cases of mortality, grouse were located and probable cause of mortality was determined.

After hatch and movement away from the nest, vegetation metrics at the nest site were assessed. In addition, for each nest 2 non-nest points were assessed using the same methods. Non-nest points were chosen by randomly selecting a bearing and distance (from 100 to 200 m). Non-nest points were searched to ensure that no other sharp-tailed grouse nests occurred in the immediate area. Brood sites were also assessed using the same vegetation assessment methods, once the hen had moved away from the site.

At each nest, non-nest, and brood site the existing vegetation was measured. Height of the residual cover, understory, and mid-story (shrub) vegetation, if present, was measured to the nearest cm or m at the plot center (the nest), at 2 m, and at 15 m from plot center in each cardinal direction. Overhead cover at plot center (within the nest bowl) was estimated using a 20 cm disc with 9 evenly spaced squares (Roersma 2001; Manzer 2004). The number of squares at least 50% visible (not obscured by vegetation) when viewed from 1 m directly overhead was recorded. Vegetation density was measured using a 2 m tall density pole divided into four 0.50 m sections (adapted from Nudds 1977). Percent obscured (in 10% increments) was determined from a height of 1 m (kneeling) for each section from 2 m and 15 m away in

each cardinal direction. The number of potential avian predator perch sites (stems > 3 m in height) within 30 m of the nest was recorded (Manzer and Hannon 2005). If the nest or point occurred under a shrub patch, the species was identified and the patch height, length, and width were measured to the nearest m. For large patches, patch height was determined by measuring the tallest stem in each cardinal direction and averaging. Patch area and volume were calculated from these values. Soil moisture level was classified as well-drained, saturated, or standing water using a scale from 1-3.

Analysis

Factors affecting lek attendance were assessed using univariate linear regression. Annual and monthly adult survival were estimated by sex using known-fate models in Program MARK (White and Burnham 1999). Monthly survival probabilities were estimated using the Kaplan-Meier product limit estimator (Kaplan and Meier 1958) with a staggered entry design (Pollock et al. 1989). Cases of mortality within 1 week of capture were removed from the survival analysis.

Nest success was evaluated using the nest-survival module in Program MARK (Dinsmore and Dinsmore 2007). Daily survival rate (DSR) was calculated and used to estimate overall nest success. Vegetation characteristics affecting nest success were assessed in MARK and R using logistic regression. Nest site selection was assessed in R using case-control conditional logistic regression using matched nest and non-nest sites. Akaike's Information Criteria corrected for small sample sizes (AICc) and likelihood values were used to compare candidate models and the top model is presented. Brood survival was not statistically analyzed due to the low sample size of broods, however apparent brood survival, brood size, and the ratio of hatch-year birds to nesting hens are reported by year. Vegetation at brood sites were characterized by comparing brood sites to nest and non-nest sites in pairwise comparisons using Tukey's Honest Significant Difference in R.

Breeding season (May-August) habitat use was assessed using compositional analysis of cover types (Aebischer et al. 1993) for all hen locations and for locations of hens with broods. Individual hen locations were compared to cover type availability surrounding the lek, or lek complex when leks overlapped. Six leks/complexes were identified and delineated using a convex hull incorporating hen locations. Cover type was classified into 6 categories: agriculture, brushland, forest, hayfield, open lowland, and open upland based on field assessment, aerial imagery, and ground-truthing at the study sites. Digitized cover type was converted to a 10m resolution grid for analysis. Developed land and open water were classified as covering <1% of the land, but were excluded from the analysis because of non-use. Use was defined as the proportion of locations in each cover type, excluding successive locations of hens on lekking grounds and nests. Availability was defined as the proportion of the lek or lek complex in each cover type.

RESULTS

Sharp-tailed grouse lek attendance was monitored at each trap site for a total of 202 observations with dates ranging from March 13 to May 16. Daily maximum attendance of females and males at leks were significantly related to each other ($P < 0.001$). Wind speed and temperature were significantly related to date ($P < 0.01$, $P < 0.001$). Wind speed was significantly related to both female ($P < 0.01$) and male ($P < 0.001$) lek attendance (Figure 2), however temperature was not related to either female or male lek attendance ($P = 0.23$, $P = 0.71$, Figure 3). Date was significantly related to female grouse attendance ($P < 0.001$, Figure 4), but not to male attendance ($P = 0.50$, Figure 5). Timing of female attendance at leks varied year to year. The first hen was observed on April 16 in 2013, April 3 in 2014, and March 27 in 2015. Spring snow melt varied year to year with 2013 being a late spring and 2015 being an early spring, however snow pack was not tracked at trapping sites.

A total of 39 female and 20 male sharp-tailed grouse were radiocollared at seven leks over the three years of the study. Average annual survival rate of radiocollared grouse was 0.41 ± 0.07 for females and 0.24 ± 0.10 for males; however survival rates differed by year (Figure 6). Monthly survival rates differed by month and between females and males (Table 1, Figure 7). The lowest monthly survival rate for females was in July, while the lowest monthly survival for males was in November. Over-winter survival from December to February was also low for males but was relatively high for females. Causes of mortality for adult grouse were difficult to determine in many cases ($n = 15$). Avian predation was the most common identifiable cause of mortality ($n = 12$). Also observed were cases of mammalian predation ($n = 6$), hunter harvest ($n = 2$), and un-depredated deaths potentially due to health or exposure ($n = 7$).

Across all years of the study, a total of 44 nests were located and monitored. This included 4 known re-nests initiated after nest loss. Following nest depredation or loss, 27% of hens re-nested. Average clutch size from nests that reached the incubation stage ($n = 35$) was 11 and ranged from 7 to 14 eggs. On average, nests were 1.2 km from the lek where trapped, but distances ranged from 60 m to 3.8 km from the lek. No nest site fidelity was observed year to year or for re-nesting hens. The top model for nest site selection, using nest sites and non-nest sites, included overhead cover, presence of shrub at the nest, vegetation density 0.5-1.0 m from 15 m, soil moisture level, vegetation density 0-0.5 m from 2m, and number of perches ($P < 0.001$, AICc = 54.77, Table 2).

Apparent nest success (≥ 1 egg hatched) was 59%. Adjusted rate of nest survival was 0.42 ± 0.09 , based on a daily survival rate of 0.976 ± 0.006 , however nest survival differed by year (Table 3). For successful nests, the rate of egg hatch was 88%, with unhatched eggs being found in a variety of stages of development. The average hatch date for first nests was 24 June. The most common reason for nest loss was depredation ($n = 14$). Also observed were cases of hen mortality ($n = 3$), prescribed fire ($n = 1$), and unknown loss ($n = 1$). The influence of vegetation metrics on nest success was modeled using 42 nests (2 nests were unable to be relocated for sampling), however vegetation variables were not able to sufficiently explain nest success despite some indication of relationships (see discussion).

Broods were followed for 26 hatched nests over 3 years of study. Seven broods were assumed to be lost following hen mortality during the brooding period. Eleven broods (42% of hatched nests, 25% of all nests) had at least one surviving chick at approximately 50 days post-hatch, with a total of 47 surviving chicks counted among the 3 years. The average number of surviving chicks per nesting hen was 1.15, and the average number of chicks per successfully nesting hen was 1.81 but varied greatly by year (Table 4). Brood sites were more similar to random non-nest sites than nest sites (Table 5). However, brood sites were similar to nest sites in that the height of shrubs at 15m was shorter than non-nest sites. Brood sites differed from both nest and non-nest sites in that they had shorter residual vegetation at the center, 2m, and 15 m, and lower density of vegetation at 15 m from 0.5-1.0m, 1.0-1.5m, and 1.5-2.0m (Table 5).

Compositional analysis was conducted using proportional cover type use of 43 hens. Results indicated that habitat selection by hens differed from random ($\lambda = 0.28$, $P < 0.01$). Cover types ranked by magnitude of selection based on use and availability were brushland, open upland, agriculture, hayfield, open lowland, and forest (Table 6). Brushland, open upland, agriculture, and hayfield were used at a greater proportion than their availability (Figure 8). Compositional analysis of cover type use by hens with broods also indicated that brood use differed from random ($\lambda = 0.18$, $P < 0.01$) with the same ranking of cover types. However, overall, hayfields were used at a noticeably higher rate by brooding hens (Figure 9).

Grouse locations (male and female, $n=1,087$) ranged from 0–4.1 km from the lek, with an average distance of 0.9 ± 0.03 km. Of these locations 36% were on public lands, and 24% were

on lands with known habitat management occurring within 10 years. Using all locations at each lek or lek complex, area of a convex hull ranged from 40–1460 ha, however avoidance of forest cover within some areas was apparent.

DISCUSSION

As expected, grouse attendance on leks was influenced by wind speed and date, consistent with other studies of lek attendance (Drummer et al. 2011). In Minnesota, annual lek surveys are to be conducted when the wind speed is <16 km/hr (Roy 2016). In this study, wind speeds as low as 5 km/hr reduced the average number of grouse present. Female grouse attendance on leks peaked later than expected. Traditionally annual lek surveys are to be conducted from April 1 to April 25, to capture the peak of lek activity (Roy, unpublished instructions). In 2013, snow remained on the ground into mid-April, and the first hen was not observed until April 16, with hen attendance peaking during the first week of May. Anecdotally, across years snow depth seemed to influence hen attendance on leks, however was not measured directly. Across all years, hen attendance was greatest from mid-April through early May. Male lek attendance peaked from early April through early May, corresponding to the survey period. Because annual lek surveys in Minnesota rely on an index of males per dancing ground they are likely not impacted by the later arrival of hens, however the finding that male and female attendance are significantly related suggests that in years when hen attendance peaks later, potentially because of late snow melt, male counts could be lower.

Average annual survival rates of 41% for females and 24% for males, were similar to the annual survival rates found in other studies across the range of sharptails. In hunted populations, survival rates ranged from 17 to 42% (Robel et al. 1972, Moyles and Boag 1981, Giesen 1987), and in an un-hunted population survival was 53% (Schroeder 1994). Hunter harvest accounted for <5% of mortality observed. For hens, a drop in survival in July corresponds with the time period when hens are caring for young broods and may be more vulnerable to predation. Similarly, Manzer and Hannon (2007) found that hen survival was lowest during the reproductive period, reporting 53% survival from May to mid-August. Hen survival during the breeding season may be an important component in understanding population trends.

Cover type and vegetation varied greatly among nest sites selected. There was no indication that hens were selecting for specific nest site characteristics, however modeling results suggested that overhead cover and shrub presence were important drivers. Similarly, a study in northeast British Columbia found that sharp-tailed grouse hens selected nest sites with greater shrub, grass, and residual cover and taller vegetation (Goddard et al. 2009). In this study, cover types and vegetation metrics at nest sites had high variability, making it difficult to detect drivers of nest success and nest site selection. Despite vegetation metrics not being strongly related to nest success, successful nests tended to have more overhead cover and higher vegetation density than unsuccessful nests. Sharp-tailed grouse in east-central MN may be showing some adaption to a less frequent disturbance regime by using a variety of nesting sites that are not typical of sharp-tailed grouse in the prairie habitats of NW Minnesota and other states.

Nest survival of $42\% \pm 9\%$ in this study was on the lower end of nest success rates of 47 to 72% found in other studies (Sisson 1976, Marks and Marks 1987, Meints 1991, Manzer and Hannon 2005). Manzer and Hannon (2005) found that nests were more likely to succeed with taller concealment cover, and that nest success was related to corvid density, which was not measured in this study. Brood survival was low with only 25% of nests having ≥ 1 chick survive to 50 days post-hatch and an average of 1.81 chicks per successfully nesting hen. Another study reporting on reproduction found 67% of broods had ≥ 1 chick survive 35 days post-hatch, with an average of 3.59 chicks per successfully nesting hen (Goddard and Dawson 2009). Goddard and Dawson (2009) found that increased travel distances were related to chick

mortality, suggesting the importance of having nesting and brood-rearing habitat in close association. Further analysis of data in this study could explore the spatial arrangement of habitat and its relationship to brood survival.

Hens with broods used hayfields more often and in greater proportion than their availability. Goddard et al. (2009) also found that brood-rearing hens primarily used hayfields and cereal crops during the brood-rearing period. A survey of hayfield mowing practices in counties associated with our study sites indicated that on average sites are first mowed early-mid June and are mowed 2-3 times per year with > 5 weeks between mowing (Corace et al. 2009). These activities overlap with the time when sharp-tailed grouse hens are raising broods. Mowing practices and an affinity for hayfields by brooding hens could be a conservation concern for the species. In this study, when brooding hens were located in hayfields, they were always in un-mowed hayfields. Freshly mowed hayfields likely do not provide sufficient cover for broods. In addition 2 mortalities were discovered in recently mowed sites, however the cause of mortality was not clear.

Berg (1997) reported that in Minnesota sharp-tailed grouse require blocks of contiguous habitat at least 5 km² (500 ha). Similarly, Sjogren (1996) reported that the home range size of grouse in the Upper Peninsula of Michigan was 641 ha. Based on locations of male and female grouse, lek complexes in this study ranged in size from 40–1460 ha. Grouse locations were as far as 4.1 km from the lek, however 94% of locations were within 2.5 km of the lek. These results suggest that habitat management efforts can be focused on lands within 4 km of the lek, with a greater priority given to lands within 2.5 km. Only 36% of locations were on public lands and 24% on lands with habitat management conducted by the MNDNR. Surrounding private lands play an important role in sharp-tailed grouse habitat.

ACKNOWLEDGMENTS

Thank you to field technicians John McLaughlin, Briana Schnelle, and Vincent Johnson who spent many hours trapping and tracking grouse, and the many volunteers and MNDNR staff that assisted with trapping efforts. Wes Bailey and Mike Larson developed and proposed this study, securing funding following an initial pilot study conducted in 2009. This work is adapted from their initial proposal.

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Table 1. Monthly and over-winter (December to February) survival rates by sex for radiocollared sharp-tailed grouse in East-Central Minnesota from 2013-2015.

Time period	Female	Male
March	$1.00 \pm 0.13 \times 10^{-4}$	$1.00 \pm 0.65 \times 10^{-7}$
April	$1.00 \pm 0.13 \times 10^{-7}$	$1.00 \pm 0.30 \times 10^{-9}$
May	0.91 ± 0.04	0.87 ± 0.07
June	0.88 ± 0.05	0.85 ± 0.08
July	0.67 ± 0.08	0.94 ± 0.06
August	0.87 ± 0.07	0.94 ± 0.06
September	0.95 ± 0.05	0.93 ± 0.07
October	$1.00 \pm 0.20 \times 10^{-4}$	0.83 ± 0.11
November	$1.00 \pm 0.11 \times 10^{-4}$	0.71 ± 0.17
Over-winter	0.94 ± 0.06	0.67 ± 0.19

Table 2. Conditional logistic regression results for the top model explaining radiocollared sharp-tailed grouse nest site selection in East-Central Minnesota from 2013-2015.

Variable	Coefficient	P-value
Overhead cover	0.57	<0.001
Presence of shrub	3.41	0.004
15m density 0.5-1.0m	-0.05	0.006
Soil moisture	-1.48	0.01
2m density 0-0.5m	0.03	0.03
Number of perches	-0.16	0.19

Table 3. Number of nests, apparent nest success, daily nest survival rate, and nest survival rate by year for radiocollared sharp-tailed grouse hens in East-Central Minnesota from 2013-2015.

Year	Total nests	Apparent nest success	Daily nest survival rate	Nest survival rate
2013	19	0.74	0.985 ± 0.003	0.60 ± 0.07

Year	Total nests	Apparent nest success	Daily nest survival rate	Nest survival rate
2014	14	0.57	0.974 ± 0.005	0.40 ± 0.09
2015	11	0.36	0.955 ± 0.01	0.20 ± 0.08

Table 4. Brood survival statistics by year for radiocollared sharp-tailed grouse hens in East-Central Minnesota from 2013-2015.

Year	Hatched broods	Hen mortality while brooding	Broods surviving	Chicks surviving	Chicks per nesting hen	Chicks per successfully nesting hen
2013	14	6	3	13	0.72	0.93
2014	8	1	5	16	1.23	2.00
2015	4	0	3	18	1.80	4.50

Table 5. Pairwise comparison (Tukey HSD) results showing the observed means and P-values comparing radiocollared sharp-tailed grouse brood sites to nest and non-nest sites in East-Central Minnesota from 2013-2015.

Variable	Brood	Nest	Non-nest	P-value brood/nest	P-value brood/non
Overhead cover	3.14	5.76	2.64	<0.001	0.4
Residual height (cm)	6.52	22.12	14.10	<0.001	<0.001
Understory height (cm)	53.86	62.64	50.92	0.08	0.63
Shrub height (m)	0.21	0.58	0.20	0.01	0.99
2m residual height	8.51	15.43	13.74	<0.001	<0.001
2m understory height	54.76	56.23	52.40	0.92	0.70
2m shrub height	0.13	0.23	0.25	0.28	0.06
2m density 0-0.5m	61.91	78.20	62.16	<0.01	1.00
2m density 0.5-1.0m	13.29	25.57	18.38	<0.01	0.17
2m density 1.0-1.5m	2.93	10.21	5.90	<0.001	0.13
2m density 1.5-2.0m	2.02	7.41	4.08	0.01	0.34
15m residual height	8.21	14.85	13.96	<0.001	<0.001
15m understory height	57.40	55.91	55.36	0.92	0.78
15m shrub height	0.22	0.40	0.44	0.10	0.01
15m density 0-0.5m	91.73	96.42	88.00	0.33	0.33
15m density 0.5-1.0m	46.20	61.03	58.31	0.05	0.04
15m density 1.0-1.5m	21.08	41.93	40.31	<0.001	<0.001
15m density 1.5-2.0m	14.92	30.54	31.57	0.01	<0.001
Number of perches	8.42	2.93	5.93	0.10	0.46
Shrub presence	0.09	0.33	0.08	<0.001	0.97
Road distance	536.37	368.62	373.74	0.16	0.07

Table 6. Compositional analysis results for radiocollared sharp-tailed grouse hen use of six cover types in East-Central Minnesota from 2013-2015. Symbols in the table indicate if the cover type in the row is used significantly more (+++), more (+), significantly less (---), or less (-) than the cover type in the column. Cover types are ordered from most to least selected for.

	Brushland	Open upland	Agriculture	Hayfield	Open lowland	Forest
Brushland		+	+	+++	+++	+++
Open upland	-		+	+++	+++	+++
Agriculture	-	-		+	+++	+++
Hayfield	---	---	-		+	+
Open lowland	---	---	---	-		+++
Forest	---	---	---	-	---	

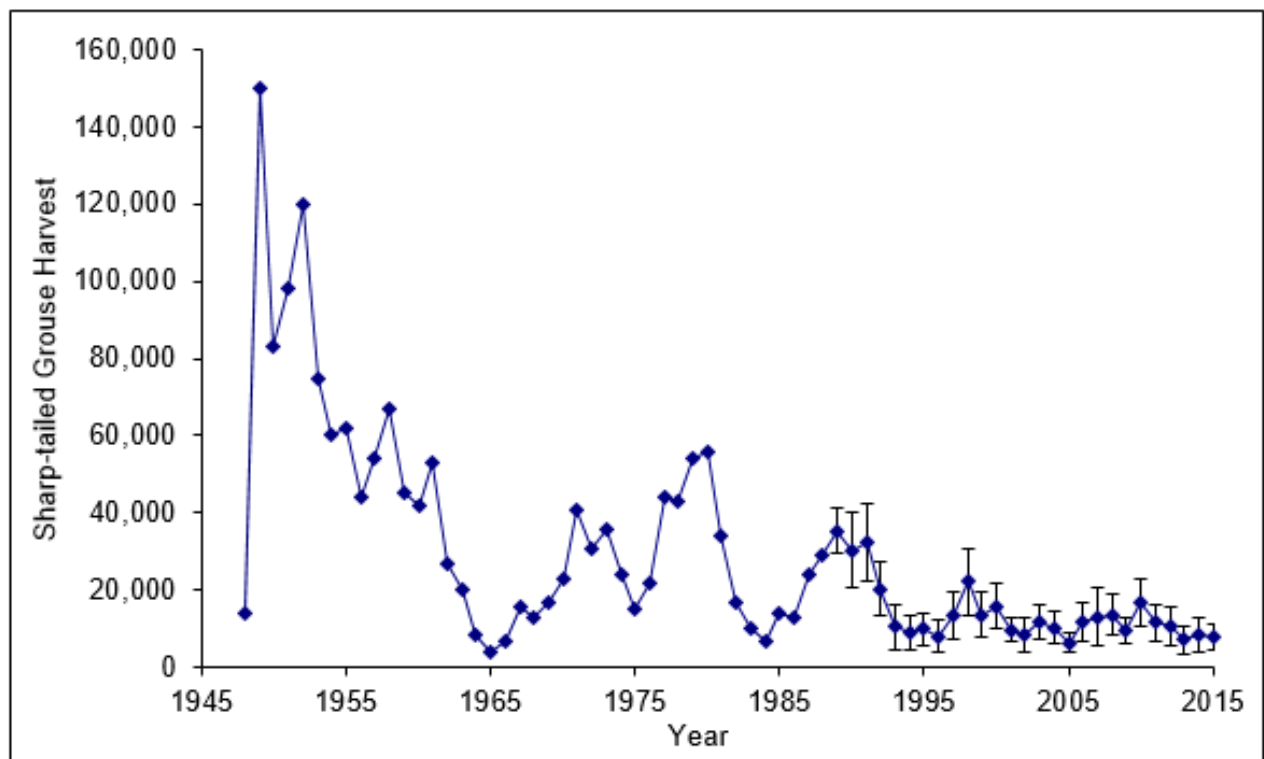


Figure 1. Sharp-tailed grouse harvest estimates for Minnesota from 1948 to 2015 from the Small Game Hunter Harvest Survey (Dexter 2015). Estimates from 1989 on show the 95% confidence interval.

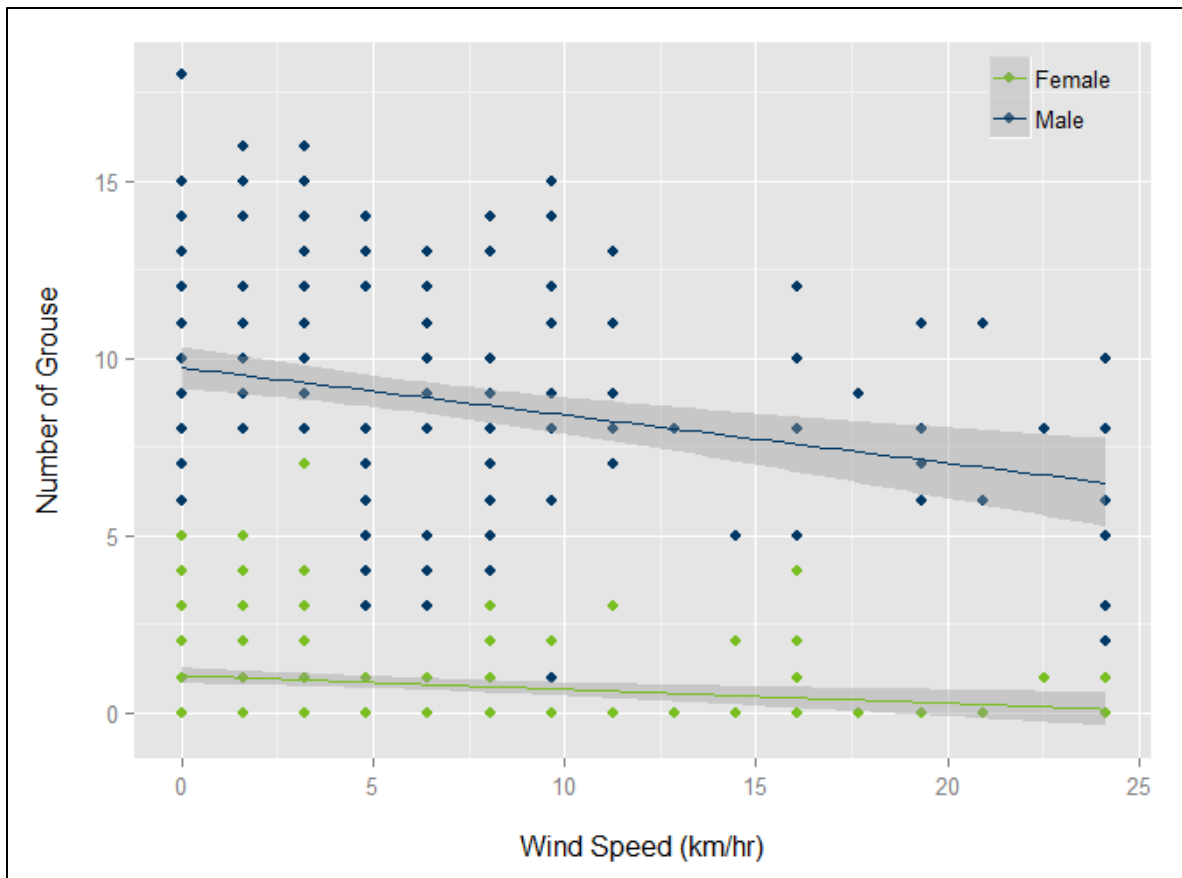


Figure 2. Sharp-tailed grouse lek attendance (number of grouse) by sex in relation to wind speed (km/hr) at trapping sites in East-Central Minnesota from 2013-2015. Linear regression (lines) and standard error (shading) are shown.

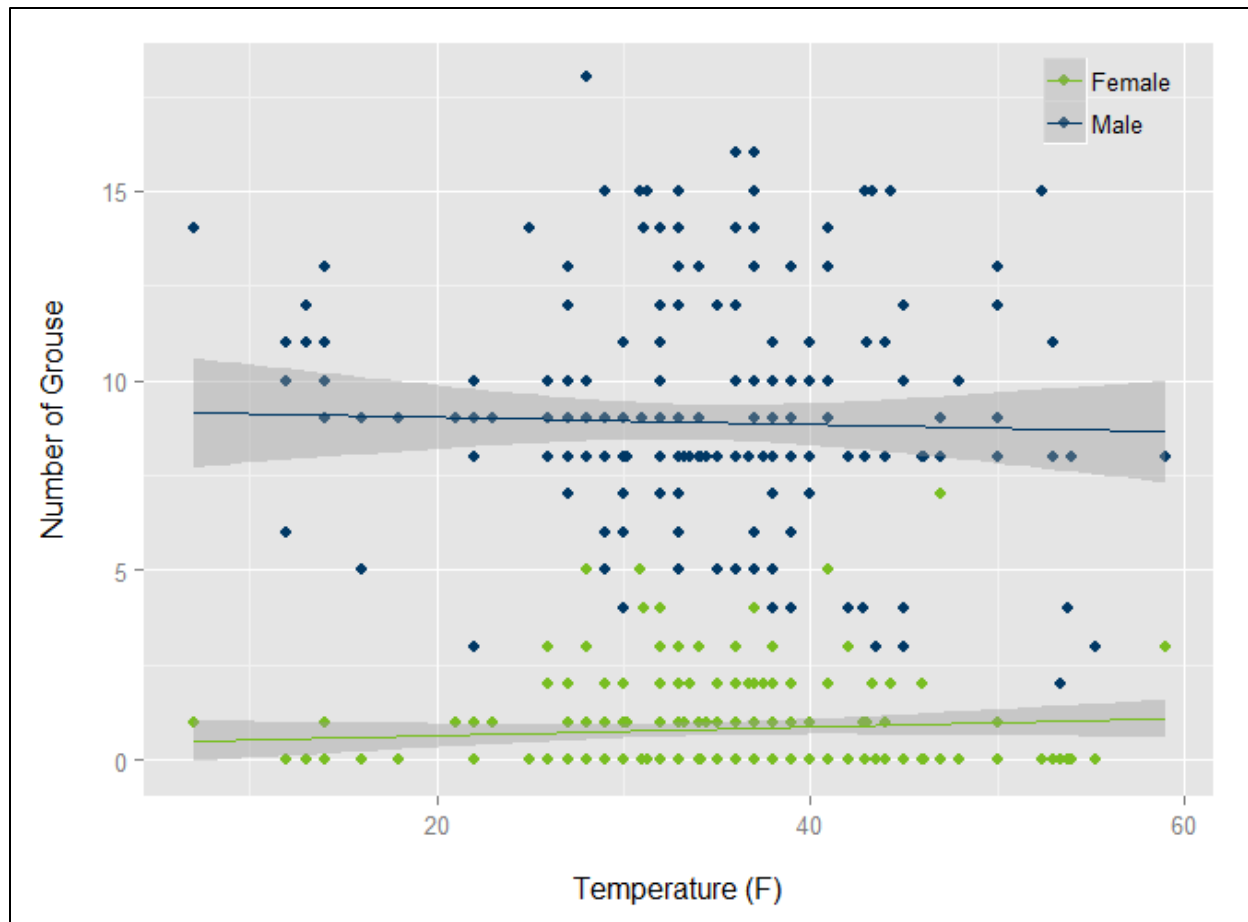


Figure 3. Sharp-tailed grouse lek attendance (number of grouse) by sex in relation to temperature (F) at trapping sites in East-Central Minnesota from 2013-2015. Linear regression (lines) and standard error (shading) are shown.

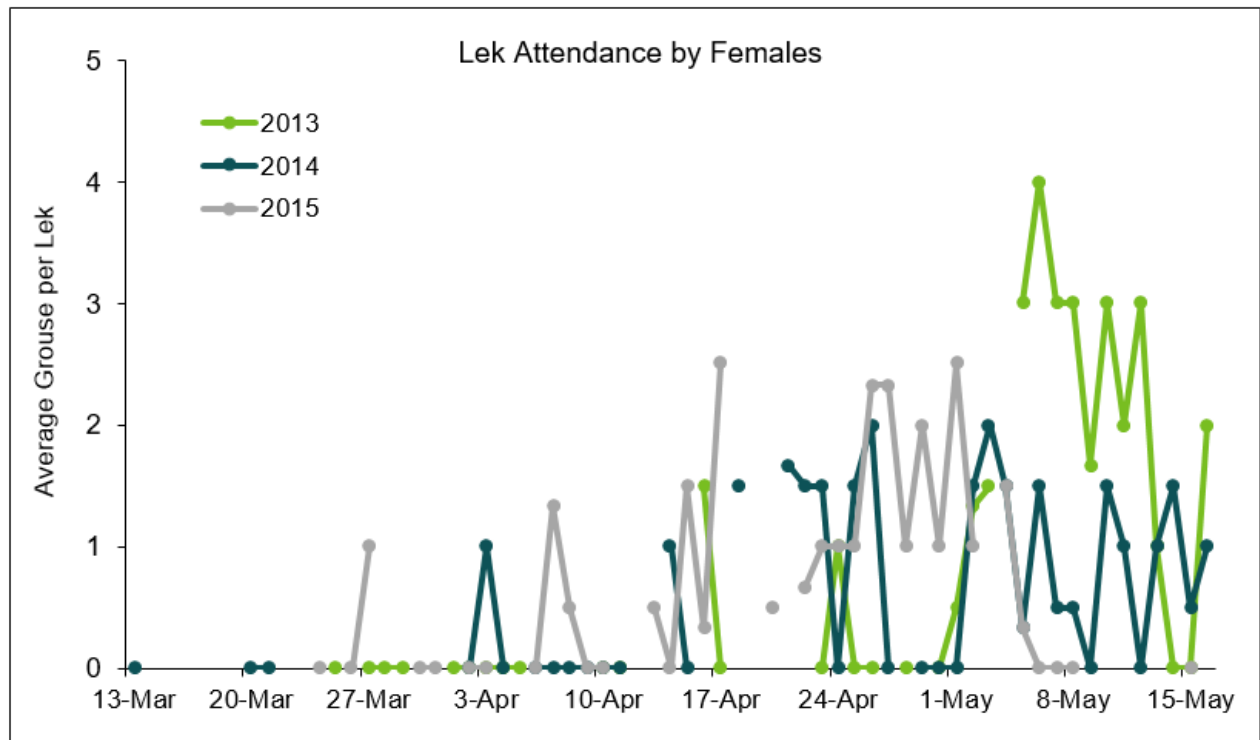


Figure 4. Female sharp-tailed grouse lek attendance (average number of grouse per lek sampled) by date at trapping sites in East-Central Minnesota from 2013-2015.

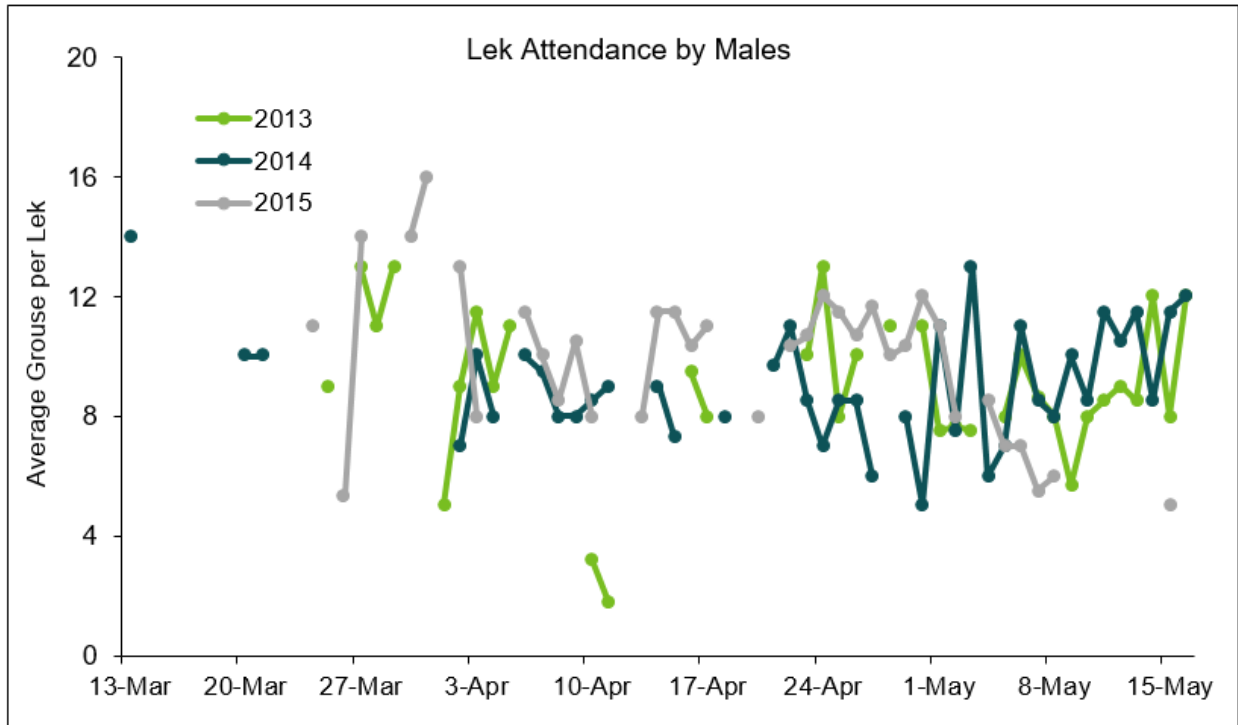


Figure 5. Male sharp-tailed grouse lek attendance (average number of grouse per lek sampled) by date at trapping sites in East-Central Minnesota from 2013-2015.

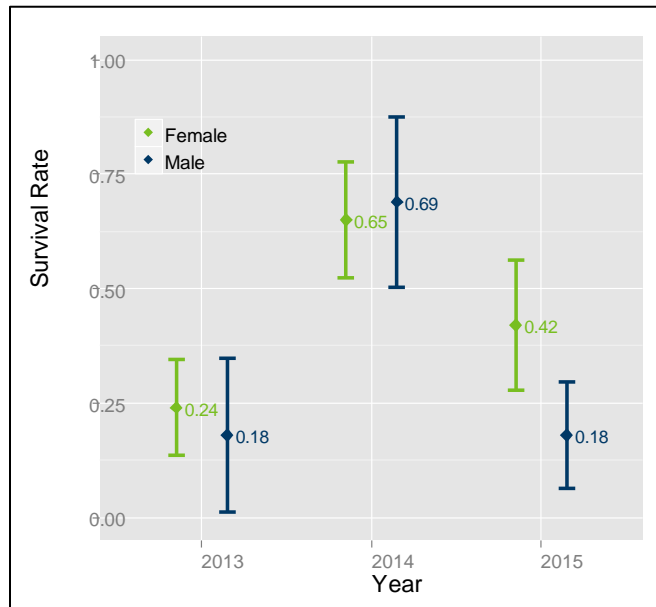


Figure 6. Annual survival rates and standard error by sex for radiocollared sharp-tailed grouse in East-Central Minnesota from 2013-2015.

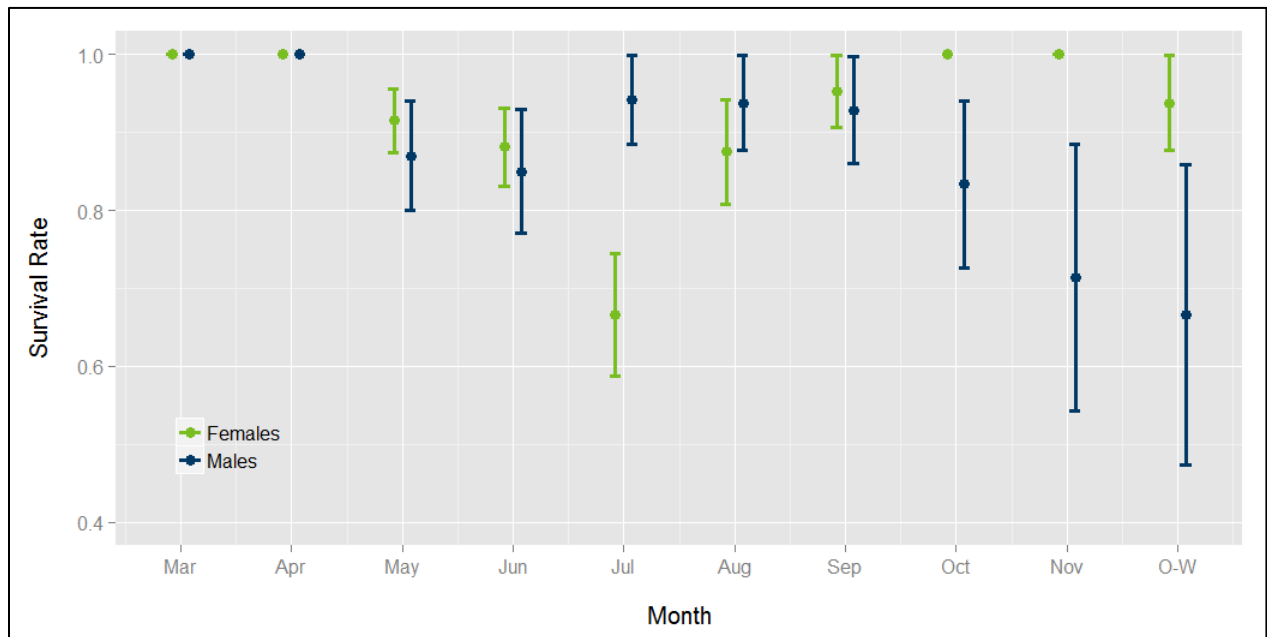


Figure 7. Monthly survival rates and standard error by sex for radiocollared sharp-tailed grouse in East-Central Minnesota from 2013-2015. O-W represents the over-winter period from December through February.

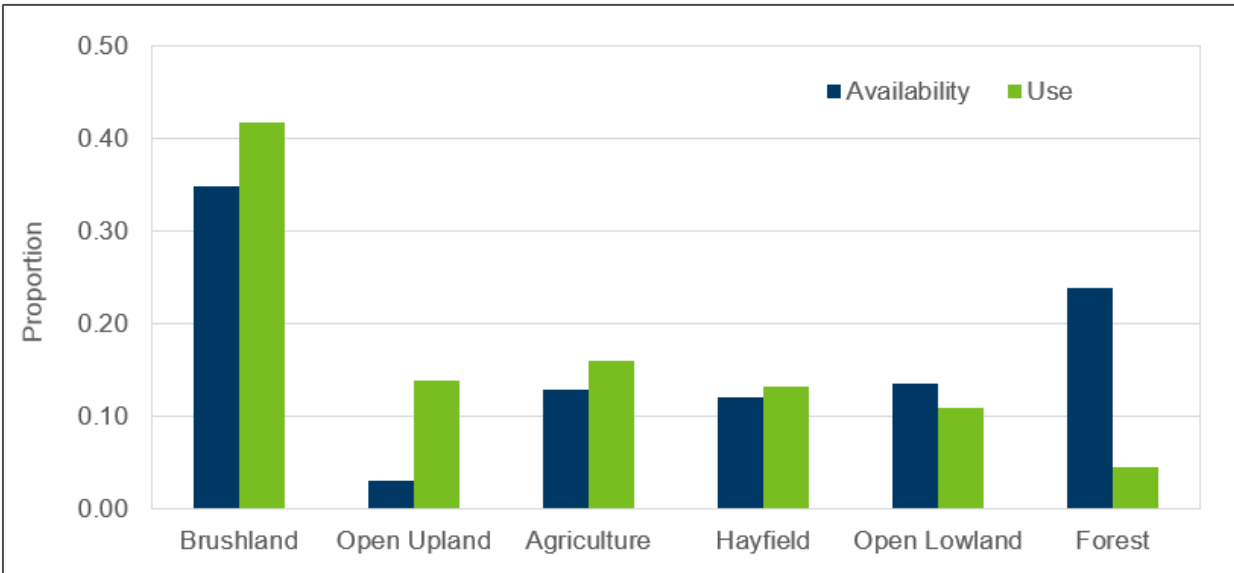


Figure 8. Availability and use of six cover types, ordered from greatest to least selected for, by radiocollared sharp-tailed grouse hens in East-Central Minnesota from 2013-2015.

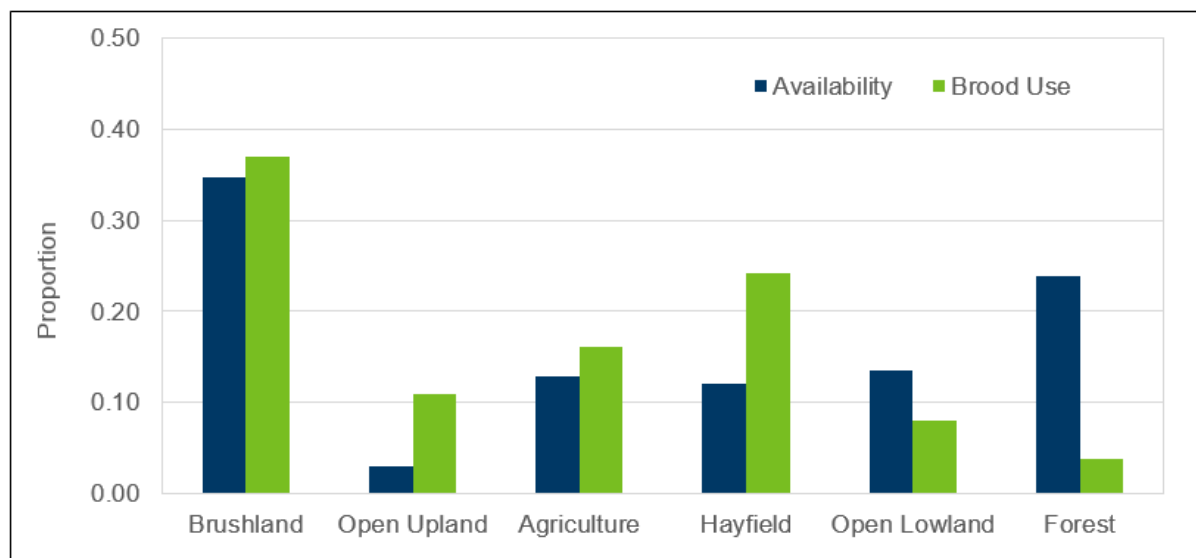


Figure 9. Availability and use of six cover types, ordered from greatest to least selected for, by radiocollared sharp-tailed grouse hens with broods in East-Central Minnesota from 2013-2015.



USE OF MANAGED FOREST WILDLIFE OPENINGS BY AMERICAN WOODCOCK

Lindsey Shartell

SUMMARY OF FINDINGS

American woodcock (*Scolopax minor*) were surveyed at permanently managed forest wildlife openings in Minnesota. Singing ground surveys conducted from mid-April through May during 2016 and 2017 indicated that 61% of openings were used by singing male woodcock. Roosting ground surveys conducted from June through August 2016 indicated that 23% of openings had confirmed roosting and 71% of openings had woodcock activity in or over the opening. In addition to woodcock surveys, vegetation data along transects were collected within openings to assess the relationship of vegetation metrics to woodcock use and management of habitat in openings. Openings used during singing ground surveys had a higher proportion of grass and lower proportions of herbaceous plants and trees. Across all openings, shrubs and trees were uncommon. Years since mowing did not seem to be a driver of woodcock use, however most openings were treated within the past 4 years. Analysis and results are preliminary. An additional year of singing ground and roosting ground surveys will be conducted at openings in 2018. The information gained from this study will guide wildlife managers in creating optimal singing and roosting habitat for woodcock in forest-dominated areas.

BACKGROUND

The American woodcock is a popular migratory game bird and a Species of Greatest Conservation Need in Minnesota (MNDNR 2015). In 2015, Minnesota had an estimated 13,500 active woodcock hunters harvesting 25,600 woodcock, ranking Minnesota third highest in the country for both woodcock hunter and harvest numbers (Seamans and Rau 2016). Annual woodcock surveys have indicated a long-term (1968-2016) decline in singing male numbers across the full breeding range (Seamans and Rau 2016). These declines have been attributed to the loss of open and early successional forest and shrub habitat due to succession, lack of disturbance, and development (Dessecker and McAuley 2001).

Woodcock require a variety of habitat components including dense young forests or shrublands and open singing and roosting grounds (Wildlife Management Institute 2009). Woodcock move frequently between these habitat types, often being found in forests during the day and open sites at night (Sheldon 1967). In the spring, male woodcock use openings as breeding sites, called singing grounds, where they perform their courtship ritual. Females nest and raise broods in the forest surrounding these openings (Sheldon 1967). Both nest and brood locations have been found to be associated with short distances to openings or forest edges (Gregg and Hale 1977, Daly 2014). In the summer, woodcock make evening crepuscular flights to open habitats to roost. Open roosting grounds provide the benefit of reduced predation risk (Masse et al. 2013). Historically, disturbance by fire, wind, Native American activities, flooding, and beavers created openings and early successional habitat for woodcock (DeGraaf and Yamasaki 2003). Many of these disturbances that created and maintained open areas are now prevented.

Pastures, fields, agricultural sites, and recent clearcuts (Hale and Gregg 1978, Long and Locher 2013) can all serve as open habitat for woodcock, but in areas dominated by forest cover, managed forest wildlife openings are often used to provide this habitat component.

The secretive nature and cryptic coloration of the woodcock makes it difficult to estimate population size and management effects. There have been past studies assessing the use of openings by woodcock, but most have been focused on wintering grounds (for example Glasgow 1958, Stribling and Doerr 1985, Berdeen and Krementz 1998). Fewer studies have explored woodcock use of summer roosting grounds in the northern part of the range (though see Sheldon 1961, Sepik and Derleth 1993, Masse et al. 2013), and even fewer have incorporated habitat characteristics and management into studies of use. Researchers have also studied the use of aspen clearcuts in Wisconsin and young pine plantations in Arkansas by woodcock in spring and summer, finding that woodcock do utilize these areas (Hale and Gregg 1978, Long and Locher 2013). Additional research comparing the use and characteristics of temporary openings such as clearcut harvests to permanent openings would improve our understanding and provide context for management in Minnesota.

The Upper Great Lakes Woodcock and Young Forest Initiative published best management practices for woodcock in 2009. Their recommendations call for establishing eight singing grounds at least 0.5 acres in size and one roosting field at least 5 acres in size per 100 acres of land (Wildlife Management Institute 2009). Open sites should cover not more than 20 percent of the area, and the remaining land should consist of abundant feeding, nesting, and brood-rearing habitat (Wildlife Management Institute 2009). The MN DNR maintains permanent forest wildlife openings to provide singing and roosting grounds for woodcock, as well as habitat for a variety of other game and non-game species such as deer and bear. These openings require regular mechanical treatment to prevent succession. Wildlife managers would like to improve their management of forest openings to maximize benefit, but do not know the optimal frequency of treatment. In addition, not all managed openings are used by woodcock. Understanding the factors that influence use, such as opening size and configuration, vegetation composition and structure, and surrounding landscape characteristics would improve the creation of forest openings and focus management on those openings expected to provide the greatest benefit. This information will allow for the development of better management practices for land managers and landowners interested in providing wildlife openings for woodcock and other wildlife.

OBJECTIVES

1. Assess woodcock use of managed forest wildlife openings with differing management history (years since mowing).
2. Relate opening size and configuration, vegetation composition and height, and surrounding landscape to woodcock use and management history.
3. Develop recommendations to improve the current management of forest wildlife openings.

METHODS

Singing ground surveys for American woodcock were conducted from mid-April through May 2016 in forest openings within the Grand Rapids, Cloquet, and Red Lake work areas and from mid-April through May 2017 in the Grand Rapids work area. Surveys followed Singing Ground Survey (SGS) protocol where possible (Seamans and Rau 2016). Surveys generally took place 15 to 60 minutes after sunset, when temperature was above 40 F, and there was no heavy precipitation or strong wind. Openings in close proximity were grouped to allow surveying multiple openings per evening. At each opening observers recorded their GPS location (UTM coordinates), time of sunset, cloud cover, temperature, wind speed, precipitation, and any noise disturbance present at the time of the survey. Observers listened for and recorded the number

of different woodcock heard peenting or observed displaying (heard and/or seen) within and over the opening during a listening period of at least 5 minutes. Observers also recorded other observations of woodcock (not within the opening) along with time and approximate location (direction and distance) of the woodcock.

Roosting ground surveys were conducted June through August 2016 using crepuscular flight surveys and spotlighting (Glasgow 1958, Berdeen and Krementz 1998). Roosting surveys were not conducted in 2017 due to funding constraints. The observer was positioned on the edge of the opening and recorded the number of woodcock observed flying into the opening, over the opening, or heard (when not seen). Surveys were conducted from 20 minutes before sunset to 40 minutes after sunset (a one hour period). Observers recorded their GPS location (UTM coordinates), time of sunset, cloud cover, temperature, wind speed, precipitation, and any noise disturbance present at the time of the survey. After the survey window, observers systematically walked openings using spotlights and recorded the number of woodcock flushed or spotted.

Vegetation characteristics were sampled using the point intersect method (Levy and Madden 1933). Two transects were sampled per opening, one placed across the widest part of the opening from edge to edge, and the second placed perpendicular to the first. Observers recorded vegetation type (i.e., grass, herbaceous, woody, shrub, tree, or bare ground) and maximum height for each type every 1.0 m along the transect. Methods in 2017 were slightly altered from the pilot study in 2016 to improve repeatability and efficiency, while still producing the same metrics for the opening as a whole (proportion and average height for each type of cover). Observers also described the habitat across the entire opening (e.g., number of trees, distribution of trees, percent shrub cover) and the surrounding habitat by type (e.g. upland forest, lowland forest, upland shrub), tree or shrub species, and coarse age class (young, middle, old). Presence of tansy, or other aggressive invasive species were recorded as approximate percent cover in 10% increments across the opening from a visual assessment. Presence of a mowed or packed trail within the opening was noted, as these may provide persistent areas of short vegetation regardless of years since mowing. To obtain an accurate estimate of opening size and shape, the edge of the opening was walked using a GPS unit to digitize the boundary of the opening.

To assess the frequency of use of openings in this study by other wildlife the presence of scat encountered within 0.5 m on either side of the transect was noted. Distance along the transect and species was recorded for each encounter.

Statistical analyses were conducted in the statistical package R (R Core Team 2014). Averages are reported with standard errors. A chi-square test was used to compare use from 2016 to 2017. Student's t-tests were used to compare variables at openings with and without woodcock use. Linear regression was used to relate vegetation variables to years since mowing. A significance level of $P < 0.05$ was used for all tests. Opening size (ac) and perimeter (m) were highly skewed toward low values, while the ratio of perimeter to area was more normally distributed and thus was used in analysis. Future work will include analysis of metrics representing the surrounding landscape, such as forest type and age, proportion of desirable habitat in area, and number of openings in area.

PRELIMINARY RESULTS AND DISCUSSION

Singing Ground Surveys

In the 2016 pilot project, singing ground surveys were conducted at 94 openings, with singing males observed at 55 openings (59%). The majority of openings with woodcock use (42 of 55) had only one male present, 10 openings had two males, and 3 openings had three males. In

2017, singing ground surveys were conducted at 64 openings, with males observed at 41 openings (64%). At 33 openings one male was observed and 8 openings had two males present. Of those openings surveyed in both 2016 and 2017 (n=51), 24 openings (47%) had woodcock present in both years. Woodcock use (presence or absence) in 2016 and 2017 was significantly related, with those openings used by woodcock in 2016 being more likely to be used in 2017 ($\chi^2 = 11.57$, $P < 0.01$). Use was not found to be related to date ($P = 0.32$), cloud cover ($P = 0.66$), temperature ($P = 0.52$), or wind speed ($P = 0.45$), and this was likely due to the restrictions placed on conditions for survey.

Openings in 2016 included some forest harvest sites, openings ranged in size from 0.4 to 16.3 ac, and forest harvest sites ranged from 3.5 to 66.3 ac. For all sites, the average size in 2016 was 5.1 ± 0.5 ac. Openings in 2017 did not include forest harvest sites, and ranged in size from 0.5 to 6.4 ac with an average size of 1.8 ± 0.2 ac. Openings used by woodcock had a significantly smaller perimeter to area ratio (i.e. shorter perimeter and larger area, $P < 0.01$).

During singing ground surveys from 2016 and 2017, the proportion of cover in 3 of 6 classes was significantly related to woodcock use. Openings used by woodcock had a significantly higher proportion of grass ($P < 0.01$) and lower proportions of herbaceous plants ($P < 0.05$) and trees ($P < 0.05$). Most openings were dominated by grasses with few shrubs and trees. It was noted anecdotally in 2016 that sites heavily invaded by common tansy (*Tanacetum vulgare*), an exotic invasive plant, tended to have no woodcock present. In 2017 presence of tansy and other invasive species was recorded; however, few sites had invasive plants dominating the opening, restricting analysis. Vegetation height in each age class was analyzed for 2017 data only, but was not found to be significantly related to woodcock use.

Number of years since mowing was not significantly related to woodcock use ($P = 0.48$). Proportion of woody cover ($P = 0.02$) and tree cover ($P < 0.01$) were significantly related to years since mowing, with both increasing with increasing years since mowing. Height of grass ($P = 0.03$), herbaceous ($P < 0.01$), woody ($P < 0.01$), and shrub ($P = 0.02$) vegetation was significantly related to years since mowing, with height increasing with increasing years since mowing for all. Thus, mowing had an effect on the vegetation within openings, but may not be a strong driver of woodcock use within established openings already free of shrubs and trees. The average cover of shrubs (12%) and trees (3%) was low across all openings. Future analysis will focus on other potential drivers of woodcock use such as the surrounding landscape composition and age, and the availability of other open areas.

Roosting Ground Surveys

Roosting ground surveys were conducted at 65 openings in 2016, but were not conducted in 2017. Roosting woodcock were spotlighted and flushed at 15 openings (23%), and woodcock were observed flying, landing in, or flushing from 46 openings (71%). Both flight and spotlighting surveys appear to provide useful information on woodcock use. Roosting surveys were not repeated due to time limits and sample size, but other research has found that the frequency of roosting field use by individual woodcock varies by month and by age and sex (Sepik and Derleth 1993). Sepik and Derleth (1993) found the highest frequencies of roosting field use in June and July. However, there was no significant relationship found between date and the number of woodcock observed at roosting openings in this study in which surveys were conducted from June through August. Roosting surveys will be conducted at openings in 2018 to further assess the importance of openings, and their management, for roosting woodcock.

Use of Openings by Other Wildlife

Other species of wildlife also benefit from the management of forest wildlife openings. In 2016, scats from bear, deer, fox, goose, porcupine, rabbit, ruffed grouse, and wolf were observed

along vegetation transects in forest wildlife openings. In 2017, scats from bear, coyote, deer, moose, rabbit, and ruffed grouse were observed, as well as a woodcock nest and mallard nest. Analysis of management effect on wildlife use will be completed in the future.

ACKNOWLEDGEMENTS

This project was made possible thanks to the dedicated fieldwork of Andrew Erickson and Nicole Benson and the cooperation of wildlife staff, especially Jeff Hines, Bruce Anderson, Charlie Tucker, and Ted Dick. Guidance on statistical design and analysis was provided by John Giudice and Véronique St. Louis. This study was funded in part through the Wildlife Restoration (Pittman-Robertson) Program.

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THE RELATIONSHIP BETWEEN GRASSLANDS, CONSERVATION RESERVE PROGRAM (CRP) ENROLLMENTS, AND GREATER PRAIRIE-CHICKEN (*TYMPANUCHUS CUPIDO PINNATUS*) POPULATIONS IN MINNESOTA

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ABSTRACT

We quantified greater prairie-chicken (*Tympanuchus cupido pinnatus*) population responses to CRP enrollments using population indices (males/lek and leks/km²) derived through annual monitoring efforts in Minnesota. We quantified land cover during the period 2004–2016 in survey blocks where systematic greater prairie-chicken surveys were conducted during the same period to evaluate the contribution of CRP enrollments to available grassland cover and estimate changes through time. We also evaluated existing vegetation characteristics of grassland CRP conservation program practices to assess how different CRP management strategies were related to greater prairie-chicken abundance and lek persistence. The best-supported model of lek density (leks/km²) included the area of CRP grasslands and wetlands; state-, federal-, and The Nature Conservancy (TNC)-managed grasslands and wetlands; and the area of “other” wetlands; the contiguity of grasslands; and the number of patches of grasslands and wetlands in each survey block. The best-supported model of the number of males/lek included the amount of CRP grassland and wetlands; state-, federal-, and TNC-managed grasslands and wetlands; and “other” wetlands; forests; developed areas; shrubs; and the contiguity of CRP grassland. These results suggest that increasing the quantity of grassland and wetland CRP contracts throughout the existing range of greater prairie-chickens in northwestern Minnesota and aggregating CRP grassland contracts in areas of known lek sites may increase greater prairie-chicken abundance. We also simulated the impact of 1) allowing CRP enrollments to expire, 2) adding CRP enrollments randomly in 1-acre blocks, and 3) adding 20-acre CRP enrollments strategically to increase contiguity. Our simulations indicated that lek density would decline by an average of 22% (2-80%) in the survey blocks and males/lek would decline by an average of 7% (0-19%) at the lek scale if CRP were allowed to expire. Greater losses occurred in areas where CRP comprised a larger component of the landscape. Comparisons between random additions and strategic additions of CRP enrollments revealed that strategic additions produced greater gains, and sometimes adding CRP randomly provided no benefit for greater prairie-chickens when contiguity decreased. Lastly, we examined vegetation characteristics associated with high-quality greater prairie-chicken breeding habitat. Many combinations of CRP contract type, age of planting, and soil type provided appropriate vegetation structure and composition for greater prairie-chickens, including low diversity introduced grass species. Our results provide guidance to the Farm Service Agency (FSA) and other organizations in targeting conservation programs in areas where they will be most effective for greater prairie-chickens.

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² Abstract modified from Master's thesis with the same title by Kalysta Adkins, University of Minnesota, December 2017.

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