

# Summaries of Wildlife Research Findings 2019



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



# **SUMMARIES OF WILDLIFE RESEARCH FINDINGS 2019**

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## ASSESSING UNMANNED AERIAL VEHICLES EQUIPPED WITH THERMAL INFRARED TO LOCATE AND CAPTURE WHITE-TAILED DEER FAWNS

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### SUMMARY OF FINDINGS

Drones are growing in popularity and are used to locate individual animals, estimate populations, and monitor species such as rhinoceros, penguins, marine mammals, and chimpanzees. However, previous research has not used drones to locate individual wildlife with the intent of capturing them. Our goal was to assess the efficacy of using drones to locate and capture neonatal white-tailed deer (*Odocoileus virginianus*). During May-June 2019, we used a drone with a thermal-infrared and Red-Blue-Green (RGB) camera to locate and confirm fawn thermal signatures in Wildlife Management Areas in Minnesota's southern farmland region. We identified 43 fawn and 117 adult deer heat signatures. We flew the drone for 47.3 hours covering approximately 792 hectares, which averaged 16.7 hectares per hour. We used 10 people to work 201.5 person-hours and spent 4.7 person-hours to locate each fawn. Flights were most efficient when flown at 6–7 m/s and at 60 m altitude; however, diurnal use of drones made identifying thermal fawn signatures difficult as the sun quickly heated vegetation reducing the temperature differential between vegetation and fawn signatures. In comparison to other common capture methods such as vaginal implant transmitters, ground searches, or doe behavior, using drones to locate fawns required up to 3.1 times less person-hours. We found this to be an efficient method to locate and capture fawns in open habitats in comparison to other capture methods, but recommend flying overnight or in cloudy conditions to avoid false positives.

### INTRODUCTION

Neonatal survival is generally the most variable demographic parameter affecting population growth in ungulates (Gaillard et al. 2000). Understanding neonatal survival provides managers important information affecting recruitment and facilitates proactive management by identifying poor recruitment classes. Fawn survival rates and cause-specific mortality, particularly during the first hunting season and winter, are largely unknown or are outdated in Minnesota (Brinkman et al. 2004, Grovenburg et al. 2011) but are used in the Minnesota Department of Natural Resources (MNDNR) annual deer population model (Michel 2019). Using outdated vital rate information directly impacts model reliability which can affect subsequent management decisions for white-tailed deer (*Odocoileus virginianus*) in Minnesota (Michel 2019). However, a major logistical challenge and financial constraint associated with establishing and subsequently monitoring juvenile survival rates is locating and marking young ungulates (White et al. 1972, Carstensen et al. 2003). If fawns can be located efficiently, marking and collaring requires minimal physical restraint and no chemical immobilization or capture traps (e.g., netted-cage traps, drop nets).

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The most common neonatal fawn capture method in farmland regions (areas with intensive row-crop agriculture) is conducting opportunistic ground searches. Strategies include systematic searches through suspected fawn rearing habitat or monitoring doe behavior as an indicator of fawn presence nearby (Downing and McGinnes 1969, Huegel et al. 1985, Carstensen et al. 2003, Grovenburg et al. 2011). Previous capture methods have required numerous personnel, coordination of large search groups, and intensive searching effort. These studies also reported significant time investments for locating fawns [e.g., 8–10 hours/fawn captured via ground searches in Michigan (Pusateri 2003); range of 5–214 hours/fawn, depending on habitat, using doe behavior (White et al. 1972, Carstensen et al. 2003, Huegel et al. 1985)].

Using vaginal implant transmitters (VITs) is generally a more efficient means of capturing fawns compared to other methods. For example, capture success rates of fawns located from VITs implanted in dams was much greater (89% versus 15%) than using doe behavior to detect fawn presence. This increased efficiency reduced capture effort by up to 3.5 times that of using doe behavior (Carstensen et al. 2003). Similarly, Bishop et al. (2007) found capture success of mule deer (*O. hemionus*) fawns increased 57% when using VIT's compared to using doe behavior. However, using VITs requires capturing adult females, which increases associated costs and may also may result in unnecessary stress and capture related mortality of adult females during capture. As a result, these techniques may be less efficient and cost effective than using ground searches.

Using thermal infrared (TIR) cameras can be efficient for identifying and subsequently capturing wildlife. One of the first wildlife studies to use TIR devices identified a polar bear (*Ursus maritimus*) and its tracks (Brooks 1972). Thermal imaging has also been used to estimate populations of ungulate species such as moose (*Alces alces*; Millette et al. 2011), bison (*Bison bison*; Chrétien et al. 2015), elk (*Cervus canadensis*; Chrétien et al. 2015), and white-tailed deer (Croon et al. 1968, Wiggers and Beckerman 1993, Haroldson et al. 2003). Ditchkoff et al. (2005a) also used TIR cameras mounted on the back of a 4-wheel drive vehicle to locate fawns. They conducted searches overnight to maximize the heat differential between fawn signatures and surrounding area. This technique required 3.3 person-hours per fawn encounter, 9.4 person-hours per fawn captured, and only 2–3 personnel to conduct the fieldwork. Although vehicle mounted TIR cameras are more effective than previous search methods, they are restricted to roads which ungulates typically avoid (Ward et al. 2004, Long et al. 2010, Anderson et al. 2013).

Unmanned aerial systems (UAS or drone) used for civil applications offers new opportunities for wildlife managers (Shahbazi et al. 2014, Whitehead and Hugenholtz 2014). Drones have been used for surveys of marine mammals (Hodgson et al. 2013, Goebel et al. 2015), to monitor rhinoceros (*Diceros bicornis* and *Ceratotherium simum*) poaching (Mulero-Pázmány et al. 2014), identify chimpanzee (*Pan spp.*) nests (Van Andel et al. 2015), and estimate colony size of chinstrap penguins (*Pygoscelis antarcticus*, Goebel et al. 2015). Recent studies show drones provide many advantages over traditional manned aerial surveys including lower disturbance, higher quality images, and lower flight altitudes, and they are safer for pilots and biologists (Jones et al. 2006, Linchant et al. 2015, Christie et al. 2016). A drone is also not spatially limited and has improved detection compared to ground-based TIR cameras because the aerial view avoids vertical obstruction from herbaceous ground cover. Using drones to locate fawns could reduce the primary cost associated with capture efforts (Kissell and Nimmo 2011, Chrétien et al. 2015, 2016, Linchant et al. 2015, Christie et al. 2016, Witczuk et al. 2018). Recently, drones with TIR detected and counted white-tailed deer in Quebec, Canada (Chrétien et al. 2016), while another study found drones with TIR provided accurate population estimates at a captive white-tailed deer facility (Beaver et al. 2020). A drone with TIR was also used to detect roe deer

(*Capreolus capreolus*) fawns in pastures to avoid them being killed by mowing machines (Israel 2011). However, no published research has identified or evaluated drone use to efficiently locate fawns or adult ungulates with the intent of capturing them.

Although the use of drone and TIR technology in wildlife research is increasing rapidly, efficacy of these technologies for locating fawns as part of capture efforts is unknown; thus, a feasibility study is needed prior to implementing these tools in large-scale research projects.

## OBJECTIVES

1. Evaluate the ability of drones equipped with TIR technology to locate fawns.
2. Compare search rate efficiency to other methods.

## METHODS

### Study Site

We collected data from May to June 2019 across 4 deer permit areas (252, 253, 296, and 299) covering 7,219 square kilometers in south central Minnesota, USA (Figure 1). Our study area was located in the North Central Glaciated Plains system (MNDNR 2019). Row crop agriculture (largely corn and soybeans) was the most abundant cover type accounting for 71% of the area, with grasslands (12%), developed (7%), wetlands (5%), forest (3%), and open water (2%) cover types encompassing the remaining area (Rampi et al. 2016). Within the study area, we located fawns at publically owned Wildlife Management Areas (WMAs). These WMAs consisted largely of wetlands (37%) and grasslands (34%), but also agriculture (corn; 12%), open water (9%), and forest (7%; MNDNR 2009). Most common graminoids included smooth brome (*Bromus inermis*), reed canary grass (*Phalaris arundinacea*), little bluestem (*Schizachyrium scoparium*), and Indian grass (*Sorghastrum nutans*). Common forbs were gray goldenrod (*Solidago nemoralis*), sweet clover (*Melilotus albus*), and Canada goldenrod (*Solidago canadensis*). Eighty-two WMAs were located within or immediately adjacent to our study area (Figure 1) and available for locating deer fawns. We determined suitability of these WMAs by analyzing aerial photos of each site for large parcels of grassland or lightly forested cover (cover types heavily used by fawns in agricultural landscapes; Grovenburg et al. 2010). We also visited 55 of the sites to confirm aerial imagery and further evaluate the habitat. We determined 53 WMAs contained suitable deer fawning habitat. Unsuitable areas were largely open water and emergent wetlands.

### Data Collection

We contracted with a drone company (PAAP Drone LLC, Apple Valley, MN, USA) to create flight paths (Figure 2) and fly their drone at each of our sites. The drone contractor created 2 flight paths for each WMA at 45 and 60 m altitude with a 10% overlap. For flights, we used a DJI Matrice 210v2 RTK UAV with a thermal (DJI Zenmuse XT-R: 640x480 13mm, 30 Hz Advanced Radiometric Thermal Camera) and Red-Green-Blue (RGB) camera (DJI Zenmuse Z30 Zoom Camera). We selected this UAS because it allows 2 camera mounts and the ability to interchange cameras while in flight. We used the TIR camera to locate thermal signatures and the RGB camera to confirm fawns. We connected the ground control system to a 50 cm screen (via HDMI) to allow more observers to monitor thermal signatures.

We recorded number of crew members, number of batteries used, temperature, wind speed, cloud cover, and precipitation at each WMA. Pilots flew drones through the preprogrammed flight path until we detected a suspected fawn. The drone pilot would then pause the preprogrammed flight path and manually direct the drone over the suspected fawn. Next, the drone pilot switched to the RGB camera and confirmed identification by modifying the zoom–

scale of the camera rather than adjusting flight altitude of the drone. This procedure minimized auditory stress, disturbance, and stress-flight behavior of the fawn. The drone contractor recorded georeferenced video footage and photo-documented fawning site characteristics. We recorded the following for each fawn detection: number of fawns, time, activity (e.g., lying, standing, moving), habitat type, doe presence, thermal obstruction (from vegetation), and the location (e.g., latitude and longitude). We also recorded similar information for other species confirmed within the WMAs.

We found diurnal conditions rapidly reduced our ability to locate thermal signatures and therefore began performing early morning (02:00 – 06:00) flights. In diurnal and sunny conditions, groundcover quickly illuminated with the TIR camera and caused many false positives. We could not confirm a suspected heat signature as a fawn during nighttime conditions (e.g., no/low sunlight for RGB camera); therefore, we recorded the coordinates of the heat signature and attempted to confirm via drone after sunrise or with a ground search. We were unable to confirm a suspected fawn in some cases, potentially because its mother may have moved the suspected fawn.

## RESULTS

We conducted drone flights for 10 days between 28 May 2019 and 11 June 2019 on 19 WMAs. We flew for 47.3 hours and covered 791.7 hectares. We identified 43 suspected fawn heat signatures and confirmed 29 heat signatures using the RGB camera, ground visualization, or the presence of a doe and/or confirmed twin (Figure 3). We identified 14 fawns before sunrise but were unable to confirm 10 with the RGB camera after sunrise. However, we were confident these unconfirmed heat signatures were fawns because of their thermal signature size.

We used 10 people to work 201.5 person-hours, with a mean crew size of 4 people. We required a mean of 4.7 person-hours to locate each fawn. The drone covered a mean of 18.4 hectares for each fawn. We also attempted to improve our efficiency by increasing our speed from 4–5 m/s and 45-meter altitude (Eff 1) to 6–7 m/s and 60-meter altitude (Eff 2). Eff 1 required 5.8 person-hours per fawn, while Eff 2 only required 2.2 person-hours per fawn (264% increase; Table 1).

We located 39 fawns (91%) in grasslands and the remaining 4 (9%) in woodlands. We found all fawns bedded (Figure 3) and observed no physical responses from the drone. Fawn obstruction (of heat signature) from vegetation was  $27.5 \pm 22.0$  (SD) % (range = 0–90%,  $n = 14$ ). The doe was present with the fawn in 45% (17/38) of observations, with the remaining 5 fawns' doe presence unknown.

We identified and confirmed heat signatures of other wildlife including coyotes (*Canis latrans*) with pups, raccoons (*Procyon lotor*), muskrats (*Ondatra zibethicus*), mallards (*Anas platyrhynchos*), ring-necked pheasants (*Phasianus colchicus*), and many small mammals and other birds. We also recorded 63 adult white-tailed deer groups (117 individuals). We found thermal signatures of coyotes and adult deer were approximately 2.7 times larger than neonatal fawns. We observed most species in grasslands (82.6%), followed by woodlands (9.3%), wetlands (3.5%), cropland (2.3%), and other (2.3%). We observed animals most often found lying (41.9%), followed by moving (34.9%), and standing (23.2%). We also found 68.6% of animals did not physically respond to the drone by altering their behavior.

## DISCUSSION

We flew at Eff 1 (4–5 m/s; 45 m) for the first several days to quantify the performance of this search method. After increasing confidence in our ability to detect fawns, we moved to Eff 2 (6–

7 m/s; 60 m) to assess whether flying at these metrics allowed us to locate fawns more efficiently. The height change increased the search swath by approximately 25%, allowing us to cover substantially more area. After we increased the speed and height, we covered approximately 53% more hectares per hour and lowered our person-hours per fawn by 264%. We ran a simulation with Eff 2 parameters with flight time kept constant and found we would be able to fly 1,352 hectares and locate 86 fawns.

Using drones equipped with TIR is more efficient than using TIR with a vehicle because we are not limited to searching near roads, which deer avoid (Ward et al. 2004, Long et al. 2010, Anderson et al. 2013). Our current drone efficiencies (Eff 1 and Eff 2) also included issues with software, sunlight, canopy cover, battery life, and confirmation of fawns. If these issues can be resolved, we could further increase efficiency. The drone with TIR method was more effective at locating fawns versus ground searches because ground searches require extensive personnel effort and likely have a lower fawn detection rate compared to our method. VITs are also less efficient because they require adult capture and ground searches to locate the fawn within a given area. When a VIT is expelled they are notified and then the capture team is launched, whereas we located and captured fawns instantaneously.

Vaginal Implant Transmitters are the most costly search method because they require adult capture to GPS-collar and insert a transmitter into the vaginal canal (Carstensen et al. 2003). Capturing and GPS-collaring of adult females is unwarranted unless specific female objectives are stated. The cost of ground searches is difficult to estimate because the unknown number of individuals required to conduct ground searches and the extensive coordination efforts pre-fieldwork conducted by personnel. However, ground searches do not require a drone contractor or expensive drone equipment to conduct the searches. The largest cost for the drone method was the drone contractor costs. We are currently not allowed to purchase drones within the MNDNR for specific projects and are required to contract for the work. If other studies have the capability to purchase their own drone and conduct flights, the method would be more cost effective than ground searches, especially after multiple years of use. Owning a drone also allows for more flying flexibility. For example, we conducted flights under poor conditions because we had designated flight dates with our contractor. We could have flown across a wider range of dates if we were not limited by these dates.

We experienced several technical difficulties while conducting flights. For example, the gimbal on the drone, which facilitates independent movement of the RGB and TIR camera, ceased function frequently. This required us to return the drone to the ground for a manual reset. We found the failed gimbal function was caused by a flaw in the software update and was resolved after completion of our study. Drone technology is expanding quickly; troubleshooting software and other potential issues should be considered while determining the best search method for your study area. Our drone contractor recommended not upgrading drones, cameras, or software shortly before the field season to avoid new issues with software updates (Steve Fines, personal communication). We also experienced difficulty switching between the TIR and RGB cameras to quickly confirm a suspected fawn's thermal signature. The drone has 2 gimbals: 1 for the TIR camera and the other for the RGB camera, but the direction between the 2 cameras was not perfectly aligned. After we located a thermal signature, we orientated the signature directly below the drone and then switched to the RGB camera to confirm the suspected fawn signature. Because of the alignment problem, we needed to slowly move the drone back and forth and modify the zoom-scale to search for the suspected fawn. This slight difference between cameras caused considerable loss of time confirming fawns. A new camera has since been developed with both TIR and RGB in 1 unit allowing for perfect alignment. This camera also has a split-screen feature to view both cameras simultaneously and allow for quicker

confirmations. Battery life was also an issue with maximum battery life of the DJI Matrice 210v2 RTK drone lasting approximately 38 minutes. Because we used 2 cameras (RGB and TIR), our battery life was reduced to approximately 25 minutes. A sufficient number of batteries and a charging source (e.g., generator) is necessary for use of drones for wildlife capture. The largest difficulty we encountered was limited flight time during daylight hours. We found the sun quickly heated the ground and washed out our ability to locate thermal fawn signatures. Detection of thermal signatures was adequate immediately after sunrise because the sun was still low and not able to penetrate the ground. However, fawn detection continually decreased until we were unable to locate thermal signatures about 3 hours after sunrise. We resolved this issue by moving our operation time to early morning (03:00 to 09:00). A part 107 federal waiver is required to fly a drone at nighttime. This waiver allows drone pilots to deviate from certain rules under part 107 by demonstrating the drone can still fly safely using alternative methods (U.S. Department of Transportation 2019). We then attempted to confirm fawns via ground or with the drone after sunrise but found some signatures were no longer present. The doe may have nursed the fawn and then moved the fawn to a new location. We plan to confirm fawns this upcoming field season by night vision technology on the drone or confirming via walking in prior to sunrise.

Canopy cover also caused reduced ground visibility because the TIR was unable to penetrate through the canopy. We also found trees held residual heat for an extended period of time from the previous day and quickly heated up in sunny conditions. Locating fawn signatures was possible in lightly wooded areas, but difficult in forested habitats. Researchers looking to use this method in forested areas may have less success.

We flew the first 7 days of drone flights at a slower speed and lower height to identify all thermal signatures and learn signature size of different species. We determined a fawn's thermal signature size and shape was unique and easy to differentiate from other signatures. We found thermal signatures of coyotes and adult deer were much larger than neonatal fawns. Fawns also curled up when bedded making their shape distinctive from other signatures. Raccoons were similar in size to fawns, but few were located during the drone flights. Fritzell (1978) found raccoons typically used developed, wooded, and wetland areas, whereas we conducted most of our flights in grasslands. We also identified raccoons did not curl their heads into their body to form a circular signature similar to fawns. We did, however, note several fawn-like signatures that were actually holes created by coyotes or small mammals that quickly heated with the sun.

We found fawn disturbance to be low with all fawns remaining bedded and only a few lifting their head in response to the drone. To further minimize disturbance, we increased the drone height from 45 m to 60 m. Linchant et al. (2015b) reviewed several drone studies and found no disturbances reported during drone flights. Although not directly related, Christie et al. (2016) showed lower disturbances compared to other aerial methods; therefore, drones may be an advantageous method for minimizing disturbance of wildlife in general.

We found drones have applicability with not only large, but also smaller mammals. We observed and confirmed a multitude of wildlife species during our flights. Although we were able to detect the thermal signatures of passerine species, we did not record them because they were not comparable to a fawn signature (i.e., there was little chance of misidentifying the thermal signature of a passerine as a fawn's thermal signature). We did detect a mallard and her ducklings at 45 m height. Another study used drones to search for fawns during mowing operations and located foxes, rabbits, and small mammals at 30 – 40 m altitude (Israel 2011). Furthermore, drones show immense promise in the future of wildlife detection and estimation, and provide a higher search efficiency compared to previous methods.

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Table 1. List of methods used to locate and capture deer fawns (*Odocoileus* spp.) across various studies. Also shown is the year of the study, sample size (N), and effort (person-hours) from each study.

Location/Capture Method	Year	N	Effort	Study
Drone w/ Thermal Imaging (Eff. 1)	2019	33	5.8	Obermoller et al. (this study)
Drone w/ Thermal Imaging (Eff. 2)	2019	10	2.2	Obermoller et al. (this study)
Vaginal Implant Transmitter	2003 - 2004	83	7 - 16	Bishop et al. 2007
Vaginal Implant Transmitter	2001	20	60	Carstensen et al. 2003
Doe Behavior	1997 - 1999	25	145 - 214	Carstensen et al. 2003
Doe Behavior	1980 -1983	58	14.5 - 43.8	Huegel et al. 1985
Vehicle w/ Thermal Imaging	2004	26	3.3 - 9.4	Ditchkoff et al. 2005
Ground Searching	1994 - 1996	35	30.6	Ballard et al. 1998

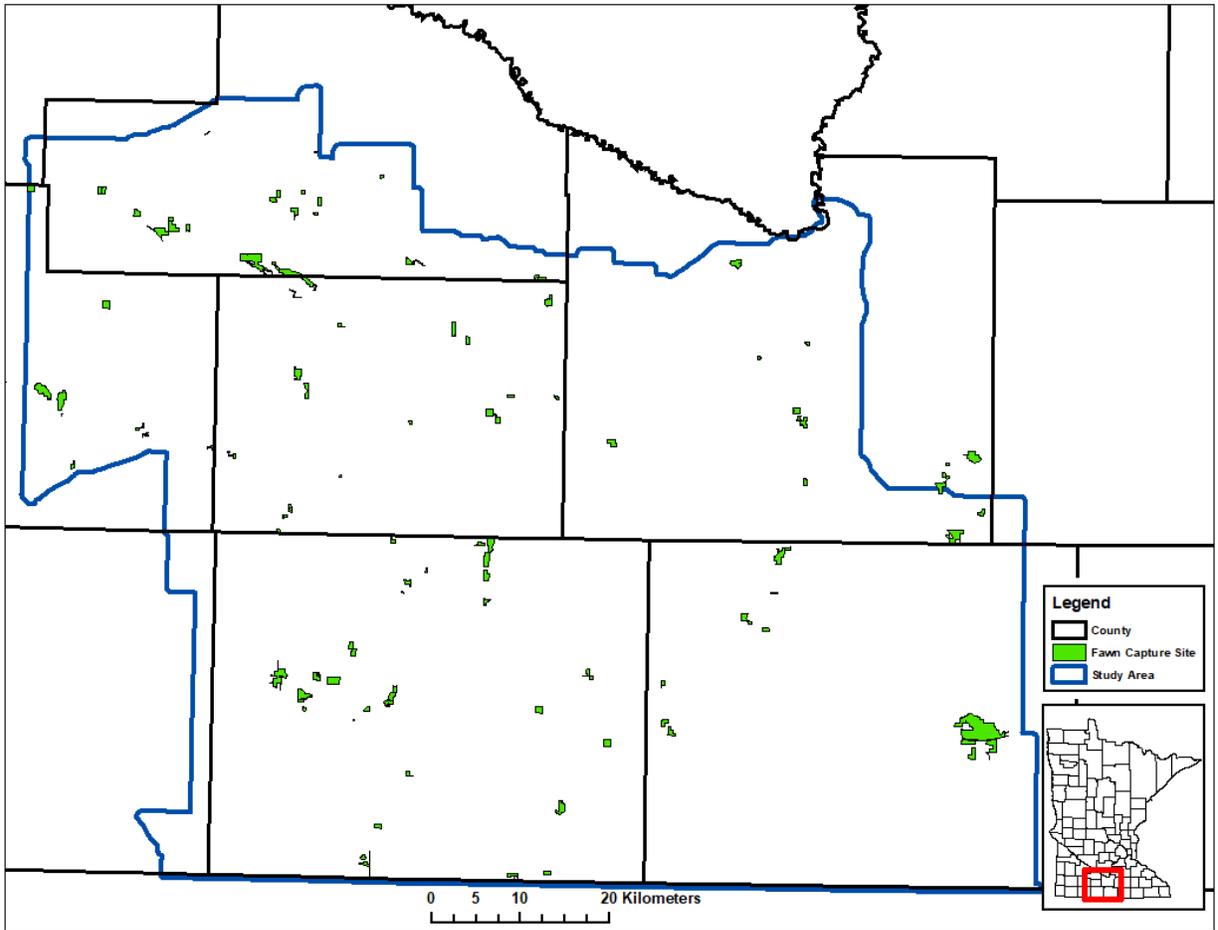


Figure 1. Study area including location of Wildlife Management Areas (green polygons) where white-tailed deer fawns were located and captured in south central Minnesota, USA from May to June 2019.



Figure 2. Flight path (yellow polylines) of the drone used to search for white-tailed deer fawns at Groebner Wildlife Management Area in south central Minnesota, USA, June 2019. This flight covered 32 hectares over 96 minutes with a 45-meter flight swath.

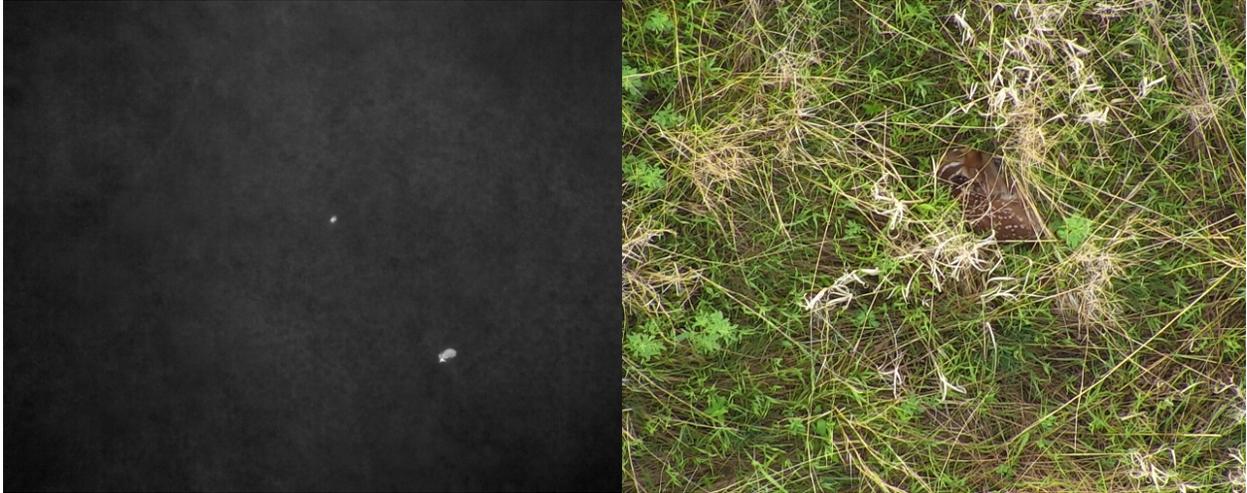


Figure 3. Thermal infrared image from a drone of a white-tailed deer fawn and her dam (left) and a zoomed-in image of the same fawn bedded in grass (right) in south central Minnesota, USA, June 2019.



## EVALUATING GRASSLAND WILDLIFE EXPOSURE TO SOYBEAN APHID INSECTICIDES ON PUBLIC LANDS IN MINNESOTA

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### SUMMARY OF FINDINGS

Increasing evidence suggests that pesticides may be an important factor explaining declines in grassland-dependent wildlife in agricultural landscapes. Minnesota Department of Natural Resource (MNDNR) wildlife managers and members of the public have reported concerns about drift from 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. Lab studies have shown chlorpyrifos (a broad spectrum organophosphate) and other insecticides used to target aphids are highly toxic to non-target organisms, including economically important game species and pollinators, but few studies have investigated the environmentally-relevant exposure of free-ranging wildlife to these chemicals. Our objectives were to assess the direct and indirect exposure of grassland wildlife to the 3 most common soybean aphid insecticides (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) along a gradient from soybean field edge to grassland interior. During summer 2017 and 2018, we sampled 5 treatment and 4 control sites across western and southern Minnesota. We detected chlorpyrifos at all distances examined (0-400 m) at both treatment and control sites, suggesting that some background level of chlorpyrifos exposure is occurring in the environment regardless of landowner activities in the adjacent row crop field. Deposition was higher near field edges than the grassland interior. We also found higher residue amounts on mid-canopy samples than ground-level samples. We detected chlorpyrifos residue amounts on arthropods that were below the acute oral LD<sub>50</sub> values for birds; however, residue amounts were above the contact LD<sub>50</sub> for honey bees up to 50 m from the row crop edge. We quantified arthropod abundance, consumable dry biomass, and family richness of insects and spiders pre- and post-spraying to evaluate the indirect effects of spraying on bird food resources. We found short-term reductions in overall arthropod abundance, bird prey abundance (specifically, individuals in the orders Araneae, Coleoptera, Lepidoptera larvae, and Orthoptera), and Coleopteran family richness in treatment sites but our other arthropod measures (i.e., overall consumable dry biomass, bird prey biomass, and richness of other families important in the diets of birds) did not differ between treatment and control sites post-spraying. Overall, our results indicate that wildlife within 25 m of a grassland/row crop edge are more likely to be exposed to drift from foliar-application insecticides, and bees within 50 m of an edge may be exposed to dosages that can cause mortality by contact. Furthermore, reductions in arthropod food abundance for grassland birds may occur up to 21 days post-spraying. Natural resource managers should try to minimize the perimeter-area ratio of grasslands to reduce wildlife exposure to drift from insecticide applications in nearby row crop fields. Additionally, management regimes that increase the percent canopy cover in grasslands also have the potential to decrease exposure of grassland wildlife to these insecticides.

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## INTRODUCTION

Grassland loss and fragmentation is a major concern for grassland-dependent wildlife throughout the Midwestern United States (U.S.). 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 mounting 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).

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). Foliar applications of insecticides using ground sprayers or airplanes are common treatment methods when chemical control of aphids is necessary. The 2 most common insecticides used are chlorpyrifos and lambda-cyhalothrin (Minnesota Department of Agriculture [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 (chlorpyrifos: 28 d; lambda-cyhalothrin: 45 d; bifenthrin: up to 14 d); thus, the timing of product use within the growing season needs to be considered. If retreatment is necessary due to a continued infestation, landowners/operators 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/operators 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.

Chlorpyrifos (common trade names: Dursban, Lorsban) is a broad-spectrum organophosphate insecticide that 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). Furthermore, MDA 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 toxic to honey bees (*Apis mellifera*) with a contact LD<sub>50</sub> of 100 ppb (Ostiguy et al. 2019), and exposure to chlorpyrifos in contaminated pollen and nectar of adult honey bees is representative of non-*Apis* bee species as well (e.g., bumblebees; Cutler et al. 2014, U.S. Environmental Protection Agency 2014). Chlorpyrifos is very highly toxic to gallinaceous bird species such as the ring-necked pheasant (*Phasianus colchicus*) with a lethal oral dose causing death in 50% of treated animals (LD<sub>50</sub>) of 12.2 (Solomon et al. 2001). Several other bird species are also particularly susceptible to chlorpyrifos, including American robins (*Turdus migratorius*), common grackles (*Quiscalus quiscula*), and mallards (*Anas platyrhynchos*; Tucker and Haegele 1971, Solomon et al. 2001, 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, impaired migratory orientation) has been documented in both lab and field studies of several avian species (McEwen et al. 1986, Richards et al. 2000, Al-Badrany and Mohammad 2007, Moye 2008, Eng et al. 2017). Thus, sub-lethal effects leading to indirect mortality (e.g., via increased predation rates) or lost breeding opportunities may be a concern for wildlife, especially birds, exposed to chlorpyrifos.

Lambda-cyhalothrin (common trade name: 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). Furthermore, 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 name: Tundra) 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 northern bobwhite (*Colinus virginianus*) and mallards (oral LD<sub>50</sub> 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).

Natural resource managers and members of the public have reported concerns about the effects of soybean aphid insecticides on non-target wildlife, including economically important game bird and pollinator species. Yet little is known about the true exposure of birds and terrestrial invertebrates to drift from these insecticides in Minnesota's grasslands. Distances reported for drift from application of foliar insecticides vary widely in the literature (1 m to 2,000 m; Davis and Williams 1990, Langhof et al. 2005, Carlsen et al. 2006, Antuniassi et al. 2014, Holterman et al. 2017, Runquist et al. 2018, Baio et al. 2019). 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 was to assess the environmentally-relevant exposure of grassland wildlife to the 3 most commonly-used soybean aphid insecticides (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin; hereafter, target chemicals) in Minnesota's farmland region. Specific objectives included:

1. *Direct and Indirect Exposure:* We quantified the concentration of target chemicals 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 these chemicals: 1a) directly via contact with spray drift, and 1b) indirectly through consumption of insect prey items exposed to the insecticides.

2. *Indirect Effects*: We quantified and compared 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 target chemicals on food availability for grassland nesting birds and other wildlife.

## STUDY AREA

We conducted our study within the southwest (SW), west central (WC), and central (C) regions of Minnesota's farmland zone (Figure 1). Corn and soybeans combined account for approximately 90%, 67%, and 71% of the landscape across these three regions, respectively [U.S. Department of Agriculture (USDA) 2019a, USDA 2019b]. Area set aside as grassland cover on public and private land accounted for 6.9%, 10.0%, and 5.6% of the landscape in these regions, respectively (Messinger and Davros 2018). 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

Our treatment study sites consisted of Minnesota Department of Natural Resources (MNDNR) Wildlife Management Areas (WMA) immediately adjacent to soybean fields that were sprayed to control for aphids. We worked closely with wildlife managers and private landowner cooperators to select treatment sites. We used 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. Predominant grass species in our sites included big bluestem (*Andropogon gerardii*), smooth brome (*Bromus inermis*), Canada wild rye (*Elymus canadensis*), and Kentucky bluegrass (*Poa pratensis*), and predominant forb species included wild bergamot (*Monarda fistulosa*), smooth oxeye (*Heliopsis helianthoides*), and Canada goldenrod (*Solidago canadensis*). We also selected control study sites with similar site characteristics except that control sites had corn as the adjacent crop and, to our knowledge, they were not sprayed with foliar chemicals to control aphids. We chose sites that were predicted to be downwind (typically east or north) from cooperators' agricultural fields based on typical wind direction patterns determined from archived daily summaries of National Weather Service data.

We sampled 5 treatment sites and 4 control sites across 2 field seasons (summer 2017 and summer 2018; Table 1). Within each treatment site prior to spraying, we established 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 was large enough, we also established a station at a distance of 400 m along each transect. This design gave us a total of 18-21 stations per site. We established transects and stations the same way within control sites. At all sites, transects ran perpendicular to the edge of the cooperator's field and were spaced 90-100 m apart to reduce the likelihood of duplicate insecticide exposure from the spraying event.

### Data Collection

Our methodology required precisely timing our field data collection. We worked closely with cooperators to find out the exact date and time that spraying would occur and we adjusted our pre- and post-spraying field schedules accordingly.

#### *Direct exposure*

To assess the potential for direct exposure of birds and other wildlife to our target chemicals, we deployed passive sampling devices (PSDs) to absorb any chemical drift that occurred. We placed PSDs in treatment fields on the day of but prior to spraying of soybeans. The PSDs were

14 cm tall by 7 cm in diameter and consisted of Whatman™ Qualitative Filter Paper (grade 2; GE Healthcare U.K. Ltd, Little Chalfont, United Kingdom) attached to 1.27-cm hardware cloth formed to a cylinder shape to approximate the size and shape of a large songbird or an approximately 3-4 week old gamebird chick. We placed the PSDs at 2 heights (ground level [0 m] and mid-canopy height [0.5 m]) at each of the 18-21 sampling stations per site for a total of 36-42 PSDs/site. Ground-level sampling represented 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 represented above-ground nesting birds, songbirds, and many species of spiders and insects. We retrieved the PSDs from the field  $\leq 2.25$  h after spraying and properly stored them for later chemical analysis. At control sites, we placed PSDs at both ground and mid-canopy levels at each of the stations. We left the PSDs on site for the same amount of time as PSDs at treatment sites before we collected and stored them for later analysis.

During 2018 only, we deployed PSDs during the pre-spraying period (i.e., 1-3 d prior to spraying) at each <1 m sampling station at 3 treatment and 2 control sites. These samples provided us with a secondary field-based control to determine if our target chemicals could be detected within a treatment site prior to known spraying events.

#### *Indirect exposure*

To assess the potential for birds and other insectivorous wildlife to be exposed to the target chemicals indirectly via consumption of prey items, we sampled invertebrates  $\leq 4$  h post-spraying at the 0 m, 5 m, and 25 m stations along each transect (total = 9 stations/site). We sampled ground-dwelling invertebrates using a hand-held suction vacuum (BioQuip Products Inc., Rancho Dominguez, CA, U.S.A.) and canopy-dwelling invertebrates using a standard 38-cm diameter sweepnet. We collected vacuum and sweepnet samples along a 30-m doubled transect (30 m x 2 = 60 m total length sampled) to the right side of the sampling stations and parallel to the soybean field. We combined vacuum and sweepnet samples taken from the same station into 1 sample and properly stored them for later chemical analysis. We sampled control sites using the same methods and timing, with the timing based on when we deployed the PSDs at these sites.

#### *Indirect effects*

To quantify and compare the effects of target chemicals on the abundance, richness, diversity, and biomass of invertebrate prey items, we collected vacuum and sweepnet samples from the 0 m, 25 m, and 100 m distances along the 3 transects at each site (total = 9 stations/site). We collected these samples 1-3 d prior to spraying and between 3-5 d and 19-21 d post-spraying at treatment sites. We collected samples along a 20-m doubled transect (20 m x 2 = 40 m total length sampled) but on the left side of the sampling stations and parallel to the soybean field. We combined vacuum and sweepnet samples into 1 sample per station per sampling period and stored them in ethanol for later sorting, identification, counting, and measuring. Each time we returned to the site, we started sampling from the endpoint of the previous 20-m sampling transect. We used the same methods and timing to collect our indirect effect samples at each of our control sites after designating an arbitrary “spray day” which occurred within the same range of dates that treatment sites received insecticide applications. During identification in the lab, we placed emphasis on 4 invertebrate orders important in the diets of grassland nesting birds (Wiens and Rotenberry 1979, Rotenberry 1980, Kobal et al. 1998, Linn 2004): Araneae (spiders), Coleoptera (beetles), Lepidoptera (butterflies and moths) larvae, and Orthoptera (grasshoppers, crickets, and katydids). We also placed emphasis on Hemiptera (true bugs) because of their diversity of body forms and trophic levels. We sorted all individuals from these orders and identified them to at least the family level for analysis. All other insects were

identified to order only. We counted and measured the body lengths of arthropods to the nearest 0.01 mm and calculated the consumable dry biomass of insects and spiders with formulas reported by Straus and Avilés (2018). We chose to use consumable dry biomass because arthropods' chitinous exoskeletons are not easily digested by birds (e.g., American robin and northern bobwhite chitin digestibility has been estimated at only 7-14%; Weiser et al. 1997). Quantifying the spider community also allowed us to examine potential impacts on an additional trophic level because spiders are an important predator of insects.

#### *Weather*

We used 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.

#### *Vegetation*

At each site, we collected vegetation data 1-3 d prior to spraying at all stations and again at 3-5 d and 19-21 d post-spraying at the reduced subset of stations (i.e., those that coincided with the indirect effects sampling efforts for invertebrates). We sampled multiple vegetation plots at each station: 1 plot at each PSD station and 1 plot at each end of the 20-m and 30-m insect sampling transects. Data collected at each plot included percent ground cover, percent canopy cover, maximum height of live and dead vegetation, litter depth, vertical density, and species richness. Using a modified point-intercept method, we categorized 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 took a nadir digital photograph of a 30 cm x 55 cm quadrat at a height of 1.5 m above the ground and used the program SamplePoint to estimate percent canopy cover (Booth et al. 2006). Canopy cover categories included grass, forb, standing dead vegetation, woody vegetation, and other. We recorded the maximum height of live and dead vegetation within each plot to the nearest 0.5 dm. We measured litter depth to the nearest 0.1 cm at 1 point within the plot that represented the average condition of the plot. We measured 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). We counted the unique number of grass and forb species in each plot to estimate species richness. Finally, we recorded the dominant grass and forb species (up to 3 species in each category) at each PSD station to obtain a qualitative assessment of the vegetation present at each site.

#### *Chemical analyses in the lab*

We sent PSD samples and invertebrate samples (indirect exposure) to the USDA Agricultural Marketing Service's National Science Lab (USDA/AMS-NSL) in Gastonia, NC for chemical residue analysis. Samples were analyzed using a solvent-based extraction method. Extracts were concentrated by evaporation and then analyzed using a gas chromatography/mass spectrometry-negative chemical ionization (GC/MS-NCI) technique or other appropriate method. The USDA/AMS-NSL equipment was capable of an extremely high degree of sensitivity in the limit of detection (LOD) and reported all results to us in parts per billion (ppb).

### **Data Analyses**

#### *Direct and indirect exposure*

We used linear mixed-effects models to assess the potential effects of distance from field edge, application spray method (i.e., ground or airplane), and PSD height on direct exposure. The

summed value (ppb) of all three target chemicals was the response variable in all models. Using data from treatment sites only, we used a hierarchical model selection approach (see Daly et al. 2015). Using this approach allowed us to examine how our primary factors influenced target chemical deposition after accounting for abiotic and biotic factors expected to affect drift. Our first set of models assessed weather covariates during the spraying event as these are known to affect drift (specifically, continuous covariates for mean air temperature [TEMP; °C] and mean wind speed [WSP; m/s] and a binary covariate for whether transects were downwind of the sprayed field [WDIR]). We retained the covariates from the model with the lowest Akaike's Information Criterion corrected for sample size ( $AIC_c$ ; Burnham and Anderson 2002) and included them in the models in the second step of analysis. Our second set added vegetation covariates to account for additional variation in the data. We used continuous covariates for maximum height of live vegetation (MHL; dm), vertical density (visual obstruction reading, VOR; dm) from the direction of the sprayed field; and percent canopy cover of live vegetation (CCLIVE; sum of percent cover of grasses and forbs). We used vegetation measurements recorded at each PSD sampling station for models explaining direct exposure, and we used the averaged vegetation measurements from the start and end of insect collection transects for models explaining indirect exposure. We retained weather and vegetation covariates from the model with the lowest  $AIC_c$  for inclusion in the final modeling step. In this final step, we incorporated distance from field edge (DIST; m), application spray method (SPRAY), and PSD height (HT). We used similar models to assess indirect exposure except that PSD height was not included as a covariate.

#### *Indirect effects*

We used linear mixed-effects models to assess the potential indirect effects of target chemical drift on the abundance, consumable biomass, and family richness of arthropods. Our 8 specific response variables were: total abundance, abundance of bird prey (i.e., individuals from the orders Araneae, Coleoptera, Lepidoptera larvae, and Orthoptera), total consumable dry biomass, consumable dry biomass of bird prey, and family richness of each of the four bird prey taxa. We again used a hierarchical model selection approach. In our first model-building step, we assessed whether vegetation and year influenced arthropod abundance, biomass, and family richness at treatment and control sites at all distances from the field edge prior to spraying. In the second step, we constructed models that included the significant covariates from step 1 and fixed effects of site type (i.e., treatment or control), sample collection timing (i.e., pre-spraying, 3-5 d post-spraying, or 19-21 d post-spraying), and the interaction between site type and sample timing. We excluded data from the 100 m distance because we measured a small difference in target chemical residues at this distance; thus, any differences in arthropod measures detected at 100 m from the field edge would not be due to the effects of target chemical application. If the site type or interaction term was significant in step 2 (i.e., 95% confidence intervals around parameter estimates did not include zero), we then tested whether distance from the field edge influenced our response variables of interest. We subset the data to include only samples that were relevant to the significant predictor in step 2. For example, if we found that samples from treatment sites collected between 3-5 d post-spraying were significantly different than measures at control sites in step 2, then we subset the data to include samples from treatment sites collected between 3-5 d post-spraying at 0 and 25 m from the field edge. Using these data, we constructed a model including distance from field edge as a continuous fixed effect in addition to any covariates that were significant in step 1.

## **RESULTS**

We sampled sites between 28 July – 14 September 2017 and 18 July – 5 September 2018, coinciding with peak activity for aphid spraying in the farmland zone (Table 2). We collected a total of 368 direct exposure PSD samples, 81 indirect exposure invertebrate samples, and 243

indirect effects invertebrate samples across both years. Additionally, we collected 30 pre-spraying PSD samples as our secondary field-based controls in 2018. Cooperators used chlorpyrifos ( $n = 4$ ) and cyhalothrin ( $n = 3$ ) on soybean fields; no cooperators used bifenthrin (Table 1). Airplanes ( $n = 3$ ) and ground sprayers ( $n = 2$ ) were both used to apply insecticides at treatment sites (Table 1). Two of 5 treatment sites were downwind at the time of spraying (i.e., the average wind direction during the spraying event was within  $62^\circ$  of the primary transect orientation).

#### *Direct exposure*

We detected target chemicals on PSDs at all distances examined (0-400 m) at both treatment and control sites (Table 3, Figure 2); however, means of target chemical deposition were lower at control sites compared to treatment sites at every distance examined except for 200 m (Table 3). Our PSDs deployed at a subset of sampling stations in 2018 as a secondary, field-based control also contained very low levels of the target chemicals within treatment sites ( $\bar{x} = 7$  ppb,  $CV = 0.58$ ) and control sites ( $\bar{x} = 6$  ppb,  $CV = 0.38$ ). Chlorpyrifos was the predominant insecticide detected in these secondary control samples.

Our best supported model of target chemical deposition on PSDs at treatment sites after accounting for weather and vegetation covariates included distance from the field edge and PSD height (Table 4). We found a significant inverse association between distance from grassland/soybean field edge and deposition ( $\beta = -63.67$ ,  $p < 0.1$ ) with target chemical deposition on PSDs being greater within 25 m of the soybean field edge (Table 3, Figure 2). Deposition was higher on PSDs placed at mid-canopy ( $\beta = 15,232.28$ ) than ground level (Table 3). Application spray method was not included in the best-supported model. Direction of the wind relative to the WMA during soybean spraying events, mean air temperature, and percent canopy cover of live vegetation were included in the best-supported weather and vegetation models. Sites that were downwind of sprayed soybean fields had a positive association with target chemical deposition ( $\beta = 12,735.63$ ). Air temperature and canopy cover of live vegetation had significant and inverse associations with deposition ( $\beta = -5,566.73$  and  $\beta = -608.95$ , respectively,  $p < 0.1$ ).

Our cooperators used chlorpyrifos more often than lambda-cyhalothrin or bifenthrin (Table 1). Additionally, chlorpyrifos had the highest mean deposition values of all chemical residues we measured at both treatment and control sites. Therefore, we analyzed chlorpyrifos residues separately to compare chlorpyrifos deposition to levels shown to be toxic to birds and pollinators (Appendix A). We found overall mean chlorpyrifos residues exceeded 100 ppb on PSDs at 0, 5, 25, 50 and 400 m from the field edge at treatment sites ( $\bar{x} = 34,875$ , 16,049, 26,489, and 699 ppb, respectively; Table 5). Mean chlorpyrifos residue values at treatment sites exceeded 100 ppb at both mid-canopy and ground heights and at sites bordered by fields sprayed by airplane at 0-50 m from the field edge (Table 5). Mean chlorpyrifos residues also exceeded 100 ppb at 0 m and 400 m from the field edge at treatment sites where bordering fields were treated with ground sprayers ( $\bar{x} = 2,509$  and 2,254 ppb, respectively; Table 5). Mean chlorpyrifos residues on PSDs did not exceed 100 ppb at any distance from the field edge at control sites ( $\bar{x} = 38$ , 20, 19, 21, 21, 18, 24 ppb; Table 5).

We found consistent but small differences in chlorpyrifos deposition associated with PSD height at treatment sites, with slightly higher deposition on mid-canopy PSDs (Table 5, Figure 3). These differences were not significant (Welch's two-sample  $t$ -test,  $p \geq 0.213$ ) and did not show a trend as distance from the field edge increased. We did not detect significant differences between chlorpyrifos residues at mid-canopy and ground height at any distance from the field edge at our control sites ( $p \geq 0.345$ ).

### *Indirect exposure*

We detected target chemicals on arthropod samples at all distances (0-25 m) at both treatment and control sites (Figure 4). We detected chlorpyrifos and cyhalothrin but not bifenthrin at treatment sites whereas we detected all 3 target chemicals at control sites.

Our best-supported model of target chemical deposition on arthropod samples at treatment sites after accounting for weather and vegetation covariates did not include distance from grassland/field edge or application spray method (Table 6). Mean air temperature and the maximum height of live vegetation were in the best-supported model. Air temperature had a significant inverse association with chemical deposition ( $\beta = -764.09$ ,  $p < 0.05$ ) whereas maximum height of vegetation had a significant positive association with deposition ( $\beta = 384.05$ ,  $p < 0.1$ ).

We found significantly higher amounts of chlorpyrifos residue on arthropods collected at 0 and 5 m from field edges at control sites than treatment sites ( $p = 0.026$ ,  $0.023$ , respectively; Table 7; Figure 5). Conversely, we found mean chlorpyrifos residue on arthropod samples collected 25 m from the field edge were higher at treatment sites ( $\bar{x} = 3,050$  ppb) than control sites ( $\bar{x} = 810$  ppb)(Table 7); however, these value were not significantly different ( $p = 0.303$ ) and were driven by outliers at the 25 m distance at treatment sites (Figure 5). The median value of chlorpyrifos residues was higher at control sites than treatment sites at the 25 m distance (494 ppb vs. 55 ppb; Figure 5).

### *Indirect effects*

We collected a total of 34,247 individuals representing 26 orders and 104 families in 2017 and 2018. By order, Hemipterans constituted 26% of the total number of individuals, followed by Coleopterans (23%), Dipterans (19%), Hymenopterans (15%), Araneae (7.2%), Orthopterans (3.5%), and Lepidopterans (2.12%). All other orders constituted 3.9% of the total number of individuals. Coleopterans and Orthopterans made up the highest percentage of total dry consumable biomass (26% each), followed by Hemipterans (16%), Dipterans (8.1%), Lepidopterans (7.6%), Hymenopterans (5.8%), and Araneae (4.8%). All other orders constituted 6.4% of the total biomass.

We found significantly lower total arthropod abundance 19-21 d post-spraying at treatment and control sites compared to pre-spraying abundance ( $\beta = -46.04$ , 95% CI = -76.81, -15.26; Table 8). Samples collected at treatment sites 3-5 d post-spraying had significantly lower measures of total abundance than samples collected in this same timeframe at control sites ( $\beta = -49.06$ , 95% CI = -89.84, -8.28; Table 8). The abundance of arthropods 3-5 d post-spraying at treatment sites was not related to distance from the field edge ( $\beta = -0.38$ , 95% CI = -1.12, 0.36).

We also found lower abundance of bird prey at treatment sites 19-21 d post-spraying than control sites in this same timeframe ( $\beta = -23.94$ , 95% CI = -44.99, -2.88; Table 8). Bird prey abundance at treatment sites 19-21 d post-spraying was not related to distance from the field edge ( $\beta = -0.02$ , 95% CI = -0.44, 0.39).

For total consumable dry biomass of arthropods, our estimates on days 3-5 and 19-21 post-spraying at treatment and control sites were significantly lower than pre-spraying biomass ( $\beta = -99.78$ , 95% CI = -169.09, -30.48 and  $\beta = -75.91$ , 95% CI = -145.28, respectively -6.55; Table 8). Total biomass was not significantly different between treatment and control sites (Table 8).

Our estimates of consumable dry biomass of bird prey collected 3-5 d post-spraying at treatment and control sites were significantly lower than samples collected before spraying ( $\beta = -62.36$ , 95% CI = -123.91, -0.81; Table 8). Consumable biomass of bird prey arthropods did not differ between treatment and control sites (Table 8).

Our estimates of family richness of Araneans did not differ between pre- and post-spraying periods or between treatment and control sites (Table 8). Coleopteran family richness was lower at treatment than control sites during the 3-5 d post-spraying timeframe ( $\beta = -0.94$ , 95% CI = -1.82, -0.06; Table 8). However, family richness of Coleopterans was not influenced by distance from field edge ( $\beta = -0.003$ , 95% CI = -0.04, 0.03; Table 8). Hemipteran family richness on days 3-5 and 19-21 post-spraying at treatment and control sites was significantly lower than pre-spraying ( $\beta = -1.04$ , 95% CI = -2.03, -0.06 and  $\beta = -1.33$ , 95% CI = -2.32, -0.33, respectively) but did not differ between treatment and control sites (Table 8). Finally, Orthopteran family richness on days 3-5 and 19-21 post-spraying at treatment and control sites was significantly lower than pre-spraying ( $\beta = -0.46$ , 95% CI = -0.83, -0.1 and  $\beta = -0.56$ , 95% CI = -0.95, -0.17, respectively; Table 8) but did not differ between treatment and control sites (Table 8).

## DISCUSSION

Our finding that detectable levels of target chemicals, particularly chlorpyrifos, were present within both treatment and control sites suggests that some background level of deposition was occurring in the environment prior to and/or during the time of our sampling regardless of spraying status of our cooperator's field. Although our control sites did not have target chemicals sprayed during our sampling timeframe, our experimental design did not control for nearby fields, including other row crop fields that were adjacent to our WMA sites but not included in our landowner coordination efforts. If insecticide application was occurring in these other fields, drift could have occurred onto our study sites. Chlorpyrifos can persist in the environment after its initial application; its half-life is 4.2 h in the atmosphere and 7-120 d in soils, and residues can remain on plant surfaces up to 14 d post-application (Solomon et al. 2001, Christensen et al. 2009). Although the target chemicals are sometimes used to treat corn pests, over 84% of corn acres in Minnesota in 2018 contained seeds genetically modified to protect against insect pests (Potter et al. 2018). With this technology, the need for foliar insecticides on corn has decreased considerably in recent years (L. Stahl, University of Minnesota Extension, personal communication). Thus, the residue amounts we measured at control sites and the extremely low residue values detected on pre-spraying PSDs suggest that target chemical deposition occurred from sprayed soybean fields beyond our cooperator's fields. Although shorter distances of 5-75 m for drift from application of foliar insecticides are reported in the literature (e.g., Davis and Williams 1990, Holland et al. 1997, Vischetti et al. 2008, Harris and Thompson 2012), a recent butterfly study in western Minnesota found insecticide drift on plants located up to 1,600 m away from potential sources (E. Runquist, personal communication). Runquist et al (2018) also found chlorpyrifos residues in all vegetation samples they collected, with the highest residue amounts measuring 2,290 ppb from a grassland edge. Their samples were collected when nearby spraying was not observed, further supporting our conclusion that measurable chlorpyrifos residues are present in grasslands even in the absence of concurrent spraying in adjacent row crop fields.

We found that distance to the soybean field edge was an important factor explaining direct exposure to insecticide drift within our grasslands. Deposition on PSDs was highest within 25 m of the field edge and decreased with increasing distance from the soybean field edge. Other studies have also found an edge effect of drift (e.g., Threadgill and Smith 1975, Langhof et al. 2005, Carlsen et al. 2006, Nsibande et al. 2015, Holterman et al. 2017, Baio et al. 2019). Furthermore, we analyzed chlorpyrifos separately from our other target chemicals because our cooperators used it more frequently and because of its reported toxicities to wildlife. The deposition amounts we measured exceeded the contact LD<sub>50</sub> for bees (100 ppb, Appendix A) on PSDs up to 50 m from the soybean field edge at both mid-canopy and ground heights. Chlorpyrifos is highly toxic to honey bees (*Apis mellifera*) and can poison non-target insects for up to 24 h after spraying (Christensen et al. 2009). We did not measure chlorpyrifos at known

contact LD<sub>50</sub> values for common farmland bird species (e.g., ring-necked pheasants, American robins, and common grackles). Notably, the [Minnesota Buffer Law](#) requiring perennial vegetative buffers calls for a minimum width of approximately 5 m along public ditches. We suggest these minimal-width buffers have the potential to be ecological traps or population sinks for wildlife vulnerable to our target chemicals, particularly pollinators such as honey bees or bumblebees.

Conversely, we did not find distance to edge to be important in our models explaining indirect exposure to target chemicals. We only collected arthropod samples at 0, 5, and 25 m from the field edge because we predicted the heaviest deposition would occur closer to the field edge. However, an edge distance up to 25 m was likely insufficient for detecting variation in deposition within this shorter range of distances. Notably, the chlorpyrifos residue amounts we detected on arthropods were below the acute oral LD<sub>50</sub> values for many of the grassland bird species commonly found in Minnesota's farmland landscape (Appendix A). Solomon et al (2001) concluded that chlorpyrifos residues on arthropods in agricultural systems are below acute oral LD<sub>50</sub> values for birds. However, comparisons to acute oral LD<sub>50</sub> values do not account for effects that can occur with chronic exposure and at sublethal doses (e.g., impaired movement, reduced foraging, lethargy, reduced body condition, impaired migratory orientation; McEwan et al. 1986, Richards et al. 2000, Al-Badrany and Mohammad 2007, Moye 2008, Eng et al. 2017). Further research is needed to determine if the widespread use of soybean aphid insecticides, particularly chlorpyrifos, which occurs in the landscape leads to chronic exposure and sublethal effects in birds.

Our PSDs at mid-canopy had higher amounts of deposition than ground-level PSDs. Additionally, canopy cover of live vegetation was inversely related to deposition on PSDs which has also been found in other studies (Pratt et al. 2000, Donkersley and Nuyttens 2011, Holterman et al. 2017). Taken together, our results suggest that wildlife that nest and forage on the ground and in thicker cover (e.g., pheasants, some songbirds, small mammals, some pollinators) may experience less direct exposure to insecticide spray drift than those that use the canopy layer of grasslands or select grassland habitat with sparser cover.

Insecticide application spray method can vary depending on field conditions at the time of spraying (e.g., fields are less accessible by tractor when soil is wet or soybeans are too tall), and members of the public perceive that airplanes are more likely than ground sprayers to produce off-target drift. We expected an interaction between spray method and edge distance would best explain drift because our mean values for target chemical deposition on PSDs were generally higher for fields sprayed by airplanes than those sprayed on the ground. Yet our best-fit models for direct drift and indirect drift did not include spray method or its interaction with edge distance. Other factors related to spraying equipment (e.g., spray droplet size, nozzle type, operating pressure, driving speed, boom height, uncontrolled boom movements) and application rate can impact drift (Threadgill and Smith 1975, Nuyttens et al. 2007, Arvidsson et al. 2011, Donkersley and Nuyttens 2011, Nsibandé et al. 2015, Nuyttens et al. 2017). Although we requested information from cooperators regarding the spraying equipment they used, we did not control for these variables in our study design or analyses. These factors likely influenced the amount of target chemical deposition on PSD and arthropod samples.

The indirect effects of insecticides, including the declines of arthropods important in bird diets, have become an increasing concern for conservation in agricultural landscapes (Campbell et al. 1997, Barker 2004, Devine and Furlong 2007, Goulson 2014). Abundance and biomass of prey items of birds are important to consider, as arthropods constitute the majority of grassland bird diets (Wiens and Rotenberry 1979) and areas with high arthropod biomass have been shown to have a strong relationship with gamebird brood use (Jamison et al. 2002, Hagen et al. 2005). Our results indicated measurable impacts on arthropods that serve as prey items for grassland

birds after insecticide spraying events. In particular, we documented short-term reductions in total arthropod abundance (3-5 d post-spraying) and bird prey abundance (19-21 d post-spraying) in grasslands bordered by soybean fields sprayed to control for aphids. We also found lower Coleopteran family richness at treatment sites 3-5 d post-spraying. Similar short-term reductions in arthropod abundance and diversity following insecticide application have been documented in other studies (Barrett 1968, Vickerman and Sunderland 1977, Longley et al. 1997, Galvan et al. 2005, Langhof et al. 2005, Devotto et al. 2006). Although overall abundance of arthropods and Coleoptera family richness were lower within a few days after insecticide application, these measures were similar to pre-spraying levels by week 3 post-spraying, indicating that arthropod populations rebounded during this period. Arthropod communities' recovery rates can vary widely between areas and with arthropods' differing dispersal capabilities, reproductive potentials, and life stages (Campbell et al. 1997, Longley et al. 1997), and arthropods could have recolonized these grasslands from nearby areas that were not treated with or impacted by insecticides during this period (Longley et al. 1997).

Interestingly, we did not detect concomitant decreases in consumable dry biomass between treatment and control sites during either sampling period post-spraying. The effects of insecticides on non-target arthropods have been shown to depend on a wide variety of factors. Toxicity of insecticides to arthropods varies widely by species (Sánchez-Bayo 2011). Different life stages of arthropods can also have different susceptibilities to insecticides, and decreases of arthropods in varying life stages can result in various effects on population growth rate (Stark et al. 2004). Larger-bodied insects which contributed more to our biomass estimates (e.g., Orthopterans) may have not have been affected as much as smaller-bodied insects. Alternatively, arthropod biomass could have been impacted regardless of spray application on the nearest field. The widespread use of soybean aphid insecticides during July-September in Minnesota combined with ubiquitous drift over longer distances that has been documented (at least 400 m, our study; up to 2,000 m, Baio et al. 2019) may have contributed to lower arthropod biomass overall across the landscape, making any differences between our treatment and control sites difficult to detect.

As part of our final LCCMR reporting, we indicated a plan to invite our landowners, cooperators, and other constituent groups (e.g., University of Minnesota's Southwest Agricultural Experiment Station personnel, Soybean Growers' Association, various natural resource professions) to a landowner appreciation seminar during early spring 2020 to engage them, update them on how their participation benefited our research efforts, and show them how the aggregated data is being shared with other groups and in reports and publications. However, the COVID-19 pandemic forced us to delay this seminar for the foreseeable future, and we may have to consider a virtual event instead. Regardless of forum, our proximate goal with this outreach event is multifold: 1) bring awareness to the issue of soybean aphid insecticide drift onto grasslands, 2) engage agricultural partners in a conversation about potential solutions and mitigation efforts to reduce the potential for drift to occur on these grasslands, and 3) promote good will and communication that could be beneficial if MNDNR conducts further pesticide research in the future. However, our ultimate goal is to provide land managers with information on patterns of soybean aphid insecticide drift onto grassland cover in the agricultural matrix of Minnesota to improve management of public and private lands set-aside for grassland wildlife conservation.

## **MANAGEMENT IMPLICATIONS**

Our findings indicate that drift from insecticide applications to control for soybean aphids is ubiquitous in small amounts even in grasslands without immediately adjacent soybean fields. Wildlife associated with row crop edges and sparser grassland vegetation are more likely to be exposed to drift directly. Additionally, less food may be available to breeding birds, their young,

and other insectivorous wildlife for up to 3 weeks after spraying operations in the area. Managers should acquire and maintain larger grassland tracts to reduce the edge effects from insecticide drift and provide refugia for arthropods to be able to recolonize affected areas. They should also use seeding mixes that create a thicker, more diverse canopy cover to prevent drift from reaching ground-dwelling wildlife and to support a more diverse arthropod community for insectivores. Further research is needed to the full impacts of insecticide drift on populations of wildlife and pollinators inhabiting narrow buffers (e.g., filter strips, grassed waterways) commonly used alongside row crop fields. Additionally, future research should also address the potential for chronic exposure and sublethal effects of these insecticides on free-ranging wildlife in Minnesota's farmland landscape.

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Table 1. Spray method and application data for soybean aphid spraying events by cooperators adjacent to Wildlife Management Areas (WMA) that were sampled for insecticide spray drift during July-September 2017 and 2018 in Minnesota's farmland zone.

Site ID <sup>a</sup>	Spray method	Insecticide trade name	Insecticide active ingredients	Insecticide application rate (L/ha)	Sprayer application rate (L/ha)	Application speed (m/s)	Boom height (m)	Tank pressure (kPa)
tA	Ground	Endigo	lambda-cyhalothrin + thiamethoxam	0.26	140.3	4.0	0.2-0.3	275.8
tB	Airplane	Bolton	chlorpyrifos + gamma-cyhalothrin	0.88	18.7	67.9	1.5	275.8
tC	Ground	Lorsban 4E	chlorpyrifos	NA <sup>b</sup>	93.5	NA	NA	137.9-206.8
tD	Airplane	Lorsban Advanced	chlorpyrifos	1.17	18.7	55.9	2.7-4.0	275.8
tE <sup>c</sup>	Airplane	Lorsban Advanced; Warrior II	chlorpyrifos; lambda-cyhalothrin	0.44; 0.22	NA	NA	NA	NA

<sup>a</sup>WMA names are not provided to protect private landowner cooperators.

<sup>b</sup>Data is not available because cooperator declined to provide this information.

<sup>c</sup>This cooperator combined two different trade name insecticides during the spraying event.

Table 2. Location, site type, year sampled, and timing of sampling for Wildlife Management Areas (WMA) sampled for insecticide drift from adjacent row crop fields sprayed for soybean aphids during July-September 2017 and 2018 in Minnesota's farmland zone.

Site ID <sup>a</sup>	Region <sup>b</sup>	County	Site type <sup>c</sup>	Year sampled	Range of dates when field sampling occurred <sup>d</sup>
tA	SW	Jackson	Treatment	2017	28 July - 18 Aug
tB	SW	Murray	Treatment	2017	9 Aug - 30 Aug
cA	SW	Jackson	Control	2017	21 Aug - 14 Sept
cB	SW	Lyon	Control	2017	7 Aug - 31 Aug
tC	WC	Lac qui Parle	Treatment	2018	10 Aug - 29 Aug
tD	C	Stearns	Treatment	2018	28 July - 16 Aug
tE	WC	Yellow Medicine	Treatment	2018	7 Aug - 28 Aug
cC	C	Kandiyohi	Control	2018	17 Aug - 5 Sept
cD	WC	Lac qui Parle	Control	2018	18 Jul - 8 Aug

<sup>a</sup>WMA names are not provided to protect private landowner cooperators.

<sup>b</sup>Regions sampled in this study include the southwest (SW), west central (WC), and central (C) regions. The boundaries for these regions follow the same boundaries as outlined in the Minnesota Department of Natural Resources' annual August Roadside Survey.

<sup>c</sup>Treatment sites had adjacent soybean fields that were sprayed for aphids; control sites had adjacent corn fields that were not sprayed for aphids.

<sup>d</sup>Includes first day of pre-spray sampling through last day of post-spray sampling for data collection activities.

Table 3. Means and coefficients of variation ( $\bar{x}$  [CV]) of target chemicals (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) detected on passive sampling devices ( $n = 368$ ) by distance from soybean field edge to grassland interior. Mean values are reported in parts per billion (ppb). Treatment sites consisted of grasslands adjacent to soybean fields that were treated with target chemicals by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. PSDs were deployed at mid-canopy height (0.5 m above ground) and ground level. Samples were collected during July-September 2017 and 2018 in Minnesota's farmland zone.

Site type	Distance from soybean field edge (m)						
	0 m	5 m	25 m	50 m	100 m	200 m	400 m
Treatment	35,322 (4.11)	16,260 (3.95)	26,712 (3.48)	385 (2.35)	40 (1.72)	14 (1.44)	699 (5.02)
<i>PSD height</i>							
Mid-canopy	61,981 (3.28)	29,310 (3.08)	38,504 (3.28)	569 (2.11)	52 (1.65)	18 (1.35)	1,387 (3.58)
Ground	8,664 (2.8)	3,210 (2.32)	14,920 (2.68)	201 (2.15)	27 (1.66)	9 (1.35)	11 (0.94)
<i>Spray application method</i>							
Airplane	57,198 (3.25)	27,080 (3.03)	44,504 (2.65)	629 (1.77)	50 (1.69)	7 (1.31)	8 (0.99)
Ground	2,510 (2.21)	30 (1.02)	25 (1.05)	19 (1.07)	24 (1.23)	23 (1.14)	2,254 (2.8)
Control	41 (1.84)	21 (0.94)	21 (0.92)	21 (0.96)	22 (1.03)	19 (0.97)	30 (1.02)

Table 4. Number of parameters (K), Akaike's Information Criterion corrected for small sample size (AIC<sub>c</sub>; n = 206), conditional R<sup>2</sup> value (variation explained by the entire model including random effects), deviance (d), and model weight (ω) for models of target chemical deposition (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) onto passive sampling devices (PSDs) at treatment study sites in the farmland region of Minnesota during July-September 2017 and 2018. PSDs were used to assess direct exposure of wildlife to drift from insecticides sprayed to control soybean aphids. A hierarchical model selection approach was used in which the first set of models assessed weather conditions during the spraying event (specifically, whether the site was downwind of the sprayed field [WDIR], ambient air temperature [TEMP], and wind speed [WSP]). The best-supported weather model was then used as a base model to assess vegetation covariates (specifically, percent canopy cover of live vegetation [CCLIVE], maximum height of live vegetation [MHL], and vertical density of vegetation from the direction of the sprayed field [visual obstruction reading; VOR]). The best-supported weather + vegetation model was then used to assess primary factors of interest: distance of the PSD from the grassland/soybean field edge (DIST), height of the PSD (i.e., mid-canopy or ground level; HT), and application spray method (i.e., airplane or ground; SPRAY). The column ΔAIC<sub>c</sub> compares models within each step of model development. Models were linear mixed-effect models, included site as a random effect, and were fit using the maximum likelihood method.

Model	K	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	R <sup>2</sup>	d	ω
Weather						
WDIR + TEMP	5	5172.26	0.00	0.10	5161.96	0.44
TEMP	4	5173.11	0.85	0.08	5164.91	0.29
WSP + WDIR + TEMP	6	5174.37	2.12	0.10	5161.95	0.15
WSP + TEMP	5	5175.12	2.86	0.08	5164.82	0.11
WDIR	4	5179.93	7.67	0.07	5171.73	0.01
WSP + WDIR	5	5182.03	9.77	0.07	5171.73	0.00
WSP	4	5182.34	10.08	0.07	5174.14	0.00
Weather + Vegetation						
WEATHER <sup>a</sup> + CCLIVE	6	5170.36	0.00	0.11	5157.94	0.31
WEATHER + MHL + CCLIVE	7	5171.67	1.30	0.12	5157.10	0.16
WEATHER + MHL	6	5172.09	1.73	0.11	5159.67	0.13
WEATHER	5	5172.26	1.90	0.10	5161.96	0.12
WEATHER + VOR + CCLIVE	7	5172.49	2.13	0.12	5157.92	0.11
WEATHER + MHL + VOR + CCLIVE	8	5173.39	3.02	0.12	5156.65	0.07
WEATHER + VOR	6	5174.05	3.69	0.10	5161.63	0.05
WEATHER + MHL + VOR	7	5174.14	3.78	0.11	5159.57	0.05
Weather + Vegetation + Primary Factors						
VEG <sup>b</sup> + DIST + HT	8	5168.69	0.00	0.14	5151.96	0.22
VEG + DIST	7	5169.31	0.62	0.13	5154.74	0.16
VEG + HT	7	5169.76	1.07	0.13	5155.19	0.13
VEG	6	5170.36	1.68	0.11	5157.94	0.10
VEG + HT + SPRAY * DIST	10	5170.44	1.76	0.15	5149.32	0.09
VEG + DIST + SPRAY + HT	9	5170.58	1.89	0.14	5151.66	0.09
VEG + SPRAY * DIST	9	5171.06	2.37	0.14	5152.14	0.07
VEG + DIST + SPRAY	8	5171.19	2.50	0.13	5154.45	0.06
VEG + SPRAY + HT	8	5171.67	2.99	0.13	5154.94	0.05
VEG + SPRAY	7	5172.26	3.57	0.12	5157.69	0.04

<sup>a</sup>WEATHER = covariates in the top-ranked Weather model (WDIR + TEMP).

<sup>b</sup>VEG = covariates in top-ranked Weather + Vegetation model (WDIR + TEMP + CCLIVE).

Table 5. Means and coefficients of variation ( $\bar{x}$  [CV]) of chlorpyrifos detected on passive sampling devices ( $n = 368$ ) by distance from soybean field edge to grassland interior. Mean values are reported in parts per billion (ppb). Treatment sites consisted of grasslands adjacent to soybean fields that were treated with target chemicals by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. PSDs were deployed at mid-canopy height (0.5 m above ground) and ground level. Samples were collected during July-September 2017 and 2018 in Minnesota's farmland zone.

Site type	Distance from soybean field edge (m)						
	0 m	5 m	25 m	50 m	100 m	200 m	400 m
Treatment	34,875 (4.15)	16,049 (3.98)	26,489 (3.5)	372 (2.36)	38 (1.7)	14 (1.44)	699 (5.02)
<i>PSD height</i>							
Mid-canopy	61,452 (3.31)	28,970 (3.1)	38,272 (3.29)	551 (2.12)	50 (1.62)	18 (1.35)	1,387 (3.58)
Ground	8,297 (2.91)	3,129 (2.34)	14,706 (2.7)	194 (2.16)	27 (1.63)	9 (1.35)	11 (0.94)
<i>Spray application method</i>							
Airplane	56,451 (3.29)	26,729 (3.06)	44,132 (2.66)	608 (1.78)	48 (1.68)	7 (1.31)	8 (0.99)
Ground	2,509 (2.21)	30 (1.02)	25 (1.05)	19 (1.07)	24 (1.23)	23 (1.14)	2,254 (2.8)
Control	38 (1.92)	20 (1)	19 (1.01)	21 (0.98)	21 (1.09)	18 (1.03)	24 (0.94)

Table 6. Number of parameters (K), Akaike's Information Criterion corrected for small sample size ( $AIC_c$ ;  $n = 45$ ), conditional  $R^2$  value (variation explained by the entire model including random effects), deviance (d), and model weight ( $\omega$ ) for models of target chemical deposition (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) on arthropod samples collected at treatment study sites in the farmland region of Minnesota during July-September 2017 and 2018. The arthropods were used to assess the potential for wildlife to be exposed to soybean aphid insecticides indirectly via consumption of invertebrate prey exposed to these chemicals via spray drift. A hierarchical model selection approach was used in which the first set of models assessed weather conditions during the spraying event (specifically, whether the site was downwind of the sprayed field [WDIR], ambient air temperature [TEMP], and wind speed [WSP]). The best-supported weather model was then used as a base model to assess vegetation covariates (specifically, percent canopy cover of live vegetation [CCLIVE], maximum height of live vegetation [MHL], and vertical density of vegetation from the direction of the sprayed field [visual obstruction reading; VOR]). The best-supported weather + vegetation model was then used to assess primary factors of interest: distance of the PSD from the grassland/soybean field edge (DIST) and application spray method (i.e., airplane or ground; SPRAY). The column  $\Delta AIC_c$  compares models within each step of model development. Models were linear mixed-effect models, included site as a random effect, and were fit using the maximum likelihood method.

Model	K	$AIC_c$	$\Delta AIC_c$	$R^2$	d	$\omega$
Weather						
TEMP	4	886.60	0.00	0.25	877.60	0.40
WDIR + TEMP	5	886.84	0.25	0.28	875.31	0.36
WSP + TEMP	5	889.06	2.47	0.25	877.53	0.12
WSP + WDIR + TEMP	6	889.51	2.91	0.28	875.30	0.09
WDIR	4	892.82	6.22	0.19	883.82	0.02
WSP	4	895.17	8.57	0.19	886.17	0.01
WSP + WDIR	5	895.36	8.76	0.19	883.82	0.01
Weather + Vegetation						
WEATHER <sup>a</sup> + MHL	5	885.22	0.00	0.31	873.68	0.30
WEATHER + MHL + VOR	6	885.40	0.18	0.35	871.19	0.28
WEATHER	4	886.60	1.38	0.25	877.60	0.15
WEATHER + MHL + CCLIVE	6	887.55	2.32	0.32	873.33	0.10
WEATHER + MHL + VOR + CCLIVE	7	888.19	2.97	0.35	871.16	0.07
WEATHER + CCLIVE	5	889.10	3.88	0.25	877.56	0.04
WEATHER + VOR	5	889.12	3.90	0.25	877.59	0.04
WEATHER + VOR + CCLIVE	6	891.74	6.52	0.25	877.53	0.01
Weather + Vegetation + Primary Factors						
VEG <sup>b</sup>	5	885.22	0.00	0.31	873.68	0.46
VEG + DIST	6	886.47	1.25	0.33	872.26	0.25
VEG + SPRAY	6	887.54	2.32	0.32	873.33	0.14
VEG + DIST + SPRAY	7	888.80	3.58	0.34	871.77	0.08
VEG + SPRAY * DIST	8	888.88	3.65	0.38	868.88	0.07

<sup>a</sup>WEATHER = covariates in the top-ranked Weather model (TEMP).

<sup>b</sup>VEG = covariates in the top-ranked Weather + Vegetation model (TEMP + MHL).

Table 7. Means and coefficients of variation ( $\bar{x}$  [CV]) of chlorpyrifos detected on arthropod samples ( $n = 81$ ) by distance from soybean field edge to grassland interior. Mean values are reported in parts per billion (ppb). Treatment sites consisted of grasslands adjacent to soybean fields that were treated with target chemicals by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. Samples were collected during July-September 2017 and 2018 in Minnesota's farmland zone.

Site type	Distance from soybean field edge (m)		
	0 m	5 m	25 m
Treatment	115 (1.89)	54 (0.73)	3,050 (2.64)
Control	628 (1.08)	711 (1.22)	810 (1.25)

Table 8. Coefficient estimates and 95% confidence intervals (CIs) for covariates included in step 2 of a hierarchical model selection approach used to examine the indirect effects of drift from target chemicals (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) used to control soybean aphids on the total abundance, bird prey<sup>a</sup> abundance, total consumable dry biomass, consumable dry biomass of bird prey, and bird prey family richness of arthropods. Arthropod samples were collected from Wildlife Management Areas (WMAs) grasslands in the farmland region of Minnesota during July-September 2017 and 2018. Bold estimates indicate significance based on non-overlapping 95% CIs.

Response variable	Cond R <sup>2b</sup>	Site type	Sample timing <sup>d</sup>		Treatment sites <sup>e</sup>	
		Treatment <sup>c</sup>	3-5 d	19-21 d	3-5 d	19-21 d
Abundance						
Total	0.43	-17.57 (-75.6, 40.46)	-25.64 (-56.35, 5.06)	<b>-46.04</b> <b>(-76.81, -15.26)</b>	<b>-49.06</b> <b>(-89.84, -8.28)</b>	-18.71 (-59.64, 22.22)
Bird prey	0.29	7.53 (-13.42, 28.49)	-11.20 (-26.9, 4.49)	-13.08 (-28.97, 2.81)	-19.38 (-40.22, 1.47)	<b>-23.94</b> <b>(-44.99, -2.88)</b>
Biomass						
Total	0.20	-44.43 (-127.27, 38.42)	<b>-99.78</b> <b>(-169.09, -30.48)</b>	<b>-75.91</b> <b>(-145.28, -6.55)</b>	14.62 (-77.44, 106.69)	-28.42 (-120.85, 64.01)
Bird prey	0.14	-17.53 (-97.68, 62.61)	<b>-62.36</b> <b>(-123.91, -0.81)</b>	-58.97 (-121.2, 3.26)	-0.43 (-82.21, 81.36)	-22.26 (-104.56, 60.04)
Family richness						
Araneae	0.05	-0.24 (-0.86, 0.39)	-0.01 (-0.57, 0.55)	0.01 (-0.56, 0.57)	-0.26 (-1.02, 0.49)	-0.22 (-0.99, 0.55)
Coleoptera	0.21	-0.21 (-1.12, 0.70)	-0.33 (-0.99, 0.33)	-0.34 (-1.01, 0.33)	<b>-0.94</b> <b>(-1.82, -0.06)</b>	0.03 (-0.85, 0.92)
Hemiptera	0.47	-1.12 (-3.55, 1.32)	<b>-1.04</b> <b>(-2.03, -0.06)</b>	<b>-1.33</b> <b>(-2.32, -0.33)</b>	-0.55 (-1.87, 0.77)	0.16 (-1.16, 1.48)
Orthoptera	0.34	-0.37 (-0.97, 0.23)	<b>-0.46</b> <b>(-0.83, -0.10)</b>	<b>-0.56</b> <b>(-0.95, -0.17)</b>	0.02 (-0.48, 0.52)	-0.17 (-0.72, 0.38)

<sup>a</sup>Bird prey includes individuals from the orders Araneae, Coleoptera, Lepidoptera (larvae only), and Orthoptera.

<sup>b</sup>Variation explained by the entire model including random effects.

<sup>c</sup>Difference from the estimate of samples collected at control sites.

<sup>d</sup>Difference from the estimate of samples collected prior to spraying event compared to either 3-5 d or 19-21 d post-spraying.

<sup>e</sup>Difference from the estimate of samples collected at control sites either at 3-5 d or 19-21 d post-spraying.

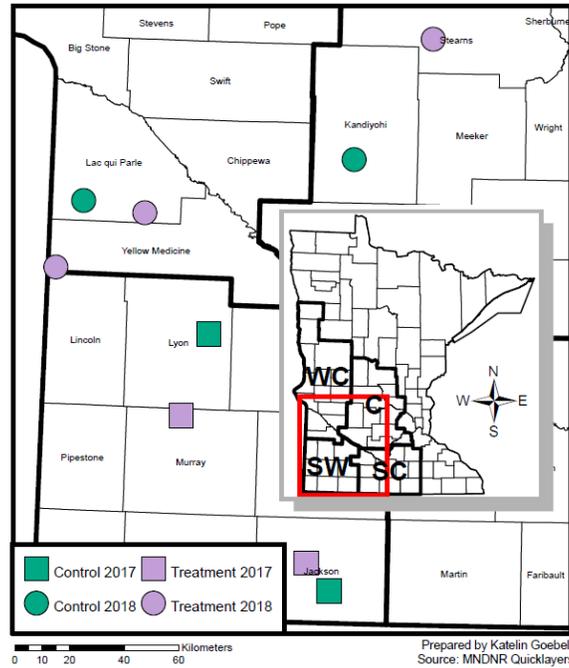


Figure 1. Location of treatment (purple symbols) and control (green symbols) sites during 2017 (square symbols) and 2018 (round symbols) sampling efforts in Minnesota’s farmland regions. Treatment sites were Wildlife Management Areas (WMA) grasslands adjacent to soybean fields sprayed for aphids; control sites were WMA grasslands adjacent to corn fields that were not sprayed with insecticides to control soybean aphids.

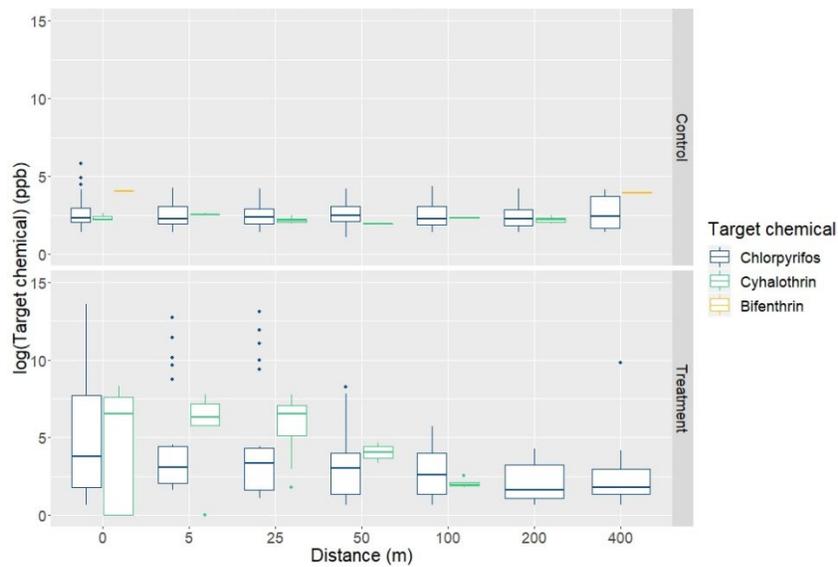


Figure 2. Target chemical deposition (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) on passive sampling devices (PSDs;  $n = 368$ ) by distance from row crop field edge (0 m) to grassland interior for control (top panel) and treatment sites (bottom panel) during July-September 2017 and 2018 in Minnesota’s farmland regions. The PSDs were used to quantify the potential for grassland wildlife to be exposed to target chemicals directly through spray drift. Spraying at treatment sites occurred on soybean fields adjacent to grasslands; control sites were grasslands adjacent to unsprayed corn fields.

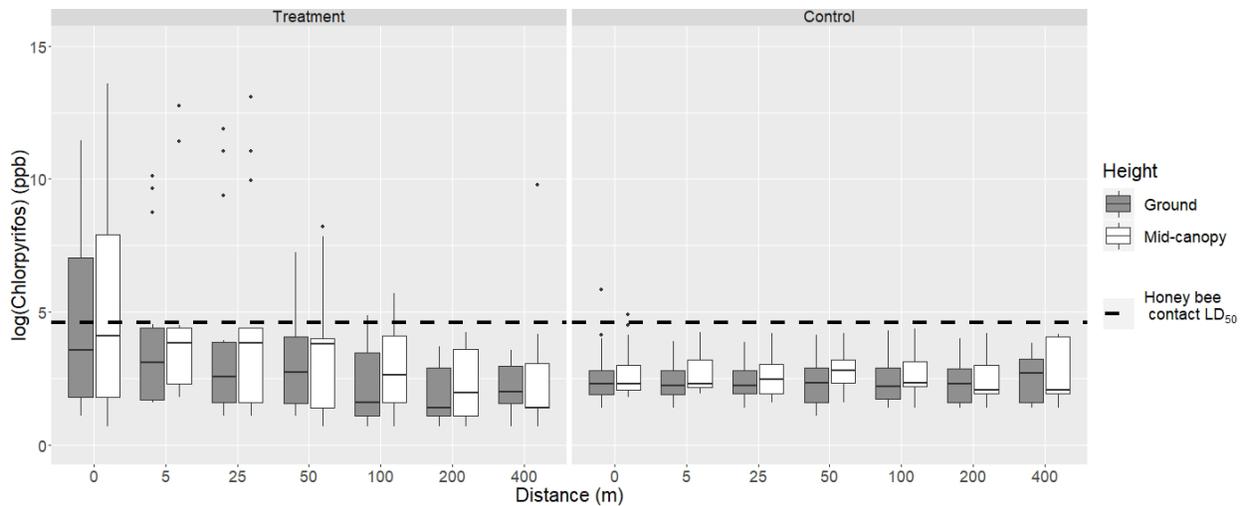


Figure 3. Chlorpyrifos deposition on passive sampling devices (PSDs;  $n = 368$ ) by distance from row crop field edge (0 m) to grassland interior for treatment (left panel) and control sites (right panel) during July–September 2017 and 2018 in Minnesota’s farmland regions. The PSDs were used to quantify the potential for grassland wildlife to be exposed to target chemicals directly through spray drift. Spraying at treatment sites occurred on soybean fields adjacent to grasslands; control sites were grasslands adjacent to unsprayed corn fields. The dashed horizontal line shows the acute contact LD<sub>50</sub> for honey bees (*Apis mellifera*).

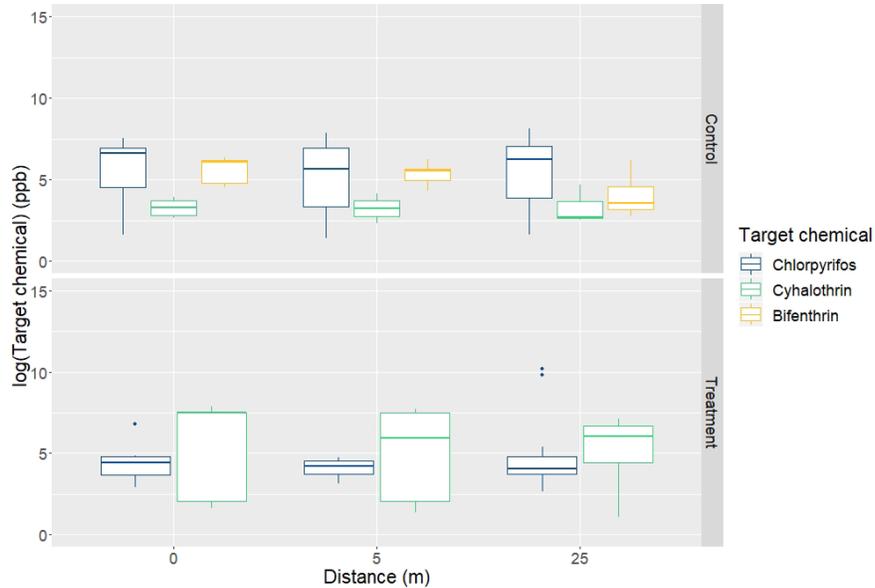


Figure 4. Target chemical deposition (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) on arthropod samples ( $n = 81$ ) by distance from row crop field edge (0 m) to grassland interior for control (top panel) and treatment sites (bottom panel) during July–September 2017 and 2018 in Minnesota’s farmland regions. Samples were collected to quantify the potential for grassland wildlife to be exposed to target chemicals indirectly through consumption of invertebrate prey exposed to drift from spraying. Spraying at treatment sites occurred on soybean fields adjacent to grasslands; control sites were grasslands adjacent to unsprayed corn fields.

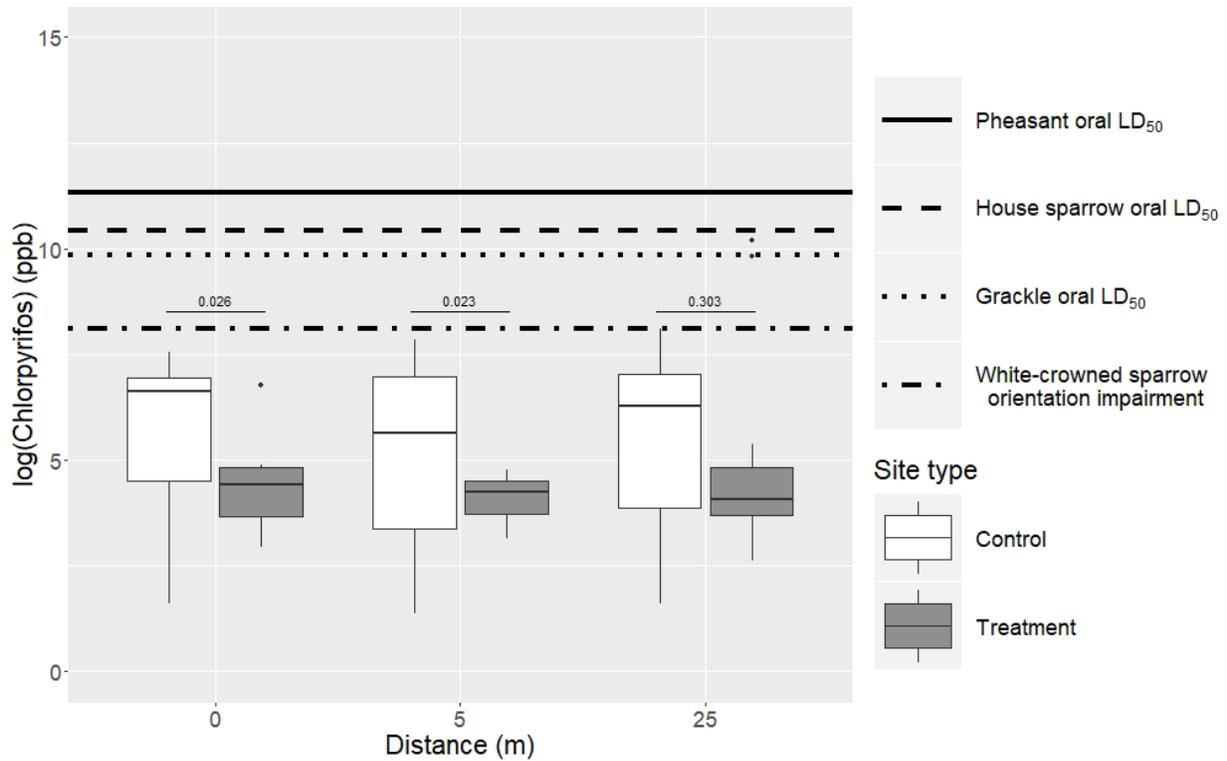


Figure 5. Chlorpyrifos deposition on arthropod samples ( $n = 81$ ) by distance from row crop field edge (0 m) to grassland interior during July-September 2017 and 2018 in Minnesota's farmland regions. Samples were collected to quantify the potential for grassland wildlife to be exposed to target chemicals indirectly through consumption of invertebrate prey exposed to drift from spraying. Spraying at treatment sites occurred on soybean fields adjacent to grasslands; control sites were grasslands adjacent to unsprayed corn fields. The thicker solid horizontal line and the dashed horizontal lines represent the acute oral LD<sub>50</sub> values for ring-necked pheasants (*Phasianus colchicus*), house sparrows (*Passer domesticus*), and common grackles (*Quiscalus quiscula*) and the acute oral dose causing migratory orientation impairment in white-crowned sparrows (*Zonotrichia leucophrys*), respectively. Values above the shorter horizontal bars are p-values resulting from Welch's two-sample *t*-tests comparing samples from control and treatment sites at each distance.

## APPENDIX A.

Acute contact toxicity (lethal does [LD<sub>50</sub>] values) of chlorpyrifos for honey bees (*Apis mellifera*) and acute oral LD<sub>50</sub> of chlorpyrifos for bird species commonly found in Minnesota's farmland landscape. The acute contact or oral LD<sub>50</sub> value is a common measure of acute toxicity and represents the lethal dose that causes death in 50% of treated animals from a single or limited exposure. The acute contact LD<sub>50</sub> for honey bees is reported in Ostiguy et al. 2019. Exposure to chlorpyrifos in contaminated pollen and nectar of adult honey bees is also representative of non-*Apis* bee species (e.g., bumblebees; Cutler et al. 2014, U.S. Environmental Protection Agency et al. 2014). Acute oral LD<sub>50</sub> (mg/kg) values and acute oral toxicity classifications are reported in Solomon et al. 2001. Acute oral LD<sub>50</sub> (ppb) values were calculated by dividing the acute oral LD<sub>50</sub> (mg/kg) by the proportion of body mass eaten as food per day (Solomon et al. 2001) and multiplying by 1,000 (Equation 4, Food Animal Residue Avoidance & Depletion Program 2016).

Species	Scientific name	LD <sub>50</sub> (mg/kg)	LD <sub>50</sub> (ppb)	Overall toxicity
Pollinator species				
Honey bee	<i>Apis mellifera</i>	59-360 ng/bee	100	Highly toxic
Avian species				
Sandhill crane	<i>Grus canadensis</i>	60	250,000	Highly toxic
Canada goose	<i>Branta canadensis</i>	60	600,000	Highly toxic
Mallard	<i>Anas platyrhynchos</i>	68	680,000	Moderately toxic
Ring-necked pheasant	<i>Phasianus colchicus</i>	12.2	122,000	Very highly toxic
Northern bobwhite	<i>Colinus virginianus</i>	32	106,667	Highly toxic
Common grackle	<i>Quiscalus quiscula</i>	8.5	28,333	Very highly toxic
Red-winged blackbird	<i>Agelaius phoeniceus</i>	13.2	44,000	Highly toxic
American robin <sup>a</sup>	<i>Turdus migratorius</i>	unknown	unknown	unknown
House sparrow	<i>Passer domesticus</i>	29.5	98,333	Highly toxic

<sup>a</sup>American robins are the most frequently reported avian species killed in field incidents; however, the LD<sub>50</sub> values are unknown (Christensen et al. 2009).



## EVALUATION OF GPS-SIZED EXPANDABLE RADIOCOLLARS DESIGNED FOR WHITE-TAILED DEER FAWNS

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### SUMMARY OF FINDINGS

During 2018-2020, we tested fit and function of 5 Global Positioning System (GPS)-sized expandable radiocollar mock-up designs on white-tailed deer (*Odocoileus virginianus*) fawns. We fitted 46 captive newborn fawns with ear tags and collars (20 Vectronic Vertex v1.0, 3 Telonics TGW v1.0, 3 Telonics Recon v1.0, 10 Vectronic Vertex v2.0, 10 Telonics TGW v2.0) and ear-tagged 15 captive control fawns without collars. We collected neck measurements from fawns at birth and at approximately 6, 9, and 12 months of age. Additionally, we conducted observations of fawns to evaluate the potential effects of collars on behavior. Vectronic collars overall accommodated the neck growth of fawns during the first 12 months of life. Telonics collars expanded prematurely resulting in loose collars, which failed (shed or removed) by  $85.5 \pm 41.1$  (mean  $\pm$  SD) days. During the first year of testing, notable effects of collars on fawn behavior included high-stepping with forelimbs during locomotion, erratic jumping behavior, and several instances of forelimbs getting caught in ill-fitting collars. Each of these atypical behaviors were most prevalent in younger collared fawns, from newborn to approximately 4 weeks of age. High-stepping was observed in fawns wearing both brands of collars, however, this behavior occurred most frequently in young fawns fitted with Vectronic collars. Collared fawns spent about 45% less time vigilant than uncollared fawns during the first 4 weeks of life. Additionally, fawns wearing Vectronic collars spent about 25% less time vigilant than fawns wearing Telonics collars. We did not observe any other significant differences in behavior (e.g., sleeping, suckling, grooming, etc.) between collared and uncollared fawns during the first 4 weeks of life. Our results suggest that the GPS-sized expandable collars tested in this study would benefit from modification before deployment in the field. We recommend modifications to each design to address collar retention issues and behavioral concerns, such as an improved stitching pattern, alternative thread and elastic materials that facilitate a more gradual elastic expansion, decreased battery housing size and weight, and improved weight distribution of the electronic components.

### INTRODUCTION

Knowledge of population parameters (e.g., sex ratio, age structure, survival, recruitment) informs decision-making for management of white-tailed deer (*Odocoileus virginianus*) populations (Jacobson et al. 1997, Keyser et al. 2005). Survival of neonatal white-tailed deer is one of the most important factors influencing population growth (Gallard et al. 2003, Chitwood et al. 2015). However, estimating survival of fawns to recruitment is logistically challenging using

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current very high frequency (VHF) collar technology (Moen et al. 1996, Rodgers et al. 1996, Bowman et al. 2000, Pusateri-Burroughs et al. 2006, Severud et al. 2015). Accurate estimation of survival requires capturing and collaring fawns soon after birth and intensively monitoring them for the first few months of life when most mortalities occur (e.g., predation, starvation, disease; Pusateri-Burroughs et al. 2006). The ideal design of radiocollars should ensure the welfare of the animal, minimize impacts on behavior and maximize collar retention (Grovenburg et al. 2014). Expandable radiocollars are designed to accommodate rapid neck growth throughout the first 12 months of life by stretching, opening at folds, deteriorating, and finally dropping from the animal (Smith et al. 1998, Grovenburg et al. 2014).

Multiple field studies have reported premature loss or failure of expandable radiocollars for neonatal deer (Vreeland et al. 2004, Pusateri-Burroughs et al. 2006, Rohm et al. 2007, Hiller et al. 2008, Grovenburg et al. 2014, Obermoller et al. 2018). Ultimately, premature loss of collars reduces the sample size and power of inference. Additional modifications to expandable collar designs have been proposed to improve retention and facilitate a more gradual collar expansion (Diefenbach et al. 2003, Cherry et al. 2014, Grovenburg et al. 2014, Obermoller et al. 2018). Some of these modifications have been deployed in the field (Diefenbach et al. 2003, Bowman et al. 2014, Cherry et al. 2014, Grovenburg et al. 2014).

Integration of GPS technology with expandable collar designs allows researchers to more efficiently and effectively investigate survival and movements of neonatal white-tailed deer (Bowman et al. 2000, McCance and Baydack 2017). The primary factor limiting use of GPS technology is the battery size and weight required to support GPS transmitters (McCance and Baydack 2017). Testing of GPS-sized expandable collars in controlled settings is warranted before extensive deployment in field studies.

Aside from small experimental deployments, GPS-sized expandable radiocollar designs have not been fitted to white-tailed deer fawns and there is currently no literature on the use of GPS technology deployed on white-tailed deer fawns. Expandable GPS collars have been deployed only within the last decade on other neonatal ungulates in the wild (moose [*Alces alces*], Severud et al. 2015, Obermoller et al. 2018; fallow deer [*Dama dama*], Kjellander et al. 2012) or in captivity (domestic horse [*Equus caballus*], Hampson et al. 2010). Utilizing animals in a captive facility allows researchers to evaluate the efficacy of GPS-sized collars over time and observe collar fit and function, the overall health and well-being of animals, and the impact GPS-sized units may have on the behavior of fawns. Also, collars that become overly restrictive on captive fawns may be safely removed. Because most prior studies have deployed GPS-sized collars on species which give birth to considerably larger young than white-tailed deer (Hampson et al. 2010, Kjellander et al. 2012, Severud et al. 2015), it is logical to assume that the relatively larger offspring would better support the weight of a GPS collar. Therefore, testing of GPS-sized collars on white-tailed deer fawns in a controlled setting is warranted to ensure animal welfare for the duration of collar deployments.

## **OBJECTIVES**

1. Evaluate the efficacy of GPS-sized expandable radiocollars designed for white-tailed deer fawns.
2. Determine the effects of GPS-sized radiocollars on the behavior of white-tailed deer fawns.
3. Provide collar companies recommendations to improve GPS-sized expandable radiocollar designs.

## METHODS

### Study Site

We conducted our study at the Whitehall Deer Research Facility on the University of Georgia campus in Athens, GA. Captive deer were held in 1-2-acre outdoor paddocks, each containing 12-14 adult does and their fawns. We provided all deer with pelleted feed (AntlerMax Breeder Textured 17-6, Purina Animal Nutrition LLC, Arden Hills, MN, USA), hay, and water *ad libitum*. All methods were approved by the University of Georgia Institutional Animal Care and Use Committee under Animal Use Proposal A2018 03-019-Y2-A0.

### Animal Capture and Handling

We captured 61 total fawns from May to July in 2018 and 2019. We searched paddocks for newborn fawns twice daily. We captured, handled, and released each fawn within the first 24 hours after birth. We collected morphometric measurements of fawns (i.e., total body length, chest girth, hindfoot length; and neck circumference at upper, middle, and lower neck), affixed individually identifying ear tags in both ears (Allflex USA Inc., College Station, TX, USA), and fitted 46 fawns with GPS-sized expandable radio-collars. During 2018, we fitted 20 fawns with Vectronic Vertex v1.0 collars (Vectronic Aerospace GmbH, Berlin, Germany), 3 fawns with Telonics TGW v1.0 collars (Telonics, Inc., Mesa, AZ, USA), and 3 fawns with Telonics Recon v1.0 collars (Telonics, Inc., Mesa, AZ, USA). We left 5 fawns uncollared to serve as experimental controls for our behavioral assessments. During 2019, we fitted 10 fawns with Vectronic Vertex v2.0 collars and 10 fawns with Telonics TGW v2.0 collars, and we left 10 fawns uncollared. After handling, we immediately returned fawns to the outdoor paddocks to be reunited with their mothers until weaned.

### Collar Fit and Function

We conducted assessments of collar fit and function 3-5 times per week on each collared fawn throughout the first 12 months of life. We remotely observed fawns in outdoor paddocks, recorded scores of collar fit and body condition, and examined each fawn's neck for signs of hair loss or lesions (Table 1). Additionally, we examined the expandable folds of each collar, recording the date at which each fold opened. We calculated collar retention by recording the date at which collars failed, dropped, or required removal to ensure animal welfare. We manually restrained fawns at approximately 6, 9, and 12 months of age to inspect the integrity of collars, evaluate the condition of fawns, and collect neck circumference measurements.

#### *Vectronic Vertex*

Vectronic Vertex v1.0 (Figure 1) collars weighed approximately 138 g, with the battery, VHF transmitter and GPS transmitter located within a single large housing at the front of the collar (dimensions = 6.2 cm x 3.9 cm x 4.4 cm). The housing was attached to the collar using high-performance glue and 2 plastic cable ties. The antenna was coated with a thin protective layer of plastic and measured 26.5 cm with 20.5 cm of its full length exposed. The neck band was 4-cm wide, composed of nylon and rubber materials with an initial circumference of 22.3 cm. The neck band included 6 expansion folds (3 sections of 2 folds each), which were 2-cm long. The section of folds furthest from the housing had a single straight stitch running through the middle of its folds, the middle section had 2 parallel straight stitches (2.4 cm apart) running through its folds, and the section closest to the housing had 2 parallel straight stitches (3.1 cm apart) through its folds. Fully expanded, the circumference of the neck band was approximately 34 cm, not including stretch of the elastic band material. The measurements of Vectronic Vertex v2.0 (Figure 2) were identical to the Vertex v2.0, except the battery housing of the updated design was slightly larger in size (dimensions = 6.5 cm x 4.2 cm x 4.4 cm). Additionally, the Vertex v2.0 (153 g) was 15 g heavier than the Vertex v1.0.

### *Telonics TGW and Recon*

Telonics TGW v1.0 (Figure 3) and Telonics Recon v1.0 (Figure 4) collars weighed about 140 g and 150 g, respectively. The primary differences between the TGW v1.0 and the Recon v1.0 designs were the battery housing material and the distribution of electronics. The Telonics TGW v1.0 battery, VHF transmitter and GPS transmitter were located in 3 housings: a polymeric housing contained the battery (dimensions = 5.5 cm x 2.9 cm x 3.3 cm) and 2 plastic housings contained the VHF (dimensions = 1.8 cm x 0.5 cm x 1.8 cm) and GPS (dimensions = 2.8 cm x 0.9 cm x 2.8 cm) transmitters. The Telonics Recon v1.0 battery, VHF and GPS transmitters were distributed between only 2 housings: an aluminum housing contained both the battery and the VHF transmitter (dimensions = 4.3 cm x 2.6 cm x 3.5 cm) and a plastic housing contained the GPS transmitter (dimensions = 2.8 cm x 0.9 cm x 2.8 cm). The battery housings of both the TGW v1.0 and the Recon v1.0 designs were attached to the collar using 4 screws and glue. The antenna of each Telonics model measured 26.8 cm with 20.9 cm of its full length exposed. The collar bands of each Telonics models were 3.8-cm wide and composed of cotton and rubber (i.e., elastic portion) sewn to a 3.7-cm wide strip of static polymer. The length of the static polymer material for each model was 15 cm and initial length of the elastic portion of each was 7.9 cm. Therefore, the initial band circumference (i.e., pre-expansion) of each Telonics model was 22.9 cm. The bands of both designs included 6 expansion folds (3 sections of 2 folds each), which were 2 cm long. The section of folds closest to the housing had a single straight stitch running through the middle of its folds, the middle section had 2 parallel straight stitches (1 cm apart) through its folds, and the section furthest from the housing had 4 parallel straight stitches (0.5 cm apart) through its folds. Fully expanded, the circumference of the band was 34.9 cm not including stretch of the elastic band material. The measurements for the Telonics TGW 2.0 (Figure 5) were identical to the TGW v1.0, except the updated model utilized 3 protruding expansion folds designed to allow more initial stretching. Additionally, the TGW v2.0 used nylon thread stitched perpendicular to the long axis, whereas the v1.0 collars used cotton thread stitched parallel to the long axis. The TGW v2.0 (136 g) was 4 g lighter than the TGW v1.0.

### **Fawn Behavior**

We conducted focal observation sessions of each fawn to evaluate effects of collars on their behavior during the first 12 months of life. During 2018-2019 the frequency of our observations decreased with fawn age:  $\leq 30$  days of age we obtained  $\geq 1$  morning and  $\geq 1$  evening session every week for each fawn; during 30-60 days of age we conducted 1 morning session per week; during 60-200 days of age we conducted 1 morning session every other week; and during 200-365 days of age we observed each fawn during 1 morning focal session every 4 weeks. We conducted focal sessions from 4.5-m stationary observation platforms within paddocks during crepuscular sampling periods: in the morning from 06:00 to 10:00 or in the evening from 17:00 to 21:00. Before conducting the first focal session of a sampling period, the observer sat quietly for 15 minutes to minimize impacts of human activity on the behavior of deer in the paddocks. During a focal session we recorded body orientation and behavior of the focal fawn each minute for 30 minutes. We recorded the overall body position, neck position, head position, and head tilt of the fawn based on a pre-determined scoring system (Figure 6). We coded all behaviors (e.g., vigilant, sleeping, foraging, suckling) in a preconstructed ethogram (Table 2). If the focal fawn moved out of sight for a period  $> 5$  minutes, we terminated the focal session and censored the data. We recorded a running tally of fawn and mother vocalizations, and tallied instances where fawns exhibited attention to collars (e.g. scratching at collar). We also monitored fawn-mother proximity using a laser rangefinder and a compass. Every 5 minutes, the observer recorded a distance (m) and compass azimuth for the fawn and mother to calculate proximity (Euclidean distance). We averaged all fawn-mother distances to obtain a mean fawn-doe

proximity for each session. If the mother of the focal fawn was not visible, the observer did not record this information during the session. During 2019-2020, we conducted focal sessions 3 times per week on each fawn. During a focal session, we located each fawn and recorded a single score for body orientation and a single score for behavior. To date, we have conducted a series of two-sample t-tests in program R (R Core Team, 2019) to compare the proportion of time collared vs. uncollared fawns spent exhibiting various behaviors. In future analyses, we will use a bootstrapping technique to further analyze behavioral data and meet assumptions of normality, and then conduct an Analysis of Variance (ANOVA) with repeated measures to compare the proportion of time collared vs. uncollared fawns spent exhibiting specific behaviors with age. We will also use an ANOVA with repeated measures to compare behaviors between collar models (e.g., Telonics vs. Vectronic, TGW v1.0 vs. Recon v1.0, etc.)

## RESULTS

During the first year of testing (2018-2019), Vectronic Vertex v1.0 collars accommodated neck growth well. As the necks of fawns grew larger (Table 3), collars initially became tight (but not restrictive) around the lower neck. This added pressure applied to the expandable materials of collars would cause 1-2 expansion folds to open, increasing the band circumference of the collar. The initial expansion resulted in some hair loss from the neck as loosened collars moved more freely along necks of fawns. This likely caused some minor discomfort; however fawns quickly grew into the expansion. We observed no effects of Vectronic collars on the body condition of fawns. Of the 20 collars deployed, 9 (45%) were retained for >365 days. No Vectronic collars exhibited premature expansion at a level that resulted in collar failure. Three (15%) Vectronic collars dropped from fawns in the outdoor paddocks at <365 days, but none were due to compromised stitching or elastic material. In 1 case, a fawn shed its collar at 256 days of age while being moved through the barn for researchers to collect neck measurements. In 2 other cases, fawns shed their collars at 261 and 270 days of age due to a large tear in the expandable material of the collars, likely caused by collars catching on fencing in outdoor paddocks. Of the 20 Vectronic collars deployed, 8 (40%) collars were retained on fawns until mortality before 365 days. Two of 8 mortalities occurred while moving deer in our captive facility, an inherent risk when handling wild animals. The other 6 fawns died from unknown causes, with 4 dying  $\leq 14$  days of life and 2 dying >100 days of life. Based on necropsy results and our own mortality assessments, these mortalities were not directly related to the collars. However, we could not discount the possibility that collars had some indirect influence on these mortalities.

During the second year of testing, we found the modified Vectronic Vertex v2.0 collars performed similarly to the Vectronic models tested the previous year. Of the 10 collars deployed, 1 collar shed prematurely in the outdoor paddocks at 109 days. This collar was loose on the fawn's neck and easily slipped over the head of the animal. The collar prematurely expanded, but the elastic material of the collar was not yet fully compromised. While moving fawns at the facility, we witnessed 1 collar drop from a fawn after snagging and tearing (at the expandable material near the housing) on a perimeter fence. The other 8 collars are still deployed on fawns at the captive facility and are sufficiently accommodating neck growth to date ( $\leq 312$  days).

During the first year of testing (2018-2019), the collar folds of all 6 Telonics v1.0 mock-ups expanded prematurely by  $75.8 \pm 27.9$  days. The ill-fitting collars caused significant hair loss on the necks of all 6 fawns but did not appear to impact their overall body condition. Fully-expanded Telonics mock-ups were a source of apparent discomfort as collars moved freely along the necks of fawns. Once the stitching on the folds was compromised and the elastic material began to degrade, all 6 fawns were able to step through collars with their forelimbs.

This displacement resulted in collars positioned around the chest or waist of fawns. One of the collars dropped 20 days after the last fold expanded at approximately 80 days of age. For another individual, the fully expanded collar was removed from a fawn's waist when it fell ill at 105 days. In the case of the other 4 fawns with Telonics mock-ups, fully-expanded collars were removed from the chest or waist of fawns at approximately 6 months of age when fawns were manually restrained to collect neck measurements. Overall, Telonics v1.0 collars (N = 6) failed (dropped or removed) by  $101.3 \pm 45.5$  (mean  $\pm$  SD) days.

During the second year of testing (2019-2020), we found that the modified Telonics TGW v2.0 collars failed even sooner than Telonics v1.0 collars from the first year. Of the 10 collars deployed, 1 shed after only 5 days because the initial band circumference of the collar was too large and the collar slipped over the animal's head. Similarly, collars expanded prematurely, resulting in ill-fitting collars and hair loss. Four collars ended up around the chest or waist of fawns after the animals stepped through prematurely expanded collars. We found 7 collars were shed prematurely by  $47 \pm 25.4$  (mean  $\pm$  SD) days. We removed 2 collars from the waist at 122 and 126 days. Lastly, we found 1 fawn suffered a leg injury and was subsequently euthanized at 17 days of age. This collar was loose on the animal and no folds were open when the collar was removed. Overall, the Telonics v2.0 collars (N = 10) failed (dropped or removed) by  $69.7 \pm 36.7$  (mean  $\pm$  SD) days.

We collected >200 hours of behavioral observations during the first year of testing. Notable effects of collars on fawn behavior included high-stepping with forelimbs during locomotion, erratic jumping behavior and several instances of forelimbs getting caught in ill-fitting collars. Each of these atypical behaviors were most prevalent in younger collared fawns, from newborn to approximately 4 weeks of age. High-stepping during locomotion can occur to some degree in newborn fawns without collars, however, this behavior was more exaggerated in GPS-collared fawns and persisted for several weeks versus several days for uncollared newborn fawns. We did not observe any uncollared fawns exhibiting high-stepping behavior during behavioral focal sessions. We observed high-stepping in fawns wearing both types of collars, but this behavior occurred most frequently in young fawns fitted with Vectronic Vertex collars. During the first year, 2 (33.3%) Telonics v1.0 collared fawns exhibited high-stepping behavior for a total of 10 occurrences. For these fawns, high-stepping behavior was not observed after an average of 7 days. We observed 12 (60%) individuals with Vectronic v1.0 collars exhibiting high-stepping behavior for a total of 71 occurrences. High-stepping behavior was not observed after an average of 18 days. The oldest collared fawn (Vectronic) to exhibit high-stepping behavior was 39 days old; however, all other fawns exhibited this behavior at  $\leq 4$  weeks of life. We observed several instances of fawns, fitted with both Telonics and Vectronic collars, getting their forelimbs caught in loose-fitting collars during the first year of observations. In these cases, a fawn's leg would remain restrained in the collar for 1-6 minutes. We observed 3 instances of young fawns (<2 weeks old), fitted with both Telonics v1.0 (2 occurrences) and Vectronic v1.0 collars (1 occurrence), getting their forelimbs caught in loose-fitting collars (pre-expansion). We found collared fawns spent ~45% less time vigilant than uncollared fawns during the first 4 weeks of life ( $p < 0.01$ ) (Figure 7). Additionally, fawns wearing Vectronic collars spent ~25% less time vigilant than fawns wearing Telonics collars ( $p < 0.01$ ) (Figure 8). We did not observe any other differences in behavior (e.g., sleeping, vigilance, suckling, grooming, etc.) between collared and uncollared fawns during the first 4 weeks of life ( $p > 0.05$ ). We have conducted approximately 150 behavioral observations per fawn during the second year of testing, and we are currently in preliminary stages of analyzing these data.

## DISCUSSION

Based on our preliminary results, we developed several recommendations for Telonics, Inc. and Vectronic Aerospace GmbH to improve their GPS-sized expandable radiocollars for neonatal white-tailed deer. Currently, we cannot recommend collar designs tested in our study for use in field studies. However, with modifications and further testing in controlled settings, researchers may have access to viable GPS fawn collar options in the foreseeable future.

We recommended Vectronic decrease the initial band circumference of their Vertex v1.0 collar, improve weight distribution, and reduce size and weight of the battery housing in order to minimize effects on behavior. Poor weight distribution, paired with an initial collar band circumference larger than the newborn fawn's necks caused the battery housing to swing freely as fawns moved. The high-stepping behavior was caused by the fawns' attempt to step around the freely moving collar to minimize contact with their forelimbs. The concern with this behavior is that a young collared fawn exhibiting high-stepping during locomotion may be more susceptible to predation than a normal fawn at that same age. If GPS-collared fawns die at a higher rate than uncollared fawns, then the results of fawn survival studies using these collars could be severely biased. We believed the erratic jumping behavior was from discomfort with the bulky and loose-fitting collars. Decreasing the initial band circumference may alleviate some behavioral issues and reduce the chance of fawns getting a forelimb caught in a loose-fitting collar. Weight of the Vectronic Vertex collar was focused at the front where a single large housing comprised all of the electronics. We believe that distributing electronics more evenly around the collar, perhaps similar to the Telonics models, would reduce the ill-effects of collars on fawn behavior. After testing the subsequent Vectronic version (Vertex v2.0), we maintain our original recommendations to improve the fit and potentially minimize impacts of collars on fawn behavior.

We recommended a slightly smaller initial band circumference for the Telonics TGW v1.0 and Recon v1.0 collars to accommodate the smaller necks of newborn fawns (Table 3). A more appropriately fitting collar may minimize issues with high-stepping and decrease the probability of fawns getting a forelimb caught in a loose-fitting collar. The primary issue with the Telonics collar was the expandable material intended to accommodate rapid growth of fawns during the first year of life. Material and stitching pattern caused collars to expand and deteriorate at an accelerated rate. Exposure to environmental elements (e.g., sunlight, temperature, humidity, precipitation) likely played a role in the rapid expansion and degradation of collar materials. We recommended incorporating an improved stitching pattern and more durable thread and elastic material to increase collar retention and promote a more gradual elastic expansion. Ideally, Telonics would use materials similar to those on the expandable band of the Vectronic Vertex collars. We recommended use of the polymeric-style housing (TGW) rather than the aluminum housing (Recon) because of lighter weight. When designing collars intended for newborn fawns, it is important to minimize weight wherever possible. Therefore, we recommended Telonics decrease collar weight to improve fit, reduce pressure on expandable materials and prevent premature expansion. After testing of the next Telonics version (TGW v2.0) during the second year of our study, we maintained our original recommendations to improve collar fit and retention. We recommended use of a fold design more similar to that of the Telonics TGW v1.0, but stitched using a more durable thread material like the nylon of the TGW v2.0. A durable elastic material more similar to that of the Vectronic collars would greatly benefit the Telonics design.

The VHF technology of fawn collars currently used in field studies limits the abilities of researchers to efficiently estimate fawn survival, recruitment, movements and habitat use. Enhancing our understanding of these factors would improve management of white-tailed deer

populations (Gingery et al. 2018, Gulsby et al. 2015). Integrating GPS technology with expandable collar designs would provide researchers with more accurate information regarding the behavior of white-tailed deer (Bowman et al. 2000, McCance and Baydack 2017). With the primary limiting factor being the size and weight of batteries required to support GPS transmitters, we believe that further testing of GPS-sized collars in controlled settings is warranted to resolve these issues before extensive deployment in field studies. The results of this study will provide important information to telemetry technology companies seeking to improve collar performance and produce less invasive collar designs.

## **ACKNOWLEDGMENTS**

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Table 1. System used for scoring collar fit and body condition of white-tailed deer (*Odocoileus virginianus*) fawns at Whitehall Deer Research Facility in Athens, GA, USA, during 2018-2020 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.

Collar fit score	Body condition score	Neck hair loss score	Neck lesions score
1 = Very loose	1 = Emaciated	0 = No hair loss	0 = No lesions
2 = Little loose	2 = Thin	1 = Coat thinning	1 = Single lesion ≤1cm
3 = Good fit	3 = Prime	2 = Single bald patch ≤1cm	2 = Multiple lesions ≤1cm
4 = Little tight	4 = Heavy	3 = Multiple bald patch(es) ≤1cm	3 = Single lesion >1cm
5 = Very tight	5 = Obese	4 = Bald patch(es) >1cm	4 = Multiple lesions >1cm

Table 2. Ethogram used for recording behavior of white-tailed deer (*Odocoileus virginianus*) fawns during focal sessions at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2018-2020 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.

Behavior	Code	Definition
Locomotion	L	Focal animal is moving forward (e.g., walking, running, jumping)
Foraging	F	Focal animal is eating or drinking (not suckling)
Suckling	S	Focal animal is actively suckling at adult doe
Grooming	GG	Focal animal is grooming another individual
Groomed	GD	Focal animal is being groomed by another individual
Grooming Self	GS	Focal animal is grooming itself
Urogenital Grooming	UG	Focal animal is being groomed by another at the urogenital region
Vigilant	V	Focal animal has eyes open and appears to be alert
Sleeping	SL	Focal animal has eyes closed and appears to be asleep
Undefined	U	Focal animal is exhibiting an undefined behavior
Out of Site	OS	Focal animal has moved out of sight

Table 3. Mean neck measurements with standard deviation collected from white-tailed deer (*Odocoileus virginianus*) fawns at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2018-2020 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.

Fawns measured	Age (months)	Mean upper neck (cm)	Mean middle neck (cm)	Mean lower neck (cm)
96	0	16.5 ± 1.5	16.7 ± 1.6	18.7 ± 1.8
50	6	25.5 ± 2.4	26.1 ± 2.7	30.9 ± 3.6
39	9	29.4 ± 3.1	30.6 ± 3.1	36.8 ± 4.3
18	12	31.4 ± 2.7	31.0 ± 2.6	38.6 ± 4.2



Figure 1. Vectronic Vertex v1.0 collar deployed on white-tailed deer (*Odocoileus virginianus*) fawns at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2018-2019 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.



Figure 2. Vectronic Vertex v2.0 collar deployed on white-tailed deer (*Odocoileus virginianus*) fawns at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2019-2020 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.

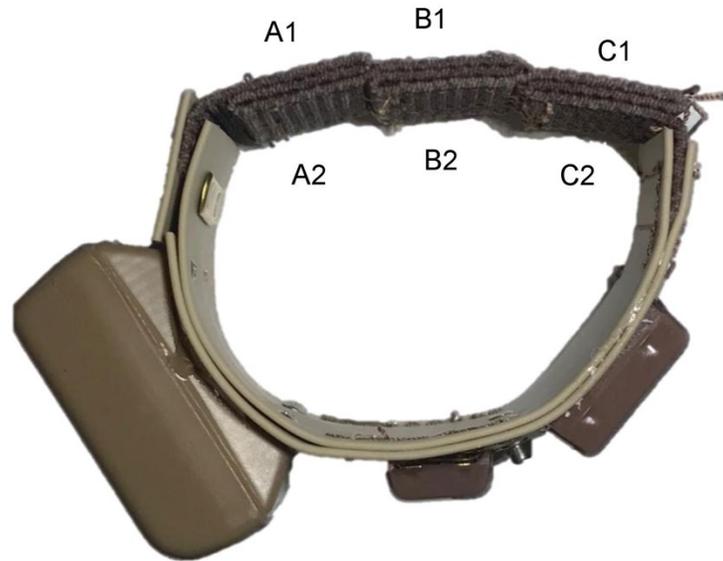


Figure 3. Telonics TGW v1.0 collar deployed on white-tailed deer (*Odocoileus virginianus*) fawns at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2018-2019 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.



Figure 4. Telonics Recon v1.0 collar deployed on white-tailed deer (*Odocoileus virginianus*) fawns at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2018-2019 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.



Figure 5. Telonics TGW v2.0 collar deployed on white-tailed deer (*Odocoileus virginianus*) fawns at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2019-2020 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.

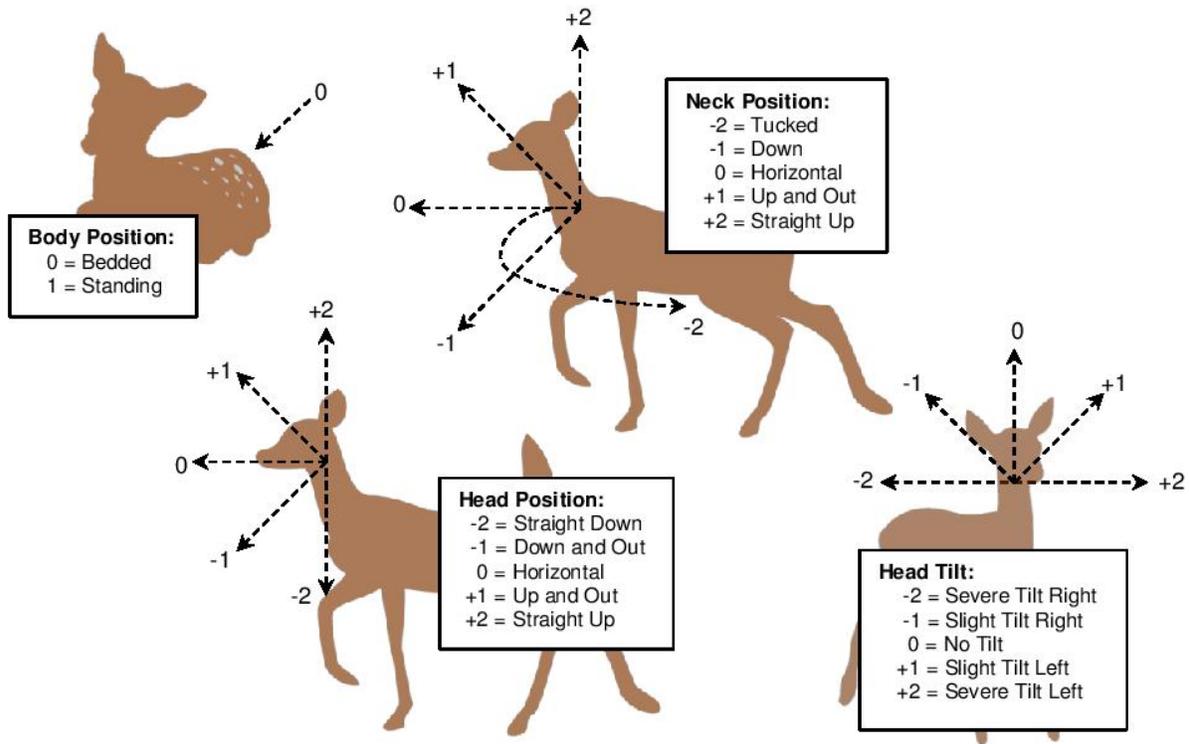


Figure 6. System for scoring body orientation during focal observation sessions of white-tailed deer (*Odocoileus virginianus*) fawns at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2018-2020 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars.

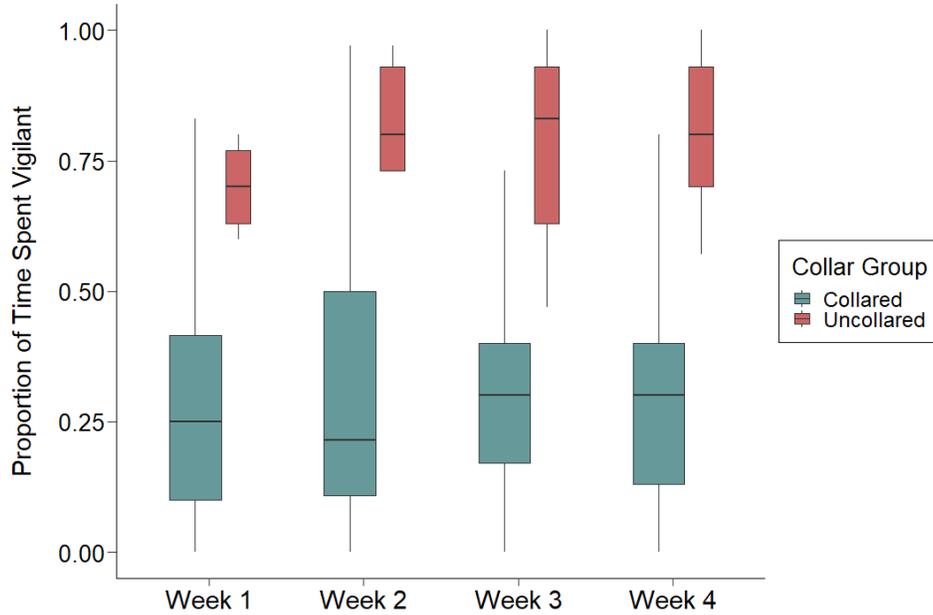


Figure 7. Proportion of time white-tailed deer (*Odocoileus virginianus*) fawns spent exhibiting vigilance during the first four weeks of life at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2018-2019 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars. The width of boxes represent differences in sample size (i.e., the wider the box, the larger the sample size).

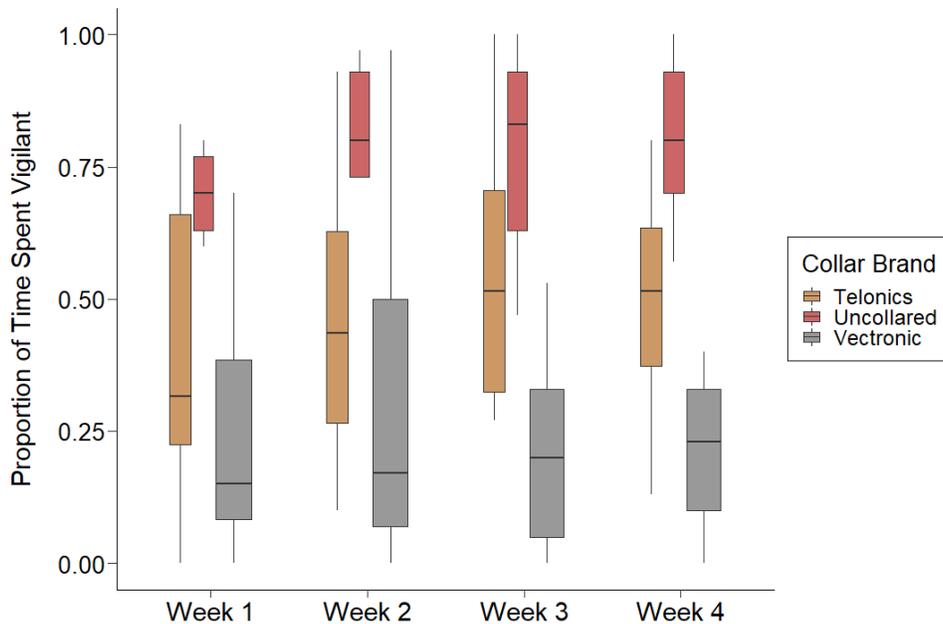


Figure 8. Proportion of time white-tailed deer (*Odocoileus virginianus*) fawns spent exhibiting vigilance during the first four weeks of life at Whitehall Deer Research Facility in Athens, Georgia, USA, during 2018-2019 for testing of experimental designs of Global Positioning System (GPS)-sized expandable radiocollars. The width of boxes represent differences in sample size (i.e., the wider the box, the larger the sample size).



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

Nicole M. Davros, Lindsey N. Messinger, and Timothy P. Lyons

### **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, individual chicks, and broods. In 2016 and 2017, we also collected data on brood-rearing habitat selection within grasslands. Overall, nest, chick, and brood survival rates were high due to the large amounts of grassland cover in the landscape. Preliminary analyses showed that the best-supported models included study area for nest survival and a constant survival model for chick survival to 21 days. The best-supported model for brood survival to 5 weeks included year and vegetation type (cool season versus forb-rich/warm-season native grass mixes). Brood survival was highest in 2015 and lowest in 2018; broods hatched in sites dominated by forb-rich, warm-season native grass mixes had higher survival than broods in cool-season sites. Based on these preliminary results, we recommend that managers continue to focus on acquiring and restoring habitat in large grassland complexes. They should also prioritize forb-rich seed mixes with native, warm-season grasses over cool-season grass mixes.

### **INTRODUCTION**

Ring-necked pheasant (*Phasianus colchicus*) 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.

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 and brood survival are vital components of pheasant population dynamics but they remain 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  $\geq 1$  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, Lyons et al. 2020). Using brood survival estimates can be unreliable because brood mixing can occur (Meyers et al. 1988; N. Davros, personal observations). Further, lack of data on individual chicks (e.g., body condition, cause of death) prevents us from understanding the role of different factors (e.g., exposure, food limitation, predation) that lead to variation in recruitment. Evidence that predation is the leading cause of chick mortality for grassland gamebirds in North America is well-established (e.g., Riley et al. 1998, Schole et al. 2011). Food availability has been implicated as an important factor explaining chick survival for many gamebird species in Europe (Green 1984, Hill 1985, Potts 2012); however, strong evidence that food is a major limiting factor for survival of chicks in North America is still lacking. Moreover, food availability and rates of predation likely interact in relation to vegetation structure and composition and confound conclusions from chick survival and food resource studies (Hill 1985). Finally, death from exposure has been shown to decrease chick survival rates, especially after periods with increased precipitation when chicks are still very young and unable to fully thermoregulate (Riley et al. 1998, Schole et al. 2011). Risk of exposure and starvation may interact to decrease chick survival, but few studies have been able to directly address this question (but see Riley et al. 1998). Therefore, additional data are needed to understand the interplay between these potential limiting factors on brood habitat selection and chick survival in different grasslands 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 habitat selection and survival in relation to prairie reconstruction and 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, reconstruction seed mixes) 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 was to evaluate the relative importance of within-patch diversity (e.g., grassland parcels dominated by cool-season grasses, warm-season grasses, and high diversity grass-forb mixtures) within MNDNR-managed Wildlife Management Area (WMA) project areas on pheasant productivity. Specifically, we:

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

## **STUDY AREA**

We conducted our study in the southwest region of Minnesota, a core region of the state's pheasant range. Topography ranged from flat to gently rolling. This region was intensively farmed, and corn and soybeans combined accounted for approximately 75% of the landscape (U.S. Department of Agriculture 2013a, U.S. Department of Agriculture 2013b). Grasslands, including those on private land (CRP, Reinvest in Minnesota [RIM], Conservation Reserve Enhancement Program [CREP], and Wetlands Reserve Program [WRP]) and public land (WMAs and U.S. Fish & Wildlife Service [USFWS] Waterfowl Production Areas [WPA]) accounted for 6.3% of the landscape in this region (Davros 2016).

For our study sites, we selected 2 WMA project areas representative of 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) (Figure 1). Each study site was about 5,760 acres in size and contained extensive amounts of grasslands and wetlands embedded in an agricultural matrix. The Lamberton WMA study site (Redwood County) was a large, nearly contiguous WMA complex with >1,100 acres of permanently protected upland and wetland habitats. The Worthington Wells study site (Nobles County) had >1,500 acres of permanently protected habitat that spanned multiple WMAs, the Okabena-Ocheda Watershed District, and USFWS lands.

## **METHODS**

### **Data Collection**

We conducted our research during the 2015-2018 breeding seasons. Our 2015 pilot season allowed us to refine methods and protocols for the study's expansion during 2016-2018. However, we also had a smaller field crew in 2018 who were time-limited due to a concurrent research project; thus, we were unable to collect some data in 2018 and we note this below, where applicable.

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). We weighed each hen to the nearest 5.0 g, measured her right tarsus to the nearest 0.5 mm, and placed a uniquely numbered aluminum leg band on her right leg. We then fitted her with a 16.0-g necklace-style very high frequency (VHF) radiotransmitter with integrated mortality switch (Advanced Telemetry Systems (ATS), Isanti, MN) before releasing her 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  $\leq 5$  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.

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). In 2015-2017 only, 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 weighed 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 we returned all captured chicks 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 monitored hens and their broods via triangulation through 5 weeks post-hatching. We triangulated hens and their broods 2-3 times daily  $\geq 3$  times per week. Each bearing-coordinate pair was taken  $\geq 100$  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. To estimate brood survival status and size, we used the homing technique to flush hens just before sunrise to detect and count chicks; however, our effort varied among years. During 2015, we flushed broods 2-3 times per week through 5 weeks. During 2016 and 2017, we flushed broods twice between days 10-14 and twice between days 28-32.

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 days of hatching for successful nests. For nests that failed, we also collected vegetation data at the nest site  $\leq 7$  days after the estimated hatch date. At each nest site, we visually estimated percent cover (Daubenmire 1959) of the upper canopy (i.e., grasses, forbs, standing dead vegetation, woody vegetation) using a 0.5 m<sup>2</sup> 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 percent cover of the ground layer (i.e., litter, bare ground, rocks/other) 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 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 vertical vegetation density 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 within grasslands during 2016-2017 only, we collected vegetation data at 5 brood locations estimated via triangulation and 10 random points outside of but within 400 m of each brood's biweekly home range. We generated biweekly home ranges twice for each brood: one home range for the first 2 weeks of age and a second home range for age 3-4 weeks. For broods not surviving a 2-week observation period, we generated home ranges and sampled vegetation if at least 1 chick from the brood survived for the first 7 days of the observation period. 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 also used GIS to generate the random points for sampling within 400 m of the biweekly home range. We restricted the selection of random points so that they were within grassland cover because our primary objective was to evaluate brood selection within this cover type. Any estimated brood locations or generated random points falling in habitat types other than grassland (e.g., row crop, wetlands, woodlots) were not sampled. We considered road right-of-ways in the grasslands category and included them in sampling efforts. We collected vegetation data within 7 days of the end of each biweekly interval. At each estimated brood point, we sampled 1 center point and 3 equidistant points (10 m north, 10 m southwest, and 10 m southeast) to capture the spatial variation associated with a brood location (i.e., hens and their broods were mobile and thus distributed around a point). We estimated percent upper canopy cover, ground layer 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 more than 50% of our brood triangulations were in habitat types other than grassland, 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.

We also qualitatively assessed vegetation within each WMA parcel (i.e., field-scale vegetation classification) and assigned it to one of the following categories based on predominant plant species present: cool-season grasses, warm-season native grasses, and forb-rich/warm-season native grass mixture (Figure 1).

## Data Analyses

To date, we have conducted preliminary analyses on hen, nest, brood, and chick survival. We also calculated basic descriptive statistics ( $\bar{x} \pm SE$ ) for nest sites and brood locations. 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. Final analyses will be incorporated into manuscripts submitted for peer-reviewed publication.

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. 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, some 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 individuals with mortality or censor events occurring outside of the analysis period from this particular analysis.

We used the logistic exposure method (Shaffer 2004) to estimate daily survival rates of nests by specifying a custom link function within R (version 3.6). We used the AICcmodavg package (Mazerolle 2019) to calculate AIC<sub>c</sub> scores, rank models, and calculate model weights and model-averaged estimates of daily survival.

We first examined univariate models that included terms related to annual and seasonal variation in daily survival rates. We considered models that included terms for different survival among years and models that included effects of month, week of the nesting season, or linear and quadratic terms for week of the nesting season. As no temporal covariates ranked better than the null (intercept-only model, constant survival), we next examined support for models with nest-site vegetation measurements only. We compared models that contained terms for visual obstruction reading (VOR), the coefficient of variation of VOR (a measure of heterogeneity in vegetation density; Bowman 1980), the additive effects of ground cover (bare ground, litter cover, litter depth), the additive effects of canopy cover (forbs, standing dead, and grass), and the total number of forb and grass species found at the nest site.

Only the model containing the effect of study area ranked higher than the null model. Because all other models that ranked lower had similar likelihoods, we inferred that these models included uninformative parameters and excluded them from the model-averaging procedure (Arnold 2010). Thus, we recalculated model weights and generated model-averaged estimates of daily survival rate from the study area and null model only. We extrapolated a 35-d survival probability (10-12 days laying, 23-25 days incubation) from daily survival rate estimates and generated 85% confidence intervals using the delta method (Powell 2007).

We used a robust-design occupancy model within program MARK (version 9.0; White and Burnham 1990) to estimate weekly survival of broods ( $\geq 1$  chick alive) from flush encounters while also accounting for imperfect detection.

We parameterized the model to estimate extinction (brood mortality), colonization, and detection, treating Psi (the probability a brood is alive) as a latent parameter. We fixed colonization to 0 to prevent zombie pheasants and only included covariates on the detection process ( $p$ ) and extinction (mortality). We fixed Psi during the first time period to 1 to ensure that all broods started out alive. We only considered two different models for detection probability, a constant detection probability among primary periods and one where we allowed detection to vary among each week post-hatch. We identified the best detection model by holding mortality constant and finding the minimum AIC<sub>c</sub>.

After identifying the best model for detection, we compared different models for brood mortality probability. Models included the effect of year, different mortality probabilities among weeks, differing mortality during the first and second week post-hatch but similar mortality among weeks 3-5, precipitation during the first 10 days post-hatch, and study area. We also examined support for field-scale vegetation composition, as well as the additive effects of year and field-scale vegetation composition, the additive effect of precipitation and field-scale vegetation, and the interaction between week-specific mortality and field-scale vegetation composition. The additive effects of year and field-scale vegetation composition were the top-ranked model and we based our inference on only this model.

We again used the logistic exposure method to estimate daily survival of radio-marked chicks and the AICcmodavg package (Mazerolle 2019) to calculate AIC<sub>c</sub> scores, rank models, and calculate model weights and model-averaged estimates of daily survival. We constructed models by first examining support for a random effect term to account for non-independent survival of marked chicks from the same brood. We then proceeded to examine support for fixed effects as single covariates. We first tested for temporal covariates, including terms for chick age (days post-hatch), year, and hatch date. We then tested whether chick mass at capture and vegetation cover type at the field-scale affected survival. The top-ranked model included the random effect of brood and vegetation at the field-scale and we computed 21-day survival estimates from the top-ranked model only.

## RESULTS AND DISCUSSION

We radiocollared 164 hen pheasants during the 6 trapping periods across our 2 study sites from spring 2015 through fall 2017. We used baited walk-in traps during spring 2015 and spring 2016 only as they were not a productive capture technique. In 2016, we set cameras at the traps and found that pheasants were not motivated to use the bait when winter conditions were mild. Only 3 hens were captured using the walk-in traps (2% of total hen captures) during those 2 seasons whereas 161 hens (98%) were captured by spotlighting across all trapping seasons. 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  $\geq 6$  inches deep and temperatures reach  $\leq 0^\circ$  F.

From 2015-2018, cumulative survival for hens during the breeding season (183-day period pooled across years) across study areas was 0.73 (95% CI: 0.66-0.81;  $n=133$ ; Figure 2). During the 4 breeding seasons, 59% of marked individuals ( $n=79$ ) suffered a known mortality event. Of these mortality events, 86% were attributed to predation events, 5% 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 are likely inappropriate given our dataset. Because nearly 32% of individuals were censored during this analysis due to unknown fates (in particular, slipped radiocollars), our subsequent survival analyses may use methods that include expert knowledge to incorporate uncertainty in fate to refine survival estimates (Walsh et al. 2018).

We monitored 132 potential nesting attempts overall but excluded some nests from analyses for various reasons (e.g., hen abandoned after initial flushing event, nest area flooded prior to researcher's visit, actual nest bowl was never found). Fifty-three percent of nests hatched ( $n=70$ ), 42% failed ( $n=56$ ), and 5% had unknown fates ( $n=6$ ).

We found that hens selected nest sites with more standing dead cover but did not select nest sites with more forb cover ( $n=107$  nests; Figure 3). 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; Table 1) whereas the percent cover of forbs was slightly lower at nest sites than random points ( $16.4 \pm 2.10\%$  vs.  $20.8 \pm 2.40\%$ , respectively; Table 1, Figure 4a).

We used 101 nests in our preliminary nest survival analyses, which provided 909 observation intervals and 1,625 exposure days for analysis (Shaffer 2004). Our best-supported model of nest survival included study site (Table 2). Nest survival was high overall but was greater at Worthington Wells ( $\bar{x} = 0.984$ , 85% CI: 0.976-0.990) than Lamberton ( $\bar{x} = 0.973$ ; 85% CI: 0.961-0.981). Extrapolating to a 35-day exposure period (laying and incubation), nest survival probability was 0.585 (85% CI = 0.438-0.719) at Worthington Wells and 0.318 (85% CI = 0.264-0.514) at Lamberton (Figure 5). Our nest success rates are comparable to the rates 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.

Although our models that included canopy cover, composition, and richness metrics were not competitive (Table 2), we note that 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 3, Figure 4b). Our preliminary canopy cover model included all canopy cover metrics (i.e., grasses, forbs, standing dead, woody), and we did not build models that incorporated cover, composition, and/or richness metrics together. Whereas hens may select nest sites for certain characteristics such as standing dead vegetation because it provides important visual concealment from predators early in the growing season, predator search efficiency and success may rely on other vegetative characteristics. Indeed, 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). Our future analyses may include a forb-only model and models that combine vegetation structure and composition metrics. Exploring these additional models may help inform management approaches that can create more productive nesting habitat.

Given annual differences in weather and the long nesting cycle and extended breeding season length of pheasants, we hypothesized that year and time of season would be important covariates in explaining patterns of nest survival. However, none of our models that included these covariates were competitive. Time-specific patterns of nest survival have been documented in several duck and passerine species (Grant et al. 2005, Grant and Shaffer 2012).

We monitored 59 broods for survival during the 4 years of our study. We documented at least 1 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). Using our field-scale classification of vegetation, our best-supported brood survival models included year and vegetation. Broods had lower survival in 2018 (Figure 6). When we averaged across years, broods associated with forb-rich and/or warm-season grass dominated fields had higher survival than broods in cool-season dominated fields (Figure 7). Our results are consistent with other studies that have shown that pheasant hens with broods selected areas with more forb-rich vegetation which, when managed frequently to reduce litter, provides the best option for mobility and food resources for pheasant chicks (Doxon and Carroll 2010, Matthews et al. 2012).

During 2016 and 2017 only, we collected vegetation data related to brood habitat selection for 40 broods. Our preliminary descriptive analyses suggest brood-rearing and nearby random locations were similar in vegetative structure and composition (Table 4). Brood and random

locations were composed primarily of litter at the ground level ( $80.2 \pm 3.05\%$  vs.  $82.9 \pm 2.40\%$ , respectively; Table 4). Grasses were the predominant canopy cover at brood and random locations ( $55.3 \pm 2.89\%$  vs.  $52.1 \pm 2.32\%$ , respectively) followed by forb cover ( $15.7 \pm 1.57\%$  vs.  $17.0 \pm 1.34\%$ , respectively). Our future analyses will incorporate this finer-scale vegetation data to evaluate brood survival.

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 7 chicks between day 12-15 and replaced their 0.65 g transmitters with sutured, 1.1-g or 1.3 g backpack-style transmitters ( $n=6$ ; ATS, Isanti, MN) or another 0.65 g transmitter ( $n=1$ ). 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.

Our best supported model of chick survival included vegetation at the field-scale (Table 2). Unlike brood survival, individual chicks survived at a higher rate on fields comprised of primarily cool-season exotic grasses (21-day survival = 0.880, 85% CI: 0.649-0.967) than fields dominated by warm-season native grasses or forb-rich warm-season grass mixes (21-day survival = 0.551; 85% CI: 0.378-0.714). One explanation for this relationship could be that chicks, especially younger ones, may have lower mobility in native stands of vegetation that have not been recently disturbed by fire, grazing, or mowing to reduce litter cover (e.g., Lyons 2017). Still, the mechanisms that influence individual chick survival may differ from those that affect survival of the brood in aggregate, and may just be another example of the importance of scale in ecology (Levin 1992, Hernández 2020). Similar to our hen survival analyses, our future chick survival analyses may use methods that incorporate expert knowledge about uncertain fates to refine estimates (Walsh et al. 2018).

## MANAGEMENT IMPLICATIONS

Based on our preliminary results to date, we recommend that managers continue to prioritize land acquisition and restoration efforts in larger grassland/wetland complexes such as those outlined in the Minnesota Prairie Plan and Pheasant Action Plan. Within grassland parcels, they should focus on reconstructions that use forb-rich seed mixes to benefit brood survival. Final results from this study are pending and will relate survival rates to nesting and brood-rearing habitat selection within prairie reconstructions. Ultimately, the data gathered will help managers better understand factors that may limit pheasant productivity so that they can prioritize their within-field management activities in an era of reduced grassland habitat acres on the landscape.

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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 ( $\leq 15$  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) <sup>a</sup>	5.2	0.21	5.3	0.22

<sup>a</sup>Vertical density is the average visual obstruction reading (VOR) as determined by using a Robel pole.

Table 2. Number of parameters (K), Akaike's Information Criterion (calculated for small sample sizes;  $AIC_c$ ) difference from  $AIC_c$  of the best-supported model ( $\Delta AIC_c$ ), model likelihood, and model weight for models explaining ring-necked pheasant nest survival and chick survival in Wildlife Management Areas (WMAs) in southwest Minnesota during 2015-2018. Also shown is the model-averaged weight for the top two models for nest survival.

Model	K	$AIC_c$	$\Delta AIC_c$	Model Likelihood	Model Weight	Model-averaged Weight
Nest survival						
Study area	2	290.47	0.00	1.00	0.53	0.79
Intercept-only	1	293.10	2.63	0.27	0.14	0.21
Chick survival						
Vegetation type <sup>a</sup>	3	152.68	0.00	1.00	0.41	
Brood effect	2	153.83	1.15	0.56	0.23	
Chick age	3	153.93	1.25	0.54	0.22	

<sup>a</sup>Based on qualitative assessment of the dominant vegetation type at the field-scale: cool-season exotic grasses, warm-season native grasses, or a forb-rich warm-season grass mix.

Table 3. 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 (n=52)		Depredated nests (n=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) <sup>a</sup>	5.4	0.26	4.5	0.60

<sup>a</sup>Vertical density is the average visual obstruction reading (VOR) as determined by using a Robel pole.

Table 4. Descriptive statistics for vegetation surveys at locations used by ring-necked pheasant broods and nearby paired random locations<sup>a</sup> in southwest Minnesota during the 2016 and 2017 breeding seasons. Vegetation data were collected biweekly up to the first 4 weeks of brood rearing and was constrained to grassland habitats [e.g., Wildlife Management Areas (WMA), roadsides, Conservation Reserve Program (CRP) fields] only.

	Brood locations (n=48)		Random locations (n=52)	
	Mean	SE	Mean	SE
% Ground cover				
Litter	80.2	3.05	82.9	2.40
Bare ground	14.6	2.91	12.3	2.27
% Canopy cover				
Grasses	55.3	2.89	52.1	2.32
Forbs	15.7	1.57	17.0	1.34
Standing dead	6.7	0.59	7.4	0.53
Woody	0.2	0.09	0.2	0.07
Other	1.9	0.76	0.3	0.10
Species richness				
Grasses	2.2	0.10	2.3	0.08
Forbs	2.0	0.17	2.0	0.16
Maximum height (cm)				
Live vegetation	61.0	6.04	57.1	5.72
Dead vegetation	36.0	4.06	36.7	3.94
Litter depth (cm)	3.6	0.26	4.1	0.21
Vertical density (dm) <sup>b</sup>	5.5	0.24	5.7	0.19

<sup>a</sup>Paired random locations were outside of but within 400 m of a brood's biweekly home range [determined by the minimum convex polygon (MCP)].

<sup>b</sup>Vertical density is the average visual obstruction reading (VOR) as determined by using a Robel pole.

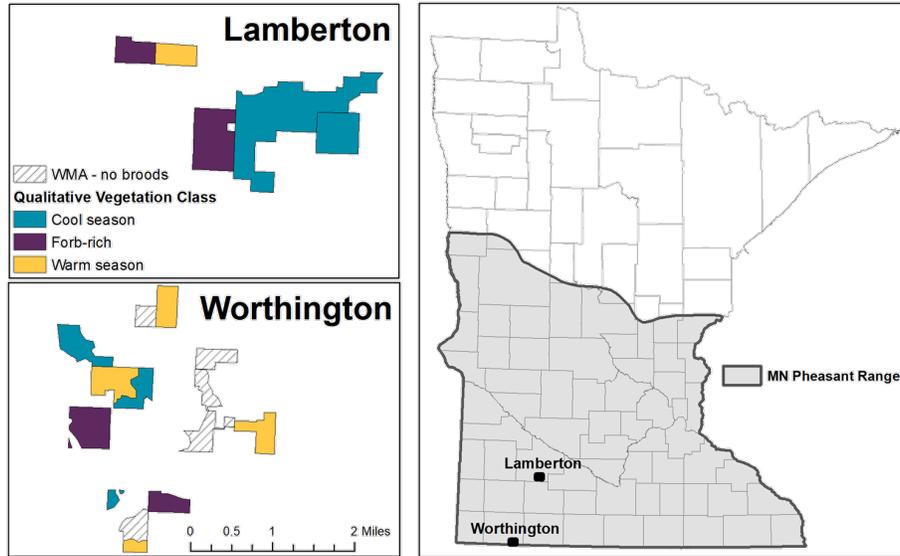


Figure 1. Location of 2 Wildlife Management Area (WMA) grassland/wetland complexes in southwest Minnesota used as study sites to evaluate ring-necked pheasant habitat selection and survival from 2015-2018. Maps on the left show individual WMA parcels that comprise the Lamberton and Worthington Wells complexes; white space depicts the agricultural matrix in which these complexes were embedded. Parcels were categorized as cool-season grasses (blue), forb-rich/warm-season native grass mixes (purple), or warm-season native grasses (yellow) based on qualitative assessment of predominant plant species present in each parcel.

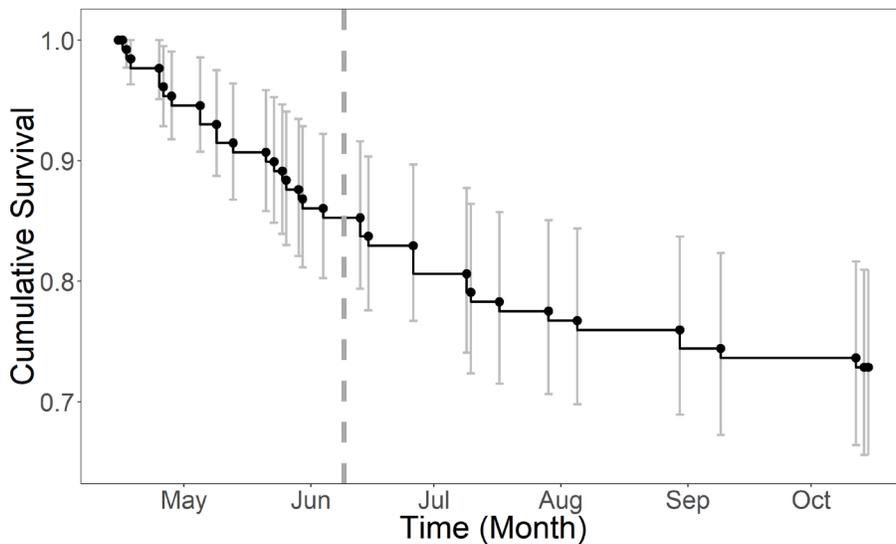


Figure 2. Cumulative survival of radiocollared ring-necked pheasant hens during the 2015-2018 breeding seasons (15 April – 15 October) in southwest Minnesota. Points represent survival estimates at intervals where mortality events took place. Error bars (vertical solid gray lines) represent the upper and lower 95% confidence interval for each survival estimate. The vertical gray dashed line shows the 10-year average (2007-2016) for peak hatch (June 12) of pheasant nests in Minnesota, as estimated by Minnesota Department of Natural Resources' annual August roadside count surveys.

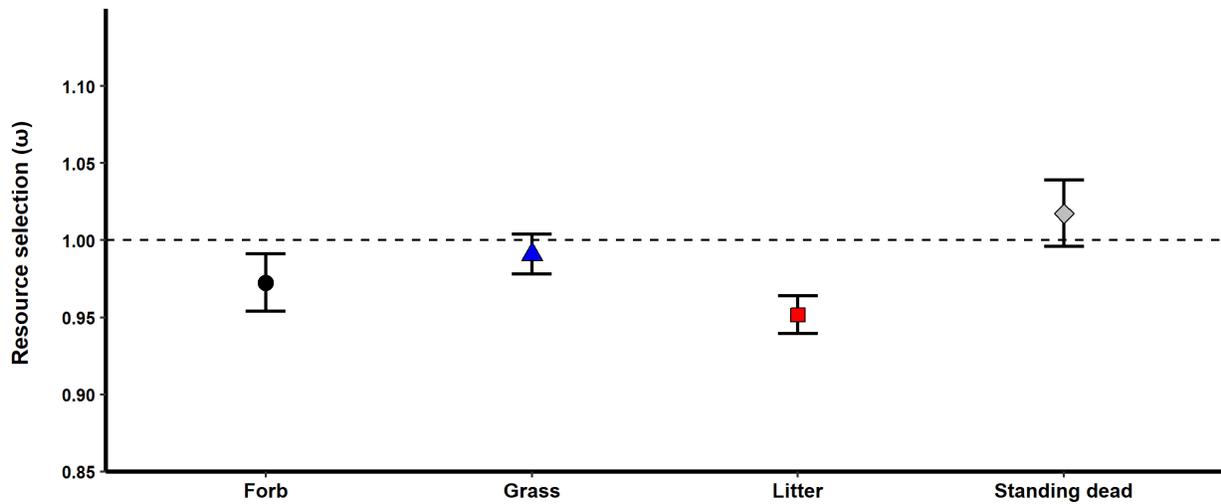


Figure 3. Resource selection of ring-necked pheasant hens for nest site characteristics including percent cover of forbs, grasses, litter, and standing dead vegetation at nests in southwest Minnesota, 2015-2017. Values falling above the dashed line at 1.00 indicate selection for a particular canopy cover metric whereas values below the dashed line indicate selection against that metric.

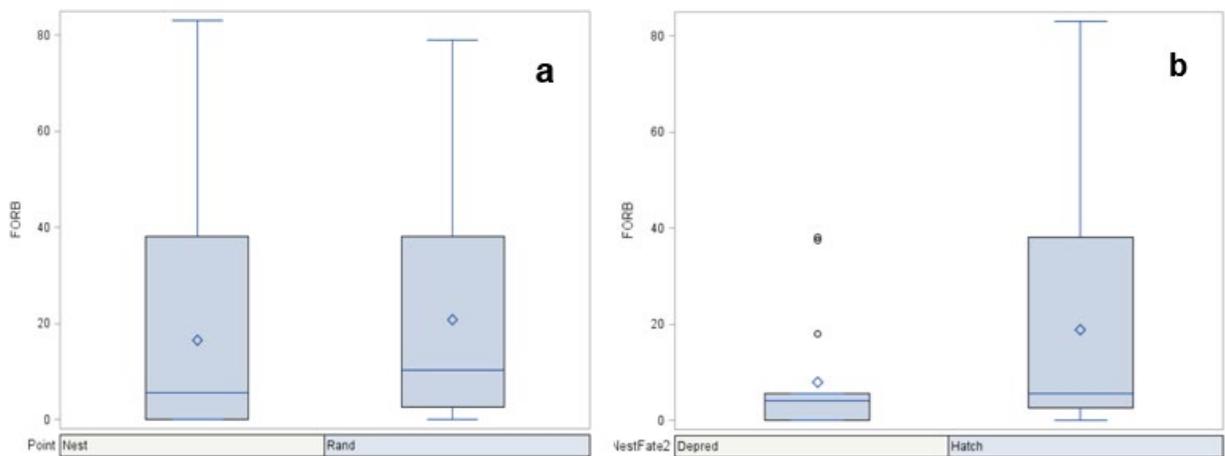


Figure 4. Box plot comparisons of estimated percent cover of forbs at (a) nest sites versus nearby random points (<15 m away) and (b) depredated versus hatched nests of ring-necked pheasants in southwest Minnesota, 2015-2017.

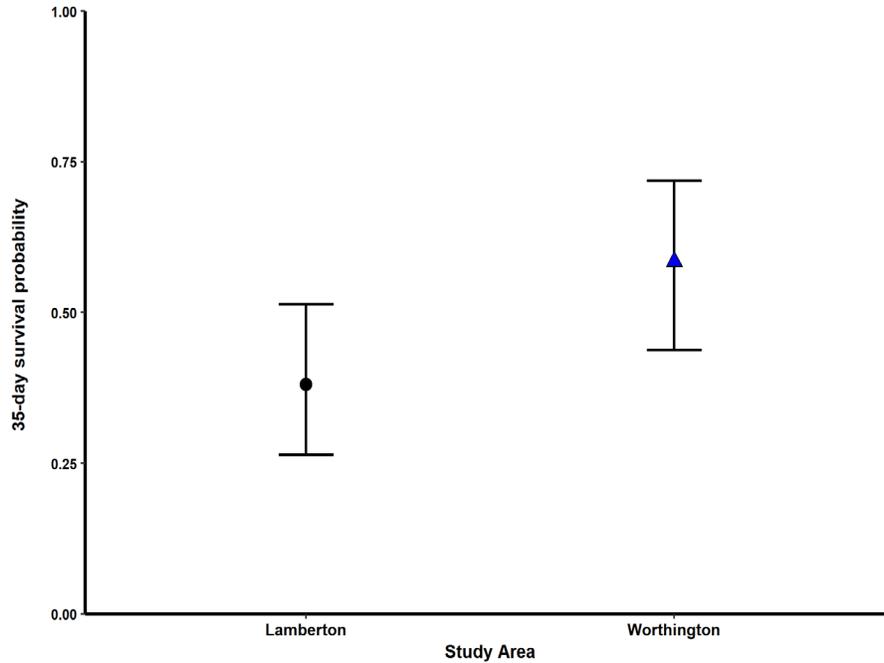


Figure 5. Nest survival probability for a 35-day exposure period (laying and incubation stages) for ring-necked pheasants in the Lamberton (black circle) and Worthington Wells (blue triangle) project areas in southwest Minnesota during 2015-2018 nesting seasons.

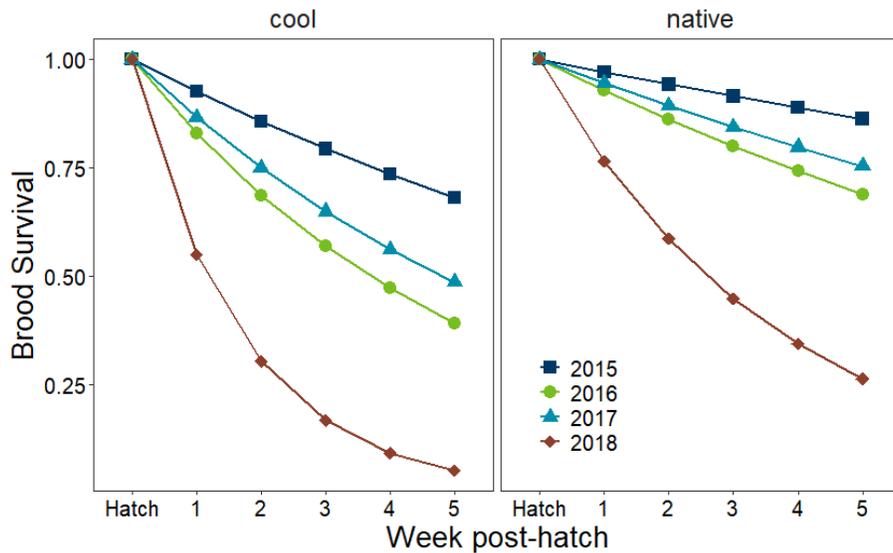


Figure 6. Survival of ring-necked pheasant broods to 5 weeks of age by year (2015-2018) and within-parcel vegetation type in southwest Minnesota. The figure on the left shows brood survival by year in Wildlife Management Area (WMA) parcels dominated by cool-season grasses. The figure on the right shows brood survival by year in WMA parcels dominated by warm-season native grasses or forb-rich mixes.

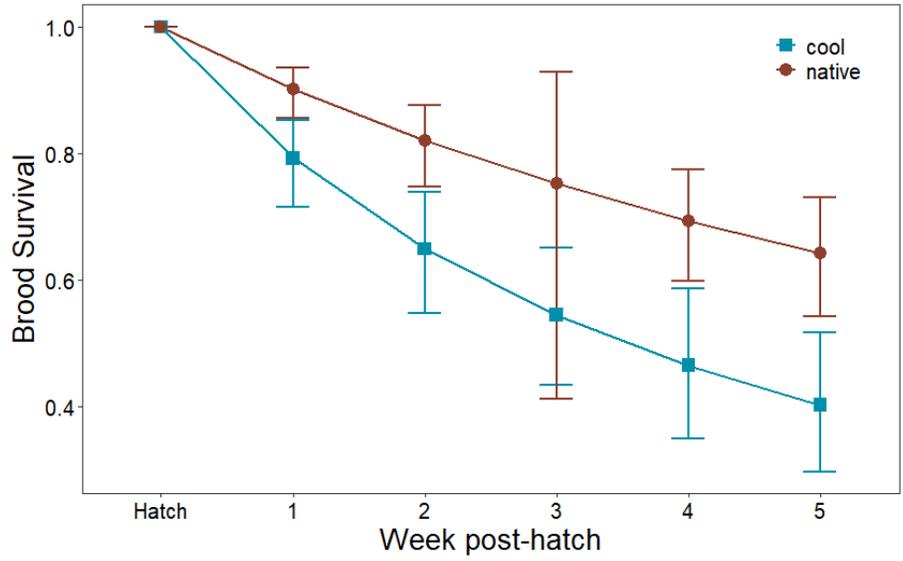


Figure 7. Survival of ring-necked pheasant broods to 5 weeks of age by within-parcel vegetation type (blue squares: Wildlife Management Area [WMA] parcels dominated by cool-season grasses; red circles: WMA parcels dominated by warm-season native grasses or forb-rich mixes); 85% confidence intervals are shown. Broods were monitored during 2015-2018 in southwest Minnesota.



## ROADSIDE DISTANCE-SAMPLING SURVEYS OF WHITE-TAILED DEER IN SOUTHERN MINNESOTA (2018-2019)

John Giudice, Brian Haroldson, Tyler Obermoller, and Eric Michel

### SUMMARY OF FINDINGS

This project was designed to evaluate the feasibility of using roadside distance-sampling surveys to generate a reliable and cost-effective population monitoring metric for white-tailed deer (*Odocoileus virginianus*) in Minnesota's farmland zone. Here we report on results from the 2018 and 2019 surveys. Our study area included 4 deer permit areas (DPAs = 252, 253, 296, and 299) in southern Minnesota's farmland zone. We used a geographic information system (GIS) to classify land-cover polygons into high and low strata based upon expected deer density. As part of this exercise, we evaluated 2 buffer sizes (250 and 500 m) around potential deer cover (woodland cover, permanent to semi-permanent grasslands, and wetland cover) to delineate high-density polygons. We then overlaid the study area with a hexagonal grid (size = 36.1 mi<sup>2</sup>), which served as our primary sampling unit (PSU). We randomly selected a spatially balanced sample of 15 PSUs and used a GIS to identify all secondary roads within each PSU. We then classified each road segment based on their juxtaposition to deer-density polygons (low, high). Finally, we randomly selected road segments (secondary sampling units) using an equal allocation of effort by stratum, which generated ~200 survey miles per stratum. We surveyed each PSU 2–4 times/year in spring and based the start of the survey season on anecdotal information on spring dispersal of deer. We began surveys approximately 1 hour after sunset and we surveyed 1–2 PSUs per night. We conducted surveys with 2-member crews using hand-held infrared sensors. For each deer group ( $\geq 1$  animal) detected, the survey team recorded perpendicular sighting distance, group size, and covariate information. In 2018, we collected survey data using the 250-m buffer design and used a post-hoc simulation to evaluate the 500-m buffer design. In 2019, we collected survey data using both the existing 250-m design plus a new 500-m design. Within the latter, we generated separate secondary sampling units (road segments) and conducted independent surveys within a subset of 10 PSUs. The approach used in 2019 allowed us to disentangle the effects of buffer size and secondary-sample allocation, which is important for guiding future design considerations. The best-supported distance-sampling models for the 250-m buffer design generated similar estimates of mean deer density in 2018 (8.2 deer/mi<sup>2</sup>; 95% CI = 5.8–11.7) and 2019 (8.0 deer/mi<sup>2</sup>; 95% CI = 5.7–11.3). Likewise, the distance model based on the 500-m buffer design generated a similar estimate of mean deer density in 2019 (8.5 deer/mi<sup>2</sup>; 95% CI = 5.5–13.2). These density estimates were slightly higher than the 2018-19 and 2019-20 winter aerial-survey estimates (6.5 deer/mi<sup>2</sup>, 95% CI = 4.9–8.1; 6.5 deer/mi<sup>2</sup>, 95% CI = 5.1–7.9, respectively), which is consistent with findings that distance-sampling estimates tend to be positively biased. However, from a management perspective, the point estimates were reasonably similar. Furthermore, annual variation in distance-sampling estimates was small (albeit we only have 2 years of data) and most (>80%) of the variation in replicate counts was due to among-PSU differences rather than day-to-day variation in the observation process. Finally, the poor precision of our distance-sampling estimates is worth noting, but we could address this through

design modifications. The simplest modification would be to increase the sample of PSUs from 15 to 25, but only conduct a single survey/year, which should produce, on average, estimates with precision similar to our aerial surveys (i.e.,  $CV \approx 13\%$ ). Design choices related to the buffer size and secondary-sample allocation are more complicated, but similar point estimates and precision would likely be obtained with 1) a 250-m buffer and 50:50 sample allocation (low:high) or 2) a 500-m buffer with 35:65 allocation. Another year of data collection with consistent protocols in the same study area will be helpful for evaluating the ultimate question of whether a distance-sampling metric can be effectively and reliably used to help monitor white-tailed deer populations in Minnesota's farmland zone.

## **INTRODUCTION**

White-tailed deer (*Odocoileus virginianus*) hunting-season recommendations should use the most reliable information available to determine the status of the deer population relative to goal. In Minnesota, estimates of deer abundance and trends are used to inform annual deer season-setting recommendations for each deer permit area (DPA). The primary source of information used by the Minnesota Department of Natural Resources (MNDNR) to inform decision-making is a harvest-based population model. Currently, the MNDNR collects annual data on winter severity, hunter-reported harvest, and hunter effort (license sales) at the DPA scale. Reliability of harvest-based models can be improved by incorporating annual information on spatial and temporal variation in vital rates and other model parameters. However, collection of such data is generally cost-prohibitive, especially at the DPA scale.

An alternative approach would be to collect independent recurrent information on population abundance or trends, which could be used to calibrate the population model. For example, the MNDNR has used winter aerial surveys to calibrate harvest model estimates. However, financial, logistical, and environmental (e.g., snow cover, conifer cover) constraints prevent recurrent use of aerial surveys for all DPAs. Moreover, comparisons involving aerial surveys may not be reliable in DPAs where seasonal migration is suspected to violate closure assumptions (e.g., when comparing winter surveys to harvest-based population models). Thus, alternative, cost-effective, large-scale monitoring methods are needed. One potential approach in the farmland zone is road-based distance-sampling surveys.

Road-based surveys (e.g., spotlight, thermal imaging) are commonly used by managers for deer population monitoring (McCullough 1982, Mitchell 1986, Focardi et al. 2001, Collier et al. 2007, DeYoung 2011, Kaminski et al. 2019). Unfortunately, the counting process can be highly variable in roadside surveys, possibly as a function of variation in deer distribution and resource use, which has limited the reliability of roadside indices. Applying distance-sampling methods (Buckland et al. 1993, 2004) to road-based surveys might provide a means to calibrate the counting process and make annual comparisons more reliable. However, some important statistical issues remain (Anderson et al. 1979, Burnham et al. 1980, Marques et al. 2010, McShea et al. 2011). For example, convenience sampling violates the assumption that transects are randomly placed (or that animals are randomly located with respect to transects), which can make it difficult to obtain unbiased estimates of abundance via distance-sampling theory. However, if that bias is relatively small and constant, then road-based distance-sampling surveys may still provide a reasonable index for population monitoring, calibrating the existing MNDNR population model, or as part of an integrated population model (IPM).

## **OBJECTIVE**

Our objective was to evaluate the feasibility of using roadside distance-sampling surveys to generate a reliable (potentially biased but reasonably precise and repeatable) and cost-effective population monitoring metric for white-tailed deer in Minnesota's farmland zone.

## **METHODS**

### **Study Area and Sampling Design**

#### *2018 (250-m buffer surveys)*

The 7,218-km<sup>2</sup> study area consisted of 4 DPAs (252, 253, 296, and 299) in southern Minnesota (Figure 1A). We used a geographic information system (GIS; ArcGIS v. 10.4, Environmental Systems Research Institute, Inc., Redlands, CA) to stratify land-cover within the sampling frame into high and low strata based upon expected deer density. We defined high-density polygons as being within a 250-m buffer of woodland, grassland (permanent to semi-permanent, excluding pasture), and wetland cover classes. Low-density polygons were the remaining areas (e.g., agricultural land, open water, and urban/developed areas). Data sources for deer-density polygons included Minnesota Land Cover Classification and Impervious Surface Area by Landsat and Lidar: 2013 update – Version 2 (woodlands), a compilation of public/private grassland layers (e.g., Waterfowl Production Areas, Wildlife Management Areas, conservation easements, etc.), and the National Wetlands Inventory for Minnesota (wetlands). We then overlaid the sampling frame with a hexagonal grid, with township-sized hexagons (size = 36.1 mi<sup>2</sup>) having >50% of their area inside the sampling frame serving as our primary sampling units (PSUs). We chose this size because it represented the approximate area that could be surveyed within a 4–6 hour period each night. We randomly selected a spatially balanced sample (Stevens and Olsen 2004) of 16 PSUs, but discarded 1 PSU that contained the city of Mankato but few rural roads. Thus, our final design contained 15 PSUs (Figure 1A). We then used a GIS to identify all secondary (e.g., county and township) roads within each PSU, defined by juxtaposition to deer-density strata (low, high). Finally, we randomly selected road segments (pooling roads >0.25 miles from all PSUs) using an equal allocation of effort by stratum (~200 miles per stratum). Thus, each PSU contained a combination of low- and high-strata road segments. We derived road data from the Roads of Minnesota, 2012 database. For the purposes of the pilot study, we were interested in obtaining sufficient observations in the low stratum to make informed decisions about the detection process and the potential to modify the stratification and allocation scheme.

#### *2019 (250- and 500-m buffer surveys)*

In 2019, we used 2 sampling frames. In addition to the existing 250-m stratification scheme described above, we also generated a 500-m stratification scheme by defining high-density polygons as being within a 500-m buffer of potential deer cover (woodland, grassland, and wetland cover classes; Figure 1B). As before, low-density polygons were the remaining areas (e.g., agricultural land, open water, and urban/developed areas). Within this new design, we retained 10 of 15 PSUs, but selected new secondary road segments. We duplicated all remaining design aspects from 2018. We added this second design to determine whether precision of the population estimate might be improved by modifying the stratification scheme.

### **Field Protocols**

We surveyed each PSU 2–4 times, with survey dates being close in time within a PSU (i.e., variation in survey dates was greater among than within PSUs). We did this because we were primarily interested in day-to-day variation in counts and wanted to separate this from variation in counts among PSUs and over the extended survey season. We based the start of the survey season on anecdotal information on spring dispersal of deer (from wintering areas to spring-summer-fall range). To be consistent among years and to match the “modeled population”, it was important that deer were on their spring-summer-fall range. We began surveys approximately 1 hour after sunset and we surveyed 1–2 PSUs per night. We conducted surveys with 2-member crews (driver and observer) using extended-cab pickup trucks. We

detected deer using FLIR Scout III (FLIR Systems, Inc., Wilsonville, OR) hand-held infrared (IR) sensors attached to the rear windows of the vehicle with window mounts. We viewed images on dual computer monitors attached to the front passenger seat using customized mounts. The vehicle's electrical system supplied power to the monitors. The observer searched for deer along both sides of the survey route within each PSU. We initially oriented sensors at 45- and 315-degree angles from the direction of travel, but we adjusted them as needed to account for visual obstruction due to variable terrain, woody cover, buildings, etc. Survey speed ranged from 8–48 km/hour depending upon vegetative cover density. When we identified a deer group ( $\geq 1$  individual), the observer directed the driver to an approximate perpendicular angle (i.e., 90 or 270 degrees) from the group to minimize sighting distance and counted group size. Then, while the observer shined the animal(s) with a spotlight, the driver measured distance and angle to the group using a laser rangefinder and digital protractor, respectively. We used a real-time, moving-map software program (DNRSurvey; Haroldson et al. 2015), coupled to a global positioning system receiver and convertible tablet computer, to guide route navigation and record survey metrics (e.g., PSU, run [survey replicate], deer and vehicle location, distance, bearing, count, cover type) to GIS shapefiles. Cover type designations included woodland, wetland, grassland, pasture, standing crop, harvested crop, other, and unknown classes. We recorded weather data (temperature, wind speed, cloud cover, precipitation) at the beginning, middle, and end of each survey route.

During the winters of 2018-19 and 2019-20, we also conducted helicopter surveys of the study area using a quadrat-based design, where quadrats were delineated by Public Land Survey (PLS) section (259 ha) boundaries. We stratified quadrats into 3 density categories (low, medium, high) using the local wildlife manager's knowledge of deer abundance and distribution. Using optimal allocation, we randomly selected a spatially balanced sample (Stevens and Olsen 2004) of 160 plots to survey. Within each plot, a pilot and 2 observers searched for deer along transects spaced 270-m intervals until they were confident all available deer were observed. To maximize sightability, we completed surveys when snow cover measured  $\geq 15$  cm and we varied survey intensity as a function of cover and deer numbers (Gasaway et al. 1986).

## **Data Analysis**

### *Data truncation*

A useful rule of thumb in distance sampling is to right truncate at least 5% of the data for robust estimation of the detection function (Buckland et al. 1993:106). The 95<sup>th</sup> percentile of our distance data was 284 m in 2018 and 327 m in 2019 (250-m and 500-m buffer surveys combined). We set the truncation distance  $w = 300$  m, which resulted in 3.7% and 6.4% of the data being truncated in 2018 and 2019, respectively. We also considered left truncation because the peak in observation distances was consistently away from the road (Figure 2). However, the peak likely reflects road avoidance rather than animal movement (e.g., due to disturbance, which is unlikely in this case because crews used IR sensors for initial detection). Thus, left-truncation methods would not resolve the underlying issue that animals are not randomly distributed with respect to the transect line. Left-truncation at some distance  $x$  from the road (e.g., 100 m), with rescaling, would improve model fit by creating the desired shoulder at distance zero. However, one would then need to generate a separate ad hoc estimate of abundance for the sampling space that is within distance  $x$  of the road transect. Thus, for this pilot-study application, it seemed prudent to set left truncation = 0 and focus on evaluating the consistency of the detection function  $g(x)$ . Although the resulting density estimate is likely biased (Stainbrook 2011, Marques et al. 2013), it may still serve as a useful monitoring index if the bias is reasonably consistent over space and time.

### *Distance-sampling models*

The half-normal and hazard-rate key functions are robust estimating functions and allow the inclusion of covariates (Buckland et al. 1993, 2004). Therefore, we focused on these 2 key functions for the initial 2018 analysis. Our base models included no adjustments or covariates. We then added a cosine adjustment to each base model. Finally, we evaluated 2 covariates (with adjustment = NULL) for detection function  $g(x)$ . The first covariate, STRATA, was used to test whether  $g(x)$  varied by deer density strata. The second covariate, COV2, was an indicator variable for tall/dense cover types (grassland, woodland, standing crop, wetland) vs short/open cover types (pasture, farmstead, harvested crop, roadsides, other). Our goal was to determine if COV2 could explain additional uncertainty in the detection function, including why  $g(x)$  might vary among strata. If COV2 could accomplish the latter, then we could pool distance data over strata to generate a more precise detection function while still generating separate density estimates for each stratum (i.e., a stratified distance-sampling estimator; Buckland et al. 1993:99–103, Miller et al. 2019). Conversely, if  $g(x)$  varied significantly by stratum, then we would need stratum-specific distance functions.

Based on what we learned in 2018, we fit all of our 2019 models using a hazard-rate function. We started by pooling the 2018 and 2019 datasets (250-m buffer surveys) and fitting some simple distance models to determine if there was evidence that  $g(x)$  varied significantly by year (annual variation), run (among-day variation), stratum, or cover type (binary indicator variable = COV2). Based on what we learned from the pooled-data analysis, we focused on fitting some simple distance models to the 2019 dataset. More specifically, we restricted our analyses to data from run 1 (similar to 2018) and used a stratified distance-sampling structure to evaluate the effect of COV2 and some new covariates that we measured in 2019 (relative topography [low, med, high], activity [lying, standing, moving], % visual obstruction [10 ordinal classes]).

### *Sources of variation*

Temporal variation is especially important in this application. If counts and resulting population estimates are highly variable over time (within and among years), then a single-effort (non-replicated) operational survey might not be reliable. Conversely, if most of the variation in counts is due to among-PSU differences, we could address this through our sampling design (e.g., by increasing PSU sample size). We used ANOVA and linear mixed-effects methods to decompose the sampling variance of raw deer counts by PSU and run to determine if run was a significant source of variation. We also compared distance-sampling density estimates by year. Harvest and population-modeling data suggested the target population was reasonably stable during the 2-yr comparison period. Thus, we expected density estimates from distance sampling to be very similar in 2018 and 2019. Large differences in density estimates would likely reflect substantial annual variation in the observation process, which would raise questions about the reliability of the method.

### *Sampling-design choices*

In 2018, we used a post-stratification analysis to examine an alternative stratification scheme based on a 500-m buffer and equal allocation of effort. However, in this application, the number of observations for estimating  $g(x)$  is fixed and sample allocation is confounded with the stratification scheme. Thus, a post-stratification analysis has limited utility for answering the primary question of interest: “which stratification scheme and allocation of effort will produce the most precise estimate?” Obtaining a reliable answer to this question requires a more sophisticated analysis that involves simulating the distribution of deer and detection distances in a computer-generated landscape (*sensu* Buckland et al. 2004:226–228). In 2019, we collected independent survey data from both a 250- and 500-m buffer design, which allowed us to construct simulated distance-sampling datasets (deer detections) drawn randomly from all

possible PSUs and road segments in the study area. Our focus was to examine the relative precision of the density estimates rather than to quantify bias because we did not know true density. That is, we only had estimates of 1) the distribution of perpendicular sighting distances, 2) mean encounter rate (deer groups per survey mile) and variance by stratum, and 3) mean group size and variance. We simulated the entire sampling and model-fitting process 500 times for both the 250- and 500-m buffer designs using  $n(\text{PSU}) = \{15, 20, 25, 30\}$  and allocation of secondary sample units (road segments) to the high stratum =  $\{0.35, 0.50, 0.65\}$ . We summarized the results graphically to illustrate how expected precision varied as a function of sampling-design choices.

## RESULTS AND DISCUSSION

### Summary Statistics

#### *2018 (250-m buffer surveys)*

We completed 48 surveys on 15 PSUs during 23 nights from 1 April to 6 May 2018. Median start time was 2058 hours (0.9 hours post-sunset) and mean survey duration was 4.1 hours. We surveyed all PSUs 3 times and we surveyed 3 PSUs 4 times. Within each PSU, we completed 3 runs within a maximum 8 days and all runs within 35 days. In total, we detected 931 deer groups (clusters) consisting of 3,194 individual deer. Of the 931 groups detected, 84% were along road segments in the high-density stratum. We observed a similar number of deer in runs 1–3 (total deer/run for all PSUs = 1,038, 1,002, and 1,082, respectively). Mean group size (observed) was 4.1 in the low-density stratum (range = 1–41, median = 3), 3.3 in the high-density stratum (range = 1–42, median = 2), and 3.4 overall. Group size was not correlated with distance ( $r = 0.025$ , 95% CI = -0.039 to 0.089), which suggests we may not need an adjustment for group-size bias in our distance-sampling estimator (a common issue in distance sampling). In the low stratum, 62% of group detections were located in harvested crop fields. Conversely, only 42% of detections were in harvested crop fields in the high stratum, with relatively more detections in grasslands (24% vs. 13%) and woodlands (12% vs. 8%). For deer groups observed along low-density road segments, the mean distance to a high-strata deer-cover polygon was 446 m (median = 345 m, range = 0–2,387 m). As expected, mean perpendicular sighting distance was greater in the low stratum (135 m; range = 0–679) compared to the high stratum (108 m; range = 0–503). Additionally, the highest density of deer detections occurred ~100 m from the road (Figure 2A). We observed a similar pattern in both strata. This could result in a biased population estimate because the mean probability of detection (the area under the detection curve) can be difficult to estimate accurately if objects are not distributed randomly with respect to transect lines, as potentially indicated by a distribution of detection distances that increases rather than decreases from the transect line. This is a common and valid criticism of convenience sampling from roadways. However, if the bias is consistent over space and time, then the distance-sampling estimator might still generate a useful long-term and large-scale monitoring metric.

#### *2019 (250-m buffer surveys)*

We completed 45 surveys on 15 PSUs during 20 nights from 2 April to 2 May 2019. Median start time was 2110 hours (1.0 hours post-sunset) and mean survey duration was 3.5 hours. Within each PSU, we completed 3 runs with 2 surveys occurring a median of 1 day apart (range = 1–4) and the third survey occurring a median of 20 days (range = 10–23) after the first survey. We detected 830 deer groups (clusters) consisting of 2,710 individual deer. Of the 830 groups detected, 83% were along road segments in the high-density stratum. The total deer count increased with each run (864, 891, 955), but mean group size was similar among runs (3.4, 3.5, 2.9) and strata (3.6 in low vs. 3.2 in high). The overall group size was 3.3 deer (range = 1–21). Group size was weakly positively correlated with distance ( $r = 0.138$ , 95% CI = 0.071 to 0.204).

In the low stratum, 63% of group detections were located in harvested crop fields. Conversely, only 37% of detections were in harvested crop fields in the high stratum, with relatively more detections in grasslands (23% vs. 7%) and woodlands (12% vs. 8%). For deer groups observed along low-density road segments, the mean distance to a high-strata deer-cover polygon was 388 m (median = 254 m, range = 0–2,666). The mean perpendicular sighting distance was greater in the low stratum (136 m; range = 1–537) compared to the high stratum (109 m; range = 0–562). Similar to 2018, the distribution of detection distances increased from the road to ~100 m and then decreased (Figure 2B). We observed a similar pattern in both strata.

#### *2019 (500-m buffer surveys)*

We completed 22 surveys on 10 PSUs during 14 nights from 6 April to 3 May 2019. Median start time was 2103 hours (0.9 hours post-sunset) and mean survey duration was 3.3 hours. All PSUs were surveyed 2 times and 2 PSUs were surveyed 3 times, with the surveys occurring a median of 1 day apart (range = 1–12). We detected 318 deer groups (clusters) consisting of 1,076 individual deer. Of the 318 groups detected, 91% were along road segments in the high-density stratum. The total deer count was slightly higher for the first run (594 vs. 482), but mean group size was similar among runs (3.5 vs. 3.2) and strata (2.8 in low vs. 3.4 in high). The overall group size was 3.4 deer (range = 1–21). Group size was weakly positively correlated with distance ( $r = 0.148$ , 95% CI = 0.038 to 0.254). Compared to the 250-m buffer surveys, there were fewer meaningful differences in the distribution of deer-group observations among cover types when using the 500-m buffer. For example, 59% and 46% of group detections in the low and high strata, respectively, were located in harvested crop fields. For deer groups observed along low-density road segments, the mean distance to a high-strata deer-cover polygon was 484 m (median = 390 m, range = 0–1,945). The mean perpendicular sighting distance was greater in the low stratum (125 m; range = 29–540) compared to the high stratum (116 m; range = 0–608). Again, the distribution of detection distances increased from the road to ~100 m and then decreased (Figure 2C); the pattern was similar in both strata.

#### **Variability in Deer Counts**

Among-plot variation accounted for 80–95% of total variation in raw deer counts. Thus, variation in counts within PSUs (due to survey day) was relatively small compared to variation among PSUs. This is important because large day-to-day variation in the observation process could result in an unreliable estimator (e.g., one that is not highly repeatable). Conversely, we can address large among-plot variation through design choices such as increasing the sample of PSUs.

#### **Model Comparisons and Density Estimates**

##### *2018 (250-m buffer, run 1)*

Raw deer counts and density estimates did not vary appreciably by run. Therefore, we focused on data from run 1 (i.e., similar to an operational survey). Our top-supported model (lowest AIC) was based on the hazard-rate key function and included the COV2 covariate (Table 1). Models with STRATA as a covariate did not fit the data well, which suggests that  $g(x)$  did not vary significantly between the 2 strata. The hazard-rate detection function is described by the following equation:

$$g(x) = 1 - \exp[-(x/\sigma)^{-b}]$$

where the parameter  $b$  is a shape parameter,  $\sigma$  is a scale parameter, and  $x$  is the perpendicular sighting distance (which may be standardized). Covariates enter the detection function via the scale parameter (e.g.,  $\sigma = \beta_0 + \beta_1 COV2$ ). The detection function parameters from our top model were  $\hat{b} = 1.000$  (SE = 0.188),  $\hat{\beta}_0 = 4.739$  (SE = 0.138), and  $\hat{\beta}_1 = 0.382$  (SE = 0.141).

Given these parameters, mean detection probability was 0.602 (SE = 0.037, CV = 6.1%), which describes the area under the detection curve (e.g., Figure 3). When adjusted for the covariate COV2, the mean predicted probability of detection was 0.596 for deer located in tall/dense cover types and 0.621 for deer in short/open cover types. The density estimate from our top model was 8.2 deer/mi<sup>2</sup> (95% CI = 5.8–11.7; Table 1). However, density estimates from the other models were similar (Table 1). More specifically, the choice of a key function and g(x) covariates did not appreciably effect the density estimate. Likewise, the density estimates when data from each stratum were analyzed separately (not shown) were nearly identical, which supports the decision to use a stratified estimator where data are pooled across strata to estimate g(x).

#### *Pooled data (2018 and 2019, 250-m buffer, runs 1-3)*

As in 2018, the detection function g(x) varied as a function of COV2, our binary indicator variable for cover type (tall vs short). Conversely, we failed to find evidence that g(x) varied by year, run, or strata (Table 2). Models based on pooled data were not useful for predicting deer density, but helped guide the 2019 analysis.

#### *2019 (250-m buffer, run 1)*

We focused on data from run 1, which is consistent with an operational survey and our 2018 and pooled-data analyses. Our top models for 2019 included COV2, but 5 additional models had  $\Delta$ AIC values <2, including the null (intercept only) model (Table 3). This may partly reflect the challenge of quantifying the suite of interacting factors that likely influence variation in the detection process (i.e., in addition to perpendicular sighting distance). This is especially true in this application because detection is based on IR imaging rather than the usual ocular process (e.g., where visual obstruction is often an important and simple detection covariate). However, more importantly, estimates of deer density did not vary appreciably among models (Table 3).

#### *2019 (500-m buffer, run 1)*

For consistency, we again focused on data from run 1 for estimating deer density. Because we had a smaller dataset ( $n = 10$  PSUs), attempts to examine detection covariates were uninformative. Therefore, we used the COV2 model for consistency. The estimated deer density was 8.5 deer/mi<sup>2</sup> (95% CI = 5.5–13.2; Table 4). For a more direct comparison, we used data from the same 10 PSUs to refit the 250-m buffer model (Table 4). The estimated deer density from this model was 8.8 deer/mi<sup>2</sup> (95% CI = 6.1–12.7). Thus, the slightly higher density estimates probably reflect sampling variation (i.e., due to surveying a subset of PSUs).

#### *2019 and 2020 (aerial survey)*

The deer density estimates from our winter aerial surveys were both the same and equaled 6.5 deer/mi<sup>2</sup> (95% CI = 4.9–8.1 in 2019; 95% CI = 5.1–7.9 in 2020; MNDNR, unpublished data), which is slightly lower than distance-sampling estimates from 2018 and 2019. This is consistent with findings by others, where distance sampling tended to generate higher density estimates compared to other methods (Beaver et al. 2014, Kaminski et al. 2019). However, from a management perspective, the difference is negligible, especially if trends from these metrics are strongly positively correlated.

### **Expected Precision vs. Design Choices**

Precision of density estimates from our 250-m buffer design were reasonably good (CV = 17–18%), but this is likely optimistic because it may not adequately reflect variation due to survey date. Not surprisingly, precision was much lower (mean CV = 25%) when we bootstrapped distance data using PSU and run (surrogate for survey date). This is probably a more realistic expectation of precision for an operational survey with a 250-m buffer,  $n = 15$  PSUs, and

approximately equal allocation of survey effort in each stratum. A common target level of desired precision for management surveys is  $CV \approx 13\%$ . To achieve this level of precision with our current design (250-m buffer and 50:50 allocation) and assuming a single (non-replicated) operational survey would require increasing the number of PSUs from 15 to ~25. However, choices related to the stratification scheme and allocation of secondary sampling units may be important too. Our Monte Carlo simulation indicated that the 250-m buffer design with 50:50 allocation (low:high) of secondary units resulted, on average, in similar precision to the 500-m buffer design with 35:65 allocation (Figure 4). Conversely, the 250-m buffer with 35:65 allocation and the 500-m buffer with 50:50 allocation tended to produce more imprecise density estimates. Increasing the buffer distance from 250 m to 500 m resulted in approximately equal stratum weights (low = 54% of study area, high = 46% of study area), but the low stratum now had significantly fewer deer-group detections (11 vs. 39) and very low estimated densities (1.1 deer/mi<sup>2</sup>; see Table 4). Thus, it makes sense to put more sampling effort into the high stratum to increase precision of the estimate. However, with so few deer-group observations in the low stratum, it becomes difficult to determine whether  $g(x)$  varies by stratum and one must pool data over strata to estimate  $g(x)$ . Conversely, the low stratum in the 250-m buffer design is relatively large (70% of study area). Deer densities are still relatively low (3.7–4.2 deer/mi<sup>2</sup>) in the low stratum (Table 4), but because of its size, it is important to put relatively more effort into surveying the low stratum. Thus, in the 250-m design, the 50:50 allocation generates a more precise estimate and provides more data to evaluate potential variation in  $g(x)$ . However, it is important to note that we are still putting relatively more effort into the high stratum with 50:50 allocation because the high stratum only comprises 30% of the sampling frame. These tradeoffs are not necessarily straightforward. However, we now have 2 years of data from the 250-m buffer design. Therefore, for consistency, we plan to continue using the 250-m buffer design with 50:50 allocation of secondary sampling units.

## **CONCLUSIONS**

The results from the first 2 years of the pilot study are encouraging. We identified and resolved several data collection and survey-design challenges and developed detailed field protocols to ensure consistency in data collection. Most importantly, density estimates were slightly higher but within a reasonable range of the aerial survey estimate, and annual variation in  $g(x)$  and the density estimates was negligible. Furthermore, we were able to use the first 2 years of data to explore questions about sampling-design tradeoffs. Another year of data collection on the same study area will strengthen our inferences and help determine if 1)  $g(x)$  and the distribution of deer relative to roads and cover is relatively consistent over time and space, and 2) the effect of variation in spring dispersal can be minimized by using observational cues to inform the start of the survey.

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Table 1. Distance sampling models used to evaluate roadside surveys of white-tailed deer in southern Minnesota, fit to the 2018 dataset. Data were collected during April and early May. For all models, we restricted survey data to the initial run (replicate) of the 15 primary sampling units, after right truncation (300 m). Analysis was restricted to the half-normal (HN) and hazard-rate (HR) key functions. Covariates included deer density strata (STRATA = low, high) and tall (i.e., grassland, woodland, standing crops, wetland) vs short (i.e., pasture, farmstead, harvested crops, roadsides, other) cover types (COV2). Number of model parameters (K), Akaike's Information Criterion (AIC) values, change in AIC values relative to the top model ( $\Delta$ AIC), AIC weights ( $\omega$ ), log likelihood (LL), density estimates (deer/mi<sup>2</sup>), and 95% confidence intervals (CI) are also presented.

Model	Key function	Covariates	K	AIC	$\Delta$ AIC	$\omega$	LL	Density	95% CI
M7	HR	~COV2	3	3103.0	0.0	0.815	-1548.5	8.2	5.8-11.7
M8	HR	~STRATA	3	3107.1	4.1	0.106	-1550.5	8.1	5.7-11.6
M5	HR	~1	2	3109.1	6.1	0.039	-1552.5	8.3	5.7-12.0
M3	HN	~COV2	2	3110.1	7.1	0.023	-1553.1	8.6	6.1-12.3
M6	HR+Cos2	~1	3	3111.1	8.1	0.014	-1552.5	8.3	5.3-13.0
M2	HN+Cos2	~1	2	3115.9	12.9	0.001	-1555.9	8.0	5.3-12.0
M1	HN	~1	1	3116.2	13.2	0.001	-1557.1	8.7	6.1-12.5
M4	HN	~STRATA	2	3116.4	13.4	0.001	-1556.2	8.5	6.0-12.1

Table 2. Distance sampling models used to evaluate roadside surveys of white-tailed deer in southern Minnesota, fit to the 2018-2019 pooled dataset. Data were collected during April and early May. For all models, we restricted data to the 250-m buffer surveys, after right truncation (300 m). Analysis was restricted to the hazard-rate (HR) key function. Covariates included year (YEAR), survey replicate (RUN), deer density strata (STRATA = low, high), and tall (i.e., grassland, woodland, standing crops, wetland) vs short (i.e., pasture, farmstead, harvested crops, roadsides, other) cover types (COV2). Number of model parameters (K), Akaike's Information Criterion (AIC) values, change in AIC values relative to the top model ( $\Delta$ AIC), AIC weights ( $\omega$ ), and log likelihood (LL) are also presented.

Model	Key function	Covariates	K	AIC	$\Delta$ AIC	$\omega$	LL
P5	HR	~COV2	3	17930	0.0	0.999	-8962.0
P4	HR	~STRATA	3	17945	14.5	0.001	-8969.3
P1	HR	~YEAR	3	17960	30.4	0.000	-8977.2
P2	HR	~RUN	4	17961	30.5	0.000	-8976.3
P0	HR	~1	2	17961	30.9	0.000	-8978.4
P3	HR	~YEAR * RUN	7	17963	33.4	0.000	-8974.7

Table 3. Distance sampling models used to evaluate roadside surveys of white-tailed deer in southern Minnesota fit to the 2019 dataset. Data were collected during April and early May. For all models, we restricted survey data to the initial run (replicate) of the 15 primary sampling units of the 250-m surveys, after right truncation (300 m). Analysis was restricted to the hazard-rate (HR) key function. Covariates included tall (i.e., grassland, woodland, standing crops, wetland) vs short (i.e., pasture, farmstead, harvested crops, roadsides, other) cover types (COV2), relative topography (TOPO = low, medium, high), deer activity (ACTIVITY = lying, standing, moving), and % visual obstruction class (OBSTR). Number of model parameters (K), Akaike's Information Criterion (AIC) values, change in AIC values relative to the top model ( $\Delta$ AIC), AIC weights ( $\omega$ ), log likelihood (LL), density estimates (deer/mi<sup>2</sup>), and 95% confidence intervals (CI) are also presented.

Model	Key function	Covariates	K	AIC	$\Delta$ AIC	$\omega$	LL	Density	95% CI
M1	HR	~COV2	3	2647.5	0.0	0.219	-1320.7	8.0	5.7-11.3
M6	HR	~COV2+TOPO	5	2648.2	0.7	0.155	-1319.1	8.1	5.7-11.5
M7	HR	~COV2+ACTIVITY	5	2648.5	1.1	0.130	-1319.3	8.1	5.7-11.4
M2	HR	~1	2	2649.3	1.8	0.088	-1322.6	8.0	5.6-11.5
M8	HR	~COV2+OBSTR	4	2649.3	1.9	0.087	-1320.7	8.0	5.7-11.3
M5	HR	~COV2+TOPO+ACTIVITY	7	2649.3	1.9	0.085	-1317.7	8.1	5.7-11.5
M9	HR	~TOPO	4	2650.0	2.5	0.061	-1321.0	8.1	5.6-11.7
M4	HR	~COV2+TOPO+OBSTR	6	2650.1	2.7	0.058	-1319.1	8.1	5.7-11.5
M11	HR	~ACTIVITY	4	2650.4	2.9	0.051	-1321.2	8.1	5.7-11.5
M3	HR	~COV2+TOPO+OBSTR+ACTIVITY	8	2651.2	3.8	0.033	-1317.6	8.1	5.7-11.5
M10	HR	~OBSTR	3	2651.3	3.8	0.033	-1322.6	8.0	5.6-11.5

Table 4. Top distance sampling models by year and buffer size used to evaluate roadside surveys of white-tailed deer in southern Minnesota, spring 2018 and 2019. For all models, we restricted survey data to the initial run (replicate) of the 10 or 15 primary sampling units (PSU), after right truncation (300 m). Summary statistics include number of PSU, stratum weight (proportion of study area defined as low deer density [Wt (L)]), transect length (Tran [mi]) by deer density strata (L=low, H=high), number of deer groups observed (Groups) by strata, mean detection probability (P), density estimates (deer/mi<sup>2</sup>) by strata, 95% confidence intervals (CI), and coefficient of variation(CV).

Year	Buffer (m)	PSU	Wt (L)	Tran (L)	Tran (H)	Groups (L)	Groups (H)	g(x)	P	Density (L)	Density (H)	Density (Total)	95% CI	CV (%)
2018	250	15	0.70	196	193	46	234	HR(COV2)	0.602	3.7	19.0	8.2	5.8-11.7	17.6
2019	250	15	0.70	196	189	50	191	HR(COV2)	0.537	4.1	17.2	8.0	5.7-11.3	17.1
2019	250	10	0.70	135	130	39	155	HR(COV2)	0.558	4.2	19.7	8.8	6.1-12.7	17.8
2019	500	10	0.54	139	144	11	143	HR(COV2)	0.532	1.1	17.2	8.5	5.5-13.2	20.1

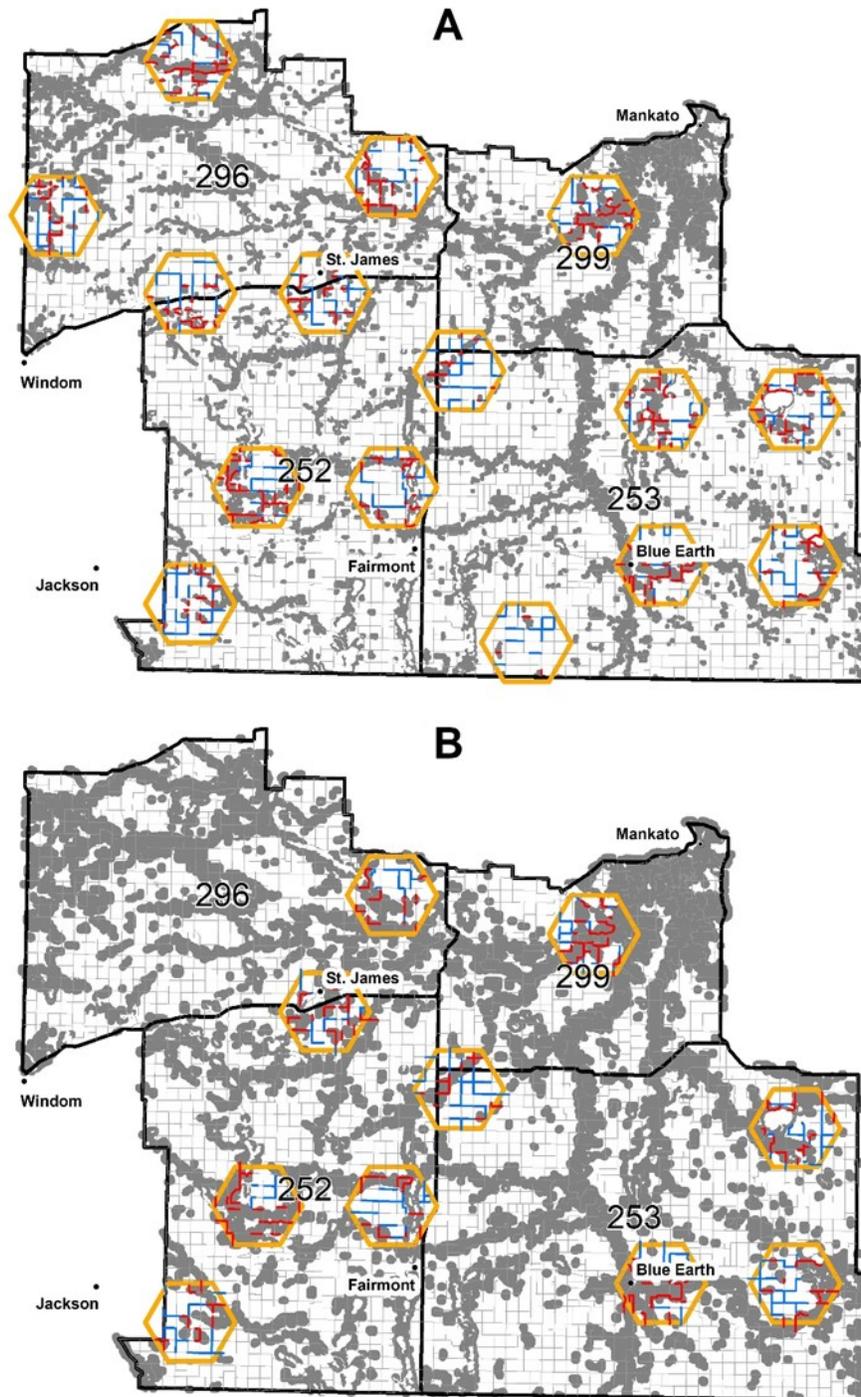


Figure 1. Sampling frame (deer permit areas 252, 253, 296, 299), primary sampling units (PSU; hexagons), and secondary sampling units (road segments; red = high-density stratum, blue = low-density stratum) for roadside distance-sampling surveys of white-tailed deer in southern Minnesota during **A**) spring 2018 and 2019 (250-m buffer surveys) and **B**) spring 2019 (500-m buffer surveys). Grey areas denote deer-cover polygons ( $\geq 2$  ac) consisting of woodland, grassland, and wetland cover types with a 250-m or 500-m buffer.

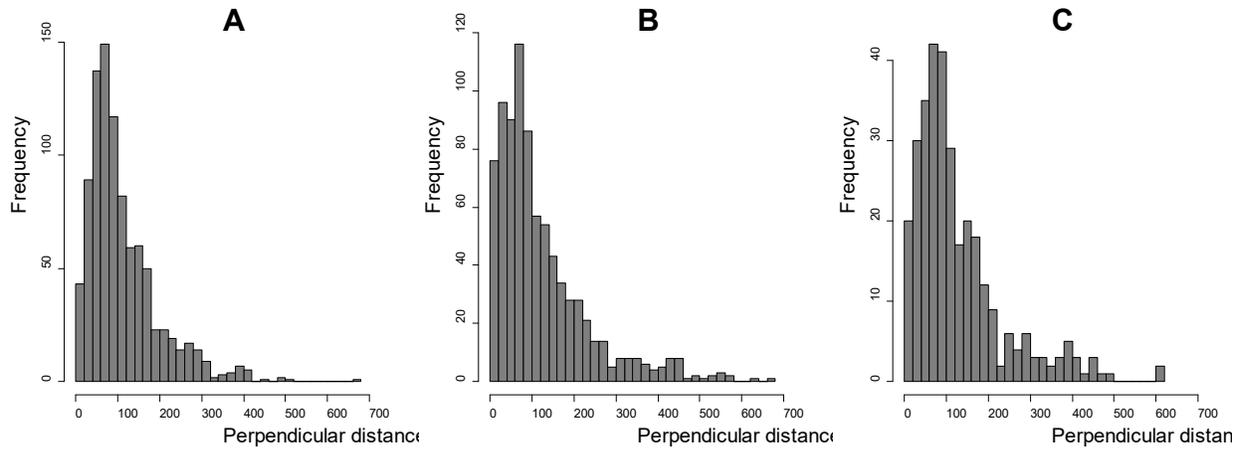


Figure 2. Distribution of perpendicular sighting distances from roadside distance-sampling surveys of white-tailed deer in southern Minnesota during **A**) spring 2018 (250-m buffer surveys), **B**) spring 2019 (250-m buffer surveys), and **C**) spring 2019 (500-m buffer surveys). Data include distance measurements collected during 2–4 replicate surveys of 10–15 primary sampling units.

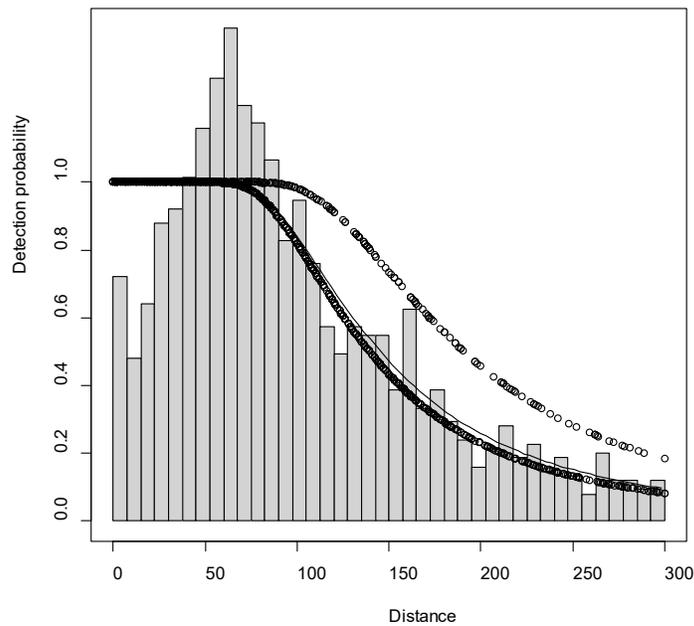


Figure 3. Estimated detection function from our top pooled distance-sampling model overlaid on a histogram of deer-group observations as a function of perpendicular sighting distance during roadside surveys of white-tailed deer in southern Minnesota, spring 2018 and 2019. The solid curved line denotes the average detection function. The lower line of circles denotes the detection curve for deer groups observed in tall/dense cover types (grassland, woodland, standing crops, wetland) and the upper line of circles denotes the detection curve for deer groups observed in short/open cover types (pasture, farmstead, harvested crops, roadsides, other).

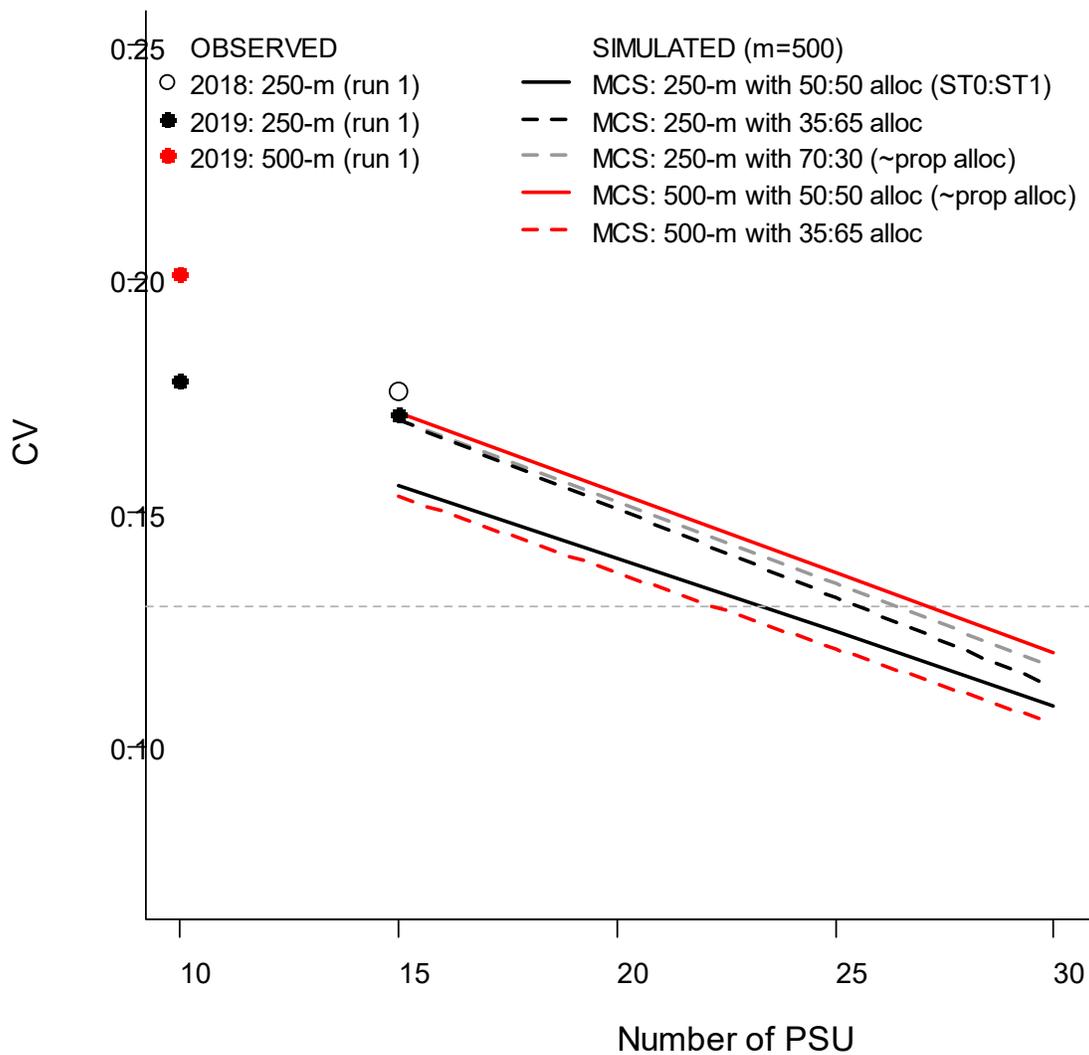


Figure 4. Expected precision of population estimates as a function of sample size (number of primary sampling units; PSU), stratification scheme (250- vs. 500-m buffer around deer-habitat polygons), and allocation of secondary sampling units (road segments) to strata. Estimates were derived from a Monte Carlo simulation with 500 replicates based on data from roadside distance-sampling surveys of white-tailed deer in southern Minnesota, spring 2018 and 2019. The gray dashed horizontal line denotes a common target level of precision for management surveys.

# **Human Dimensions Summaries**





# MINNESOTANS' ATTITUDES TOWARD WOLVES AND WOLF MANAGEMENT

Susan A. Schroeder<sup>1</sup>, Adam C. Landon<sup>2,3</sup>, Leslie McInenly<sup>2</sup>, and Daniel Stark<sup>2</sup>

## SUMMARY OF FINDINGS

The Minnesota Department of Natural Resources, in partnership with the Minnesota Cooperative Fish and Wildlife Research Unit, conducted a survey of Minnesota residents', deer hunters', and livestock producers' values, beliefs, attitudes, and behaviors toward wolves and wolf management. Findings demonstrate a diversity of perspectives regarding stakeholders' attitudes toward wolves, priorities for wolf management, preferences for future wolf populations and distribution, and support for the establishment of regulated wolf hunting and trapping seasons, among other variables measured. Residents, on average, expressed positive attitudes toward wolves, preferred to maintain current (2019) wolf populations and distribution, and opposed lethal solutions to human-wolf conflict. Residents were split in their support or opposition for establishing wolf hunting and trapping seasons. Deer hunters and livestock producers held similar positions on most variables. A majority of deer hunters and livestock producers expressed a negative attitude toward wolves, preferred to reduce current (2019) wolf populations, and supported killing wolves as a solution to conflict. Deer hunters differed from livestock producers regarding preferences for wolf distribution, with roughly 42% preferring to maintain the status quo. Majorities of both deer hunters and livestock producers supported establishing wolf hunting and trapping seasons in Minnesota.

## INTRODUCTION

Gray wolf (*Canis lupus*) management is a topic of concern for diverse stakeholders given the cultural, ecological, and economic significance of the species (Kellert et al 1996; Landon et al 2019; Carlson et al 2019). In 2019, the U.S. Fish and Wildlife Service introduced a rule proposing to remove the gray wolf from the U.S. endangered species list. If adopted, individual U.S. states will assume management authority for wolves. Minnesota is the only state in the lower 48 that has maintained a continuous breeding population of wolves within its borders since European colonization, and has a history of wolf management (Mech 1995). In 2001, Minnesota adopted a wolf management plan, and this document has since served to guide state wolf policy during periods of state control. In the face of substantial uncertainty regarding federal endangered species status, and owing to the dated nature of the extant management plan, the Minnesota Department of Natural Resources (MNDNR) initiated a process to update its wolf management plan in 2019. Part of this process involved the collection of information about stakeholders' values, beliefs, attitudes, and behaviors toward wolves and wolf management. Generalizable information about controversial issues like wolf management is critical for developing fair and equitable policies that reflect the needs of diverse stakeholders (Nie 2010). Scientific survey methods are one tool used to obtain information of this nature. To that end, we

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conducted a survey of Minnesota residents, deer hunters, and livestock producers to assess attitudes toward wolves and wolf management, during the fall and winter of 2019.

## **OBJECTIVES**

To survey Minnesota residents, deer hunters, and livestock producers to assess values, beliefs, attitudes, and behaviors toward wolves and wolf management. Specific topics assessed included individuals:

1. Experiences with wolves.
2. General attitudes and emotions associated with wolves.
3. Values for wolves.
4. Wildlife value orientations.
5. Tradeoffs between wolf, deer, and moose.
6. Preferences for wolf populations and distributions.
7. Preferences for wolf management .
8. Support for establishing regulated wolf hunting and trapping seasons, and
9. Demographics.

## **METHODS**

### **Data Collection**

We randomly selected livestock producers, deer hunters, and residents from independent sample frames for inclusion in the study. The Minnesota Board of Animal Health provided a list of known livestock producers, and we selected 2,000 cattle producers and 500 sheep producers for participation. We purchased a sample of Minnesota household residential addresses from the survey firm Marketing Systems Group. Households were selected using address based sampling from the U.S. Postal Service Master Delivery Sequence File (n=5,250). Stratification for the resident sample was by DNR administrative region, plus an additional strata corresponding to Hennepin and Ramsey counties, to ensure representation. For the resident sample, the individual in the household aged 18+ with the birthdate nearest receipt of the solicitation was directed to complete the questionnaire. Adult (18 and older) firearm deer hunters comprised the hunter sample frame, and we randomly selected 2,000 individuals from the DNR electronic licensing system to receive a questionnaire, from those with a valid 2018 license.

Data collection occurred through postal-mail, following the recommendations of Dillman et al. (2014). Personally addressed cover letters inviting participation, survey questionnaires, and postage-paid return envelopes were furnished to subjects at 3-week intervals. Responding parties were removed from mailing lists and a maximum of 3 solicitations were sent. After accounting for non-deliverable mail, and invalid cases (e.g., deceased), response rates were 33%, 47%, and 53% for the resident (n=1,466), deer hunter (n=895), and livestock producer (n=1,139) samples respectively.

We constructed post-stratification weights for statewide estimates for the resident sample. Weights adjusted estimates to account for study strata population, gender, age, and hunting participation, given the sampling design and response propensity. Hunting participation was calculated using Minnesota DNR electronic license records.

### **Selected Measures**

We measured respondents' general attitude toward wolves with 4 items on a semantic differential scale. Item pairs included bad/good, negative/positive, harmful/beneficial, dangerous/harmless,

and responses were recorded in reference to each polar anchor; very (1), moderately (2), slightly (3), neither (4), slightly (5), moderately (6), and very (7). Items were averaged to create an attitude scale variable and interpreted relative to the original response scale.

We assessed respondents' perception of the importance of wolves in Minnesota with a single item; "it is important to maintain a wolf population in Minnesota." Responses were recorded on a 7pt likert-type scale where 1 = strongly disagree, 2 = moderately disagree, 3 = slightly disagree, 4 = neither, 5 = slightly agree, 6 = moderately agree, and 7 = strongly agree.

Respondents' preferences for future wolf populations and range were also assessed. Preference for future wolf population was measured using the question: "there were an estimated 2,655 wolves in MN in winter 2017/18. In the future, I would like to have \_\_\_\_\_ wolves in Minnesota." Response options included 1 = zero, 2 = many fewer, 3 = fewer, 4 = about the same number, 5 = more, and 6 = many more. A similar question assessed preference for geographic range; "compared to today, I would like to see wolves occupy \_\_\_\_\_ territory in MN." Response options included 1 = no, 2 = much less, 3 = about the same amount of, 4 = more, and 5 = much more.

Respondents' preferences for wolf management actions were assessed with 11 items. Actions were presented following the stem "how important do you personally think it is that the Minnesota DNR do each of the following concerning wolves in Minnesota?" Items included "protect individual wolves," "compensate livestock producers for animals lost to wolves," and "study wolf populations," among others. Responses were recorded on a scale where 1 = not at all important, 2 = slightly important, 3 = somewhat important, 4 = moderately important, and 5 = very important.

We assessed respondents values for wolves using 10 items adapted from Kellert's (1996) typology. Items were presented following the stem "people value having wolves in MN for a number of reasons, how much do you agree or disagree with the following statements? I value having wolves in Minnesota..." Individual items included "...because they have a right to exist," "...because they are an important part of the ecosystem," and "...because they are a symbol of wilderness" among others.

Finally, respondents' support for establishing regulated hunting and trapping seasons was assessed with two items following the stem "some Minnesotans want the opportunity to hunt and trap wolves, while others feel the hunting and trapping of wolves is wrong. If wolves were removed from the endangered species list and management authority moves to the state of Minnesota, how much do you support or oppose the following? a) establishing a regulated wolf hunting season, b) establishing a regulated wolf trapping season? Responses were recorded on a scale where 1 = strongly oppose, 2 = moderately oppose, 3 = slightly oppose, 4 = neither, 5 = slightly support, 6 = moderately support, 7 = strongly support.

## **RESULTS**

### **Attitudes toward Wolves**

Residents, on average, expressed positive attitudes toward wolves, with 69% reporting average attitudinal scale scores greater than neutral. A minority (20%) evaluated wolves negatively (scale score less than 4), or possessed a neutral attitude toward wolves (scaled score of 4). This pattern was reversed for deer hunters (52%) and livestock producers (62%), among whom a majority expressed a negative attitude toward wolves. Nearly 32% of livestock producers held a positive attitude toward wolves, while a small minority (8%) were neutral. Similarly, 39% of

deer hunters held a positive attitude toward wolves, and roughly 10% were neutral. Results are presented in Figure 1.

### **Importance of Wolves**

When asked about the importance of maintaining a wolf population in Minnesota, both residents and deer hunters responded in the affirmative. The vast majority (87%) of residents and a clear majority of deer hunters (67%) either slightly agreed, moderately agreed, or strongly agreed with the statement “it is important to maintain a wolf population in Minnesota.” Agreement with this statement was less strong among livestock producers. Individuals in this group were split between agreement (47%) and disagreement (43%) about the importance of maintaining a wolf population in the state. A further 10% were neutral toward the position. Results are presented in Figure 2.

### **Wolf Population and Distribution**

On average, residents preferred maintaining wolf populations (44%) and geographic distribution (56%) “about the same” compared to conditions in 2019. Around 33% and 28% of residents expressed a preference for increasing wolf populations and geographic distribution in the state respectively. Deer hunters, on average, preferred a reduction in wolf populations, with 59% expressing a desire for fewer wolves. A further 30% indicated a preference for maintaining wolf populations “about the same,” and the remainder preferring increase. A similar pattern emerged for preferences for the geographic distribution of wolves in Minnesota, with around 42% of deer hunters preferring “about the same” and 48% preferring a reduction. Nearly 73% of livestock producers expressed a preference for having fewer wolves in Minnesota in the future. Similarly, 67% preferred that wolves occupy less range than the current distribution. Results summarizing respondents’ preferences for wolf populations and distribution are presented in Figures 3 and 4 respectively.

### **Values for Wolves**

Respondents were asked their agreement with a number of statements about the values they may assign to wolves. Livestock producers, on average, did not agree with any of the statements they were asked to evaluate. Among value items evaluated, livestock producers were roughly neutral toward the belief that wolves “have a right to exist”, “for the opportunity to hunt and trap them,” and “because they are an important part of the ecosystem.” Among the items evaluated, livestock producers expressed the strongest disagreement with the belief that they have an emotional connection to wolves. Deer hunters assigned a greater diversity of values to wolves than did livestock producers. On average, deer hunters agreed that wolves “have a right to exist,” and “are an important part of the ecosystem,” among other values. Like livestock producers, deer hunters also disagreed most strongly with a belief that they have an emotional connection to wolves. Residents, unlike deer hunters and livestock producers, agreed with the majority of the value items they evaluated, on average. The two items with the strongest agreement among these individuals were “because they have a right to exist,” and “because they are an important part of the ecosystem.” Residents expressed the strongest disagreement that wolves are valuable in Minnesota because they provide an opportunity for hunting or trapping. Results summarizing respondents’ values for wolves are presented in Figure 5.

### **Preferences for Wolf Management**

We assessed respondents’ priorities for potential management actions for wolves with 11 items. Livestock producers placed the greatest priority on “kill wolves in areas where they are attacking domestic livestock,” “kill wolves that show aggression or threatening behavior toward people,” and “compensate livestock producers for animals lost to wolves” as actions DNR should take

with respect to wolf management. These same individuals felt that “protect individual wolves” should be the lowest priority for the agency. Deer hunters placed the highest importance on lethal management of problem wolves, rating “kill wolves that show aggression or threatening behavior toward people,” and “kill wolves in areas where they are attacking domestic livestock” as the 2 highest items. Deer hunters also placed the least importance on “protect individual wolves.” Residents’ priorities for wolf management were different from both livestock producers and deer hunters. These individuals, on average, placed the greatest importance on “educate livestock producers about best management practices to prevent conflict,” “educate people about wolves,” and “study wolf populations as actions DNR should take. Residents placed the least importance on “reduce wolf populations to address concerns about deer and moose populations.” Results summarizing respondents’ wolf management preferences are presented in Figure 6.

### **Wolf Hunting and Trapping**

Respondents were asked to evaluate the prospects of establishing regulated wolf hunting and trapping seasons in Minnesota, in the instance that management authority is returned to the state and the species is removed from federal endangered species protection. The vast majority of both livestock producers supported establishing both regulated wolf hunting (88%) and trapping (84%) seasons. A very similar result was found among deer hunters with 88% supporting establishing a wolf hunting season, and 80% supporting establishing a wolf trapping season. Among residents, attitudes toward wolf hunting and trapping were mixed. Roughly, 41% of residents supported establishing a wolf hunting season, whereas 30% held a favorable view of establishing a wolf trapping season. A majority of residents (58%) opposed the prospect of wolf trapping, while a near majority (49%) indicated the same with respect to hunting. Results are presented in figures 7 and 8.

### **DISCUSSION**

Results of this study inform recommendations formulated by technical and citizen advisory committees convened to support the wolf management plan revision. Salient results include residents’, deer hunters’, and livestock producers’ attitudes toward wolves, priorities for management, preferences for future wolf populations and distribution, and attitudes toward the establishment of wolf hunting and trapping seasons. A plurality of individuals in each group agree that maintaining a wolf population in Minnesota is important. However, residents diverge from deer hunters and livestock producers about how to manage that population, especially in their support for the establishment of hunting and trapping seasons. These data serve to demonstrate the diversity of perspectives held by stakeholder groups that experience wolves in very different ways. Policy makers, and citizen and technical advisory committees, should heed these results in seeking solutions to conflict that protect individuals’ interests when faced with conflict, while respecting the values of society at-large whenever possible.

### **ACKNOWLEDGMENTS**

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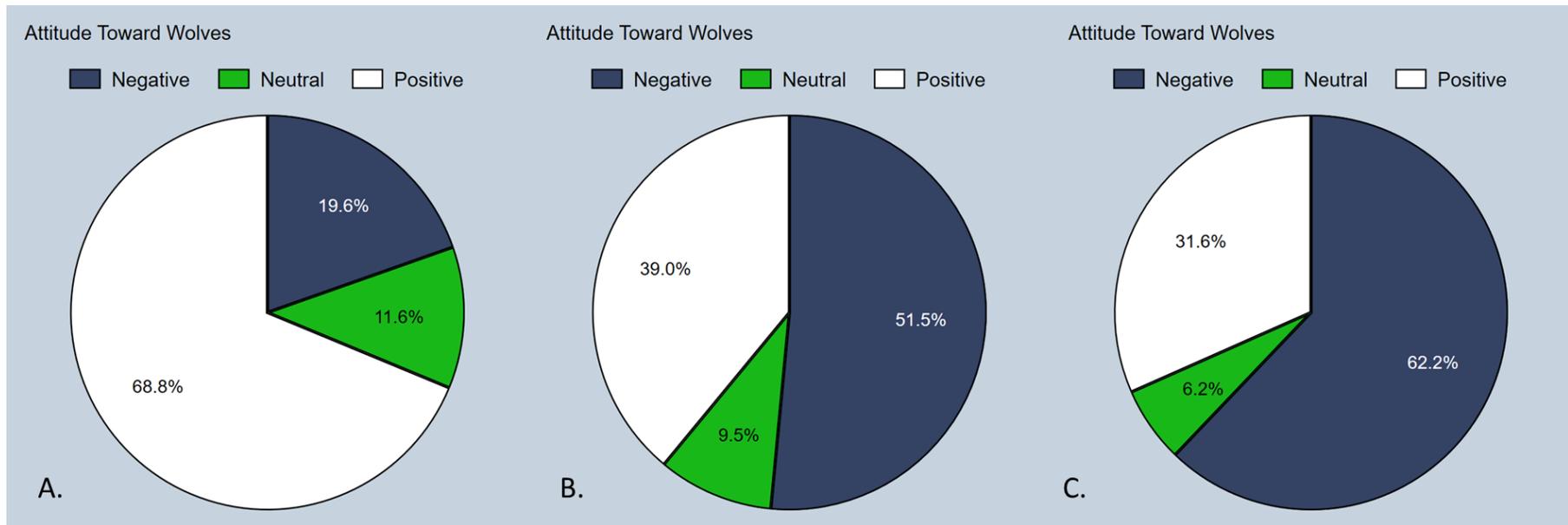


Figure 1. Summary of 2019 Minnesota A) Residents', B) Deer hunters', and C) Livestock producers' general attitude toward wolves. Percent of respondents scoring greater than 4 (positive), 4 (neutral), or less than 4 (negative), on attitude scale variable where 1 = negative anchor, 7 = positive anchor, and 4 = neutral.

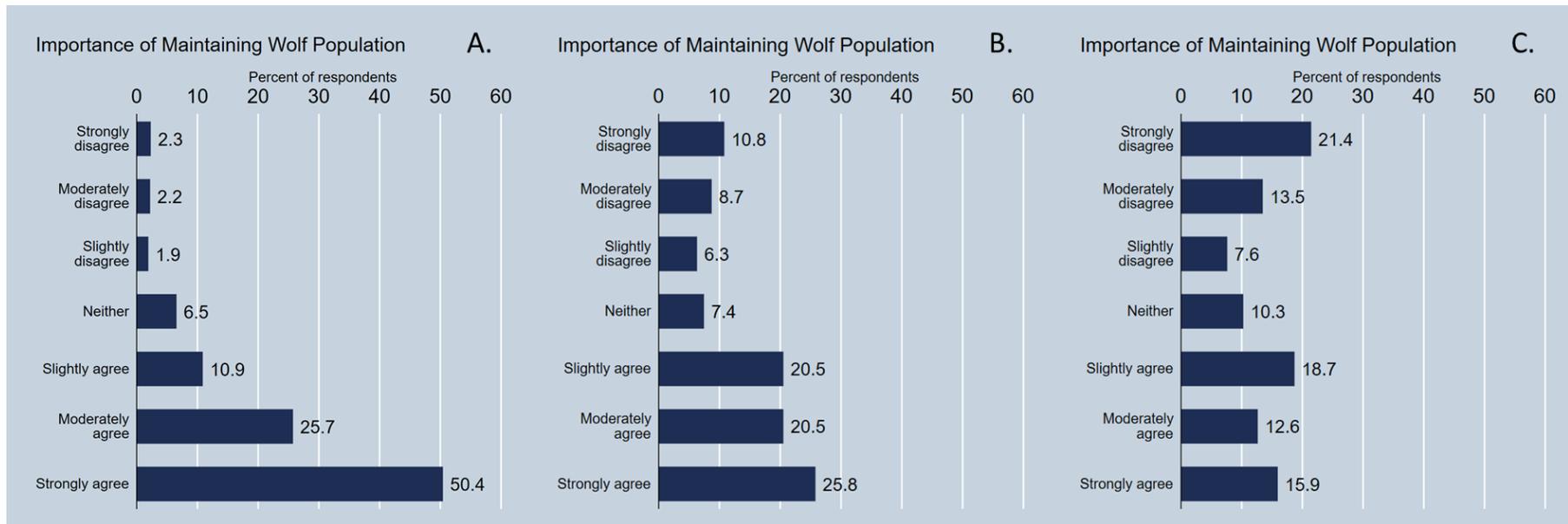


Figure 2. Summary of 2019 Minnesota A) Residents', B) Deer hunters', and C) Livestock producers' agreement with the statement "it is important to maintain a wolf population in Minnesota.

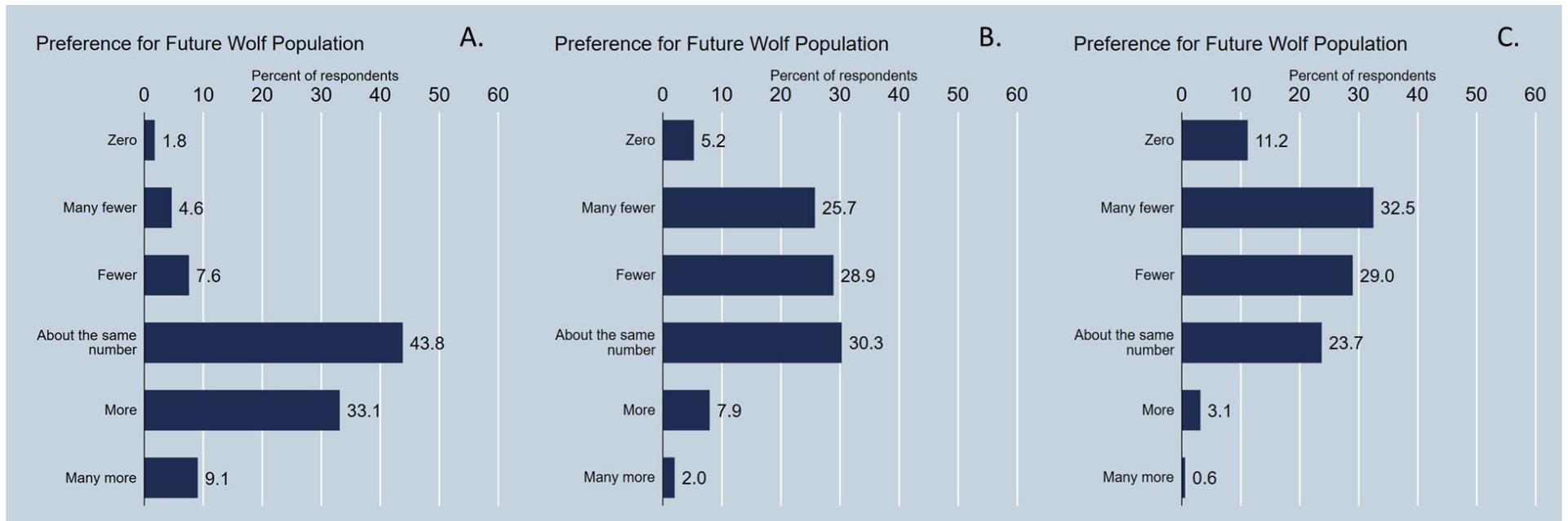


Figure 3. Summary of 2019 Minnesota A) Residents', B) Deer hunters', and C) Livestock producers' preference for future deer populations in Minnesota.

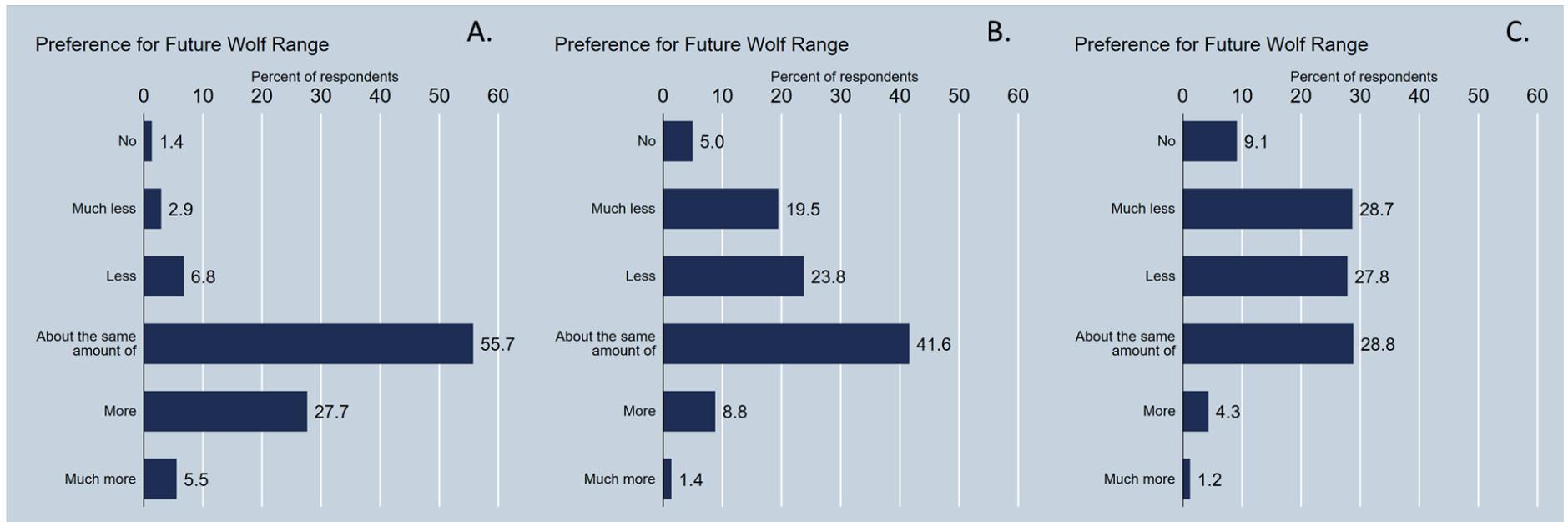


Figure 4. Summary of 2019 Minnesota A) Residents', B) Deer hunters', and C) Livestock producers' preference for future deer distribution in Minnesota.

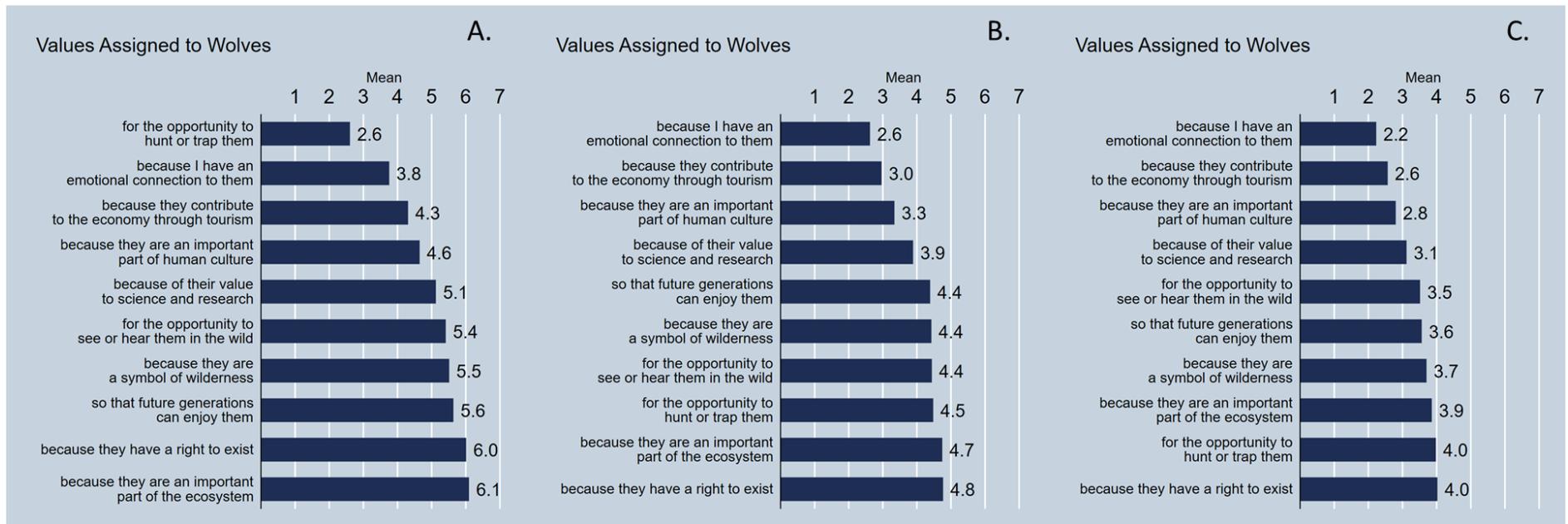


Figure 5. Summary of 2019 Minnesota A) Residents', B) Deer hunters', and C) Livestock producers' values for wolves in Minnesota.



Figure 6. Summary of 2019 Minnesota A) Residents', B) Deer hunters', and C) Livestock producers' preferences for DNR wolf management.

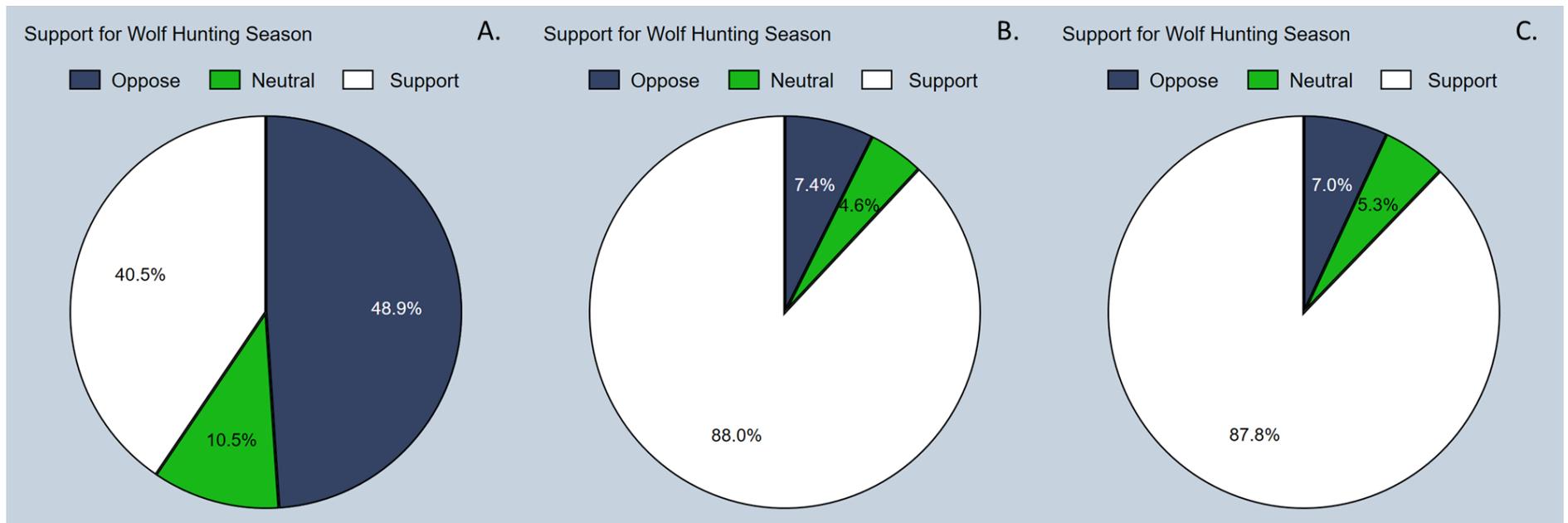


Figure 7. Summary of 2019 Minnesota A) Residents', B) Deer hunters', and C) Livestock producers' support or opposition to establishing a regulated wolf hunting season in Minnesota.

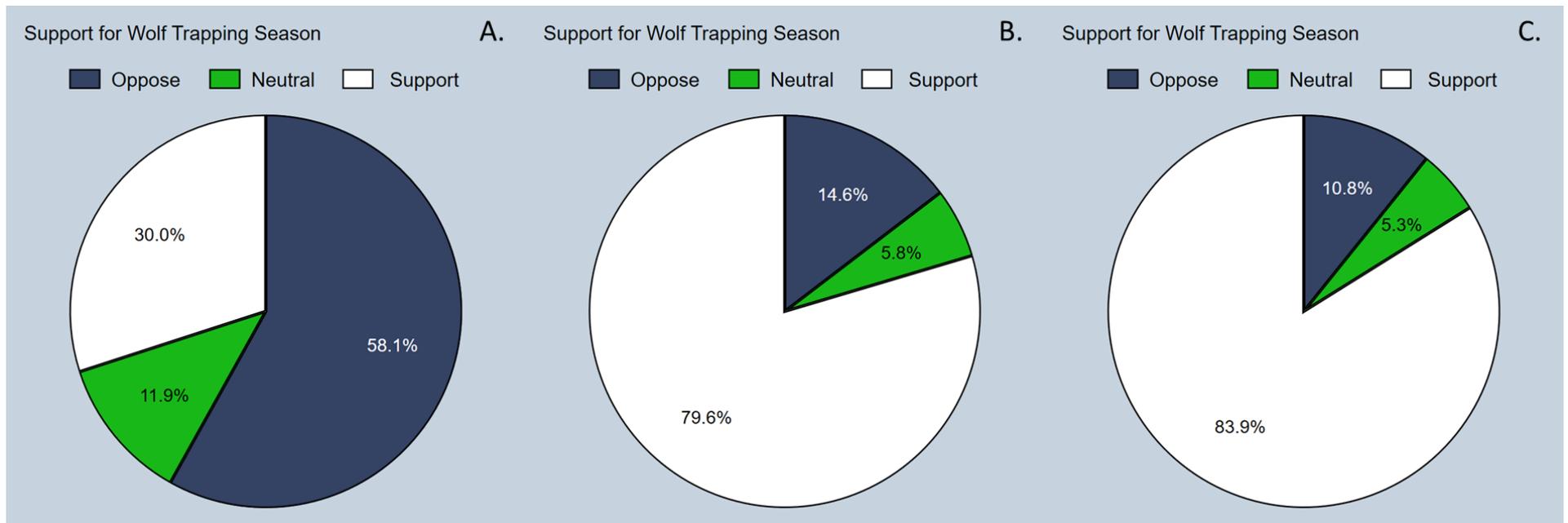


Figure 8. Summary of 2019 Minnesota A) Residents', B) Deer hunters', and C) Livestock producers' support or opposition to establishing a regulated wolf trapping season in Minnesota.

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## BEHAVIORAL RESPONSES OF AMERICAN BLACK BEARS TO REDUCED NATURAL FOODS: HOME RANGE SIZE AND SEASONAL MIGRATIONS

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### SUMMARY OF FINDINGS

American black bears (*Ursus americanus*) in the Chippewa National Forest demonstrated appreciable fat reserves and stable reproduction despite a substantial decline in natural food availability over a 30-year period. Here we investigated potential strategies that bears may have employed to adapt to this reduction in food. We hypothesized that bears increased their home range sizes to encompass more food and/or increased the frequency, duration and distance of large seasonal migrations to seek out more abundant food resources. We estimated home range sizes using both Minimum Convex Polygon and Kernel Density Estimate approaches and developed a method to identify seasonal migrations. Male home range sizes in the 2010s were approximately twice the size of those in the 1980s; whereas, female home ranges tripled in size from the 1980s to the 2010s. We found little difference in migration patterns with only slight changes to duration. Our results supported our hypothesis that home range size increased in response to declining foods, which may explain why body condition and reproduction has not changed. However, these increased movements, in conjunction with bears potentially consuming more human-related foods in the fall, may alter harvest vulnerability, and should be considered when managing the bear hunt.

### INTRODUCTION

As a large generalist omnivore, American black bears (*Ursus americanus*; henceforth black bear or bear) demonstrate exceptional plasticity in response to changes in food availability. One way in which black bears cope with changing resources is through their mobility. Summer and fall natural bear foods (e.g., fruits, nuts) in Minnesota often fluctuate substantially on a seasonal and yearly basis (Noyce and Coy 1990). Bears have adapted to these vagaries in food availability by moving around their home range and using their keen sense of smell to find local patches of food. In a largely agricultural region of northwestern Minnesota, for example, some black bears exhibited the largest home ranges (>2700 km<sup>2</sup>) ever reported for the species (Ditmer et al. 2018) because patches of food are widely spaced. In northcentral Minnesota, Noyce and Garshelis (2011) reported that in any given year, ~40% of black bears make large seasonal migrations in fall, and ~10% migrate in spring, to seek out patches of more abundant foods, such as acorns (*Quercus spp.*), which may be lacking in their home ranges. While these movement patterns were in response to short-term changes in food, few studies have explored how black bear populations respond to diminishing natural food availability over multiple decades.

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We documented a ~70% decline in natural food abundance for bears over the course of 3 decades (1980s – 2010s) on a study area in northcentral Minnesota, likely attributable to landscape-level changes in forest management practices and climate change (Rettler 2018). During this time, the bear population also drastically declined in this area (Garshelis and Tri 2018). It is unclear whether this decline in the population was attributable solely to over-hunting, or if the reduction in food availability also played a role. Contrary to our expectations, we have found little or no changes in weights, reproduction, or cub survival across the decades (Garshelis et al. 2020). These preliminary results leave us with many questions, and new hypotheses about how this bear population has responded to changes in food availability.

One possible reason for the lack of physical or biological changes in the bears is that they managed to obtain sufficient food by travelling more, either expanding their home ranges or going on excursions outside their home ranges more frequently or for longer durations. Several studies have reported that home range size in black bears, especially adult females, is inversely related to natural food abundance and quality – abundant food and high quality habitat often results in reduced home range size (Lindzey and Meslow 1977, Lindzey et al. 1986, Powell et al. 1997, Koehler and Pierce 2003). Home ranges of adult females should be large enough to encompass sufficient resources across many seasons and years, and also accommodate their female offspring, which ultimately will inherit some of it. Males, by contrast, establish home ranges that include multiple breeding females, so the position and size of female home ranges, as well as female density, would, besides food conditions, also affect male home range size.

We used location data obtained from radiocollared bears to test the hypotheses that home ranges in the 2010s for females increased in size since the 1980s due to reduced food, and that male home ranges increased from the combination of reduced foods, larger female home ranges, and reduced female density. We further hypothesized that seasonal migrations of both sexes outside their home ranges were more frequent in the 2010s.

## OBJECTIVES

1. Determine whether home range size has changed since the 1980s.
2. Determine whether seasonal migrations have changed since the 1980s.

## STUDY AREA

Located in northcentral Minnesota, the Chippewa National Forest (CNF) study area falls in the transition zone between the boreal forests to the northeast and the temperate forests in the central part of the state. Our 620-km<sup>2</sup> study area was dominated (42% of area) by the eastern extent of the CNF; the remainder included part of the George Washington State Forest and other state-owned land (11%), county land (6%), private land (18%), commercial timber industry (8%) and open water (15%). The eastern two-thirds of the study area were upland forests dominated by various combinations of aspen (*Populus tremuloides*, *P. grandidentata*), maple (*Acer spp.*), red pine (*Pinus resinosa*), paper birch (*Betula papyrifera*), and balsam fir (*Abies balsamea*). The western third was largely lowlands dominated by speckled alder (*Alnus incana*), black spruce (*Picea marina*), tamarack (*Larix laricina*), black ash (*Fraxinus nigra*), northern white-cedar (*Thuja occidentalis*), quaking aspen and balsam poplar (*P. balsamifera*). Many lakes, forest roads, and recreational trails occur throughout the public land. This area was heavily hunted for bears due to the large extent of easily accessible public land. Larger oak stands and agricultural landscapes do not exist on the study area, but can be found to the south and west.

## METHODS

Location data were collected during 2 sampling periods: 1981–1989 and 2015–2019 (henceforth, 1980s and 2010s respectively). We captured bears using either barrel traps or Aldrich foot snares. While bears were anesthetized with a mixture of ketamine–xylazine, we fit them with VHF radiocollars (Telonics Inc., Mesa, AZ;  $n=220$  bear-years) during the 1980s, and Iridium GPS radiocollars (Telonics Inc., Mesa, AZ;  $n=67$  bear-years) or store-on-board GPS pods (Telemetry Solutions, Concord, CA;  $n=3$  bear-years) during the 2010s. We defined a bear-year as 1 year of data from an individual bear between post-den emergence and pre-den entrance (i.e., approx. mid-April to late-September). We collected location data on VHF radiocollared bears during weekly telemetry flights during the 1980s. With the GPS radiocollared bears, we programmed the collars to record data every 2-hours. We downloaded data from store-on-board GPS pods during winter den visits.

For the home range analysis, we subsampled the 2010s GPS data to match the frequency of the 1980s data. We selected unique bear-years from the 1980s that had a minimum of 15 locations (during approx. 3.5 months). We calculated the median time between locations within these datasets (4–10 days) to guide subsampling the 2010s data. We randomly subsampled the 2010s data at these intervals ( $n=100$  iterations/bear-year), restricted to the hours sampled in the 1980s (0600–2100 hours), which yielded  $\sim 15 - 30$  locations per bear-year.

In addition to subsampling the data, we also had to account for large seasonal migrations, which occur commonly in this population (Garshelis and Noyce 2011). We removed these from the calculation of home range area, and quantified them in a separate analysis. We identified migrations in a 3-step process (Figure 1). First, we assessed whether a step length between consecutive locations exceeded the median step length for that given bear-year. Second, we calculated the distance from this location to all previous locations: if it was  $>5$  km for males or  $>2$  km for females from any previous location, we considered it a possible migration and moved to step 3. Finally, if this location and the next 2 consecutive locations exceeded the median net squared displacement (NSD; Singh et al. 2016) for that given bear-year, we then categorized this progression of movements as a migration. The migration ended when the NSD of a bear location dropped below the median NSD, suggesting that it returned to its typical home range. After partitioning out migrations, we omitted bear-years with  $<15$  relocations.

We recognize that with such few relocations per year, we may not be capturing the true area that a bear used; however, our goal was to determine whether the dispersion of locations had changed over time by using home range estimates, with approximately equal sampling, as an index of change (Signer et al. 2015). We used 2 different home range estimators: Minimum Convex Polygons (MCP; Mohr 1947) and ad-Hoc scaled Kernel Density Estimators (KDE; Kie 2013). The MCP approach generated the smallest convex polygon enclosing all locations; whereas, the ad-Hoc scaled KDE decreased the reference smoothing factor by increments of 0.01 until the smallest contiguous polygon was achieved. The purpose behind using 2 estimators was to test how sensitive our results were when analyzed in different ways.

To test for changes in home range size between the 2 sampling periods, we used linear mixed-effects models with a log-transformed response variable. We used the area of each estimated home range at the 95% and 50%-level as the response variable. For the 2010s rarified data, we averaged across the 100 subset iterations for a given bear-year to produce a home range estimate. Covariates included the sampling period (1980s or 2010s), demographic group (females with cubs, females without cubs, and males) and age (continuous variable). Furthermore, we added an interaction term between sampling period and demographic group to determine if home range size had changed differently among demographic groups. We included

a random intercept of bear ID to account for repeated measurements on the same bears over multiple years.

We created a binary variable for whether each bear-year contained a migration (1=yes; 0=no). In some instances with the rarified data, different subset iterations resulted in the same bear-year with and without migrations. We examined the proportion of subset iterations within each bear-year that identified the occurrence of a migration, and coded it as a migrating year if it was identified as such by >50% of the iterations. Additionally, we visually examined the full 2010s data to confirm that migrations occurred for bear-years that were questionable. We categorized migrations as occurring in either spring (i.e., April–June) or fall (July–October), and also measured the duration and maximum distance of the movement (measured from the first location of the bear-year after den emergence).

We used a generalized linear mixed-effects model (GLMM) with a binomial distribution and a linear mixed-effect model with a log-transformed response to test whether the probability of bears making a migration had changed, as well as patterns in duration and distance, since the 1980s. We used sampling period and demographic group as covariates and included an interaction term. We also controlled for the age of the bear in the model. A random intercept for bear ID was included to account for repeated measurements on the same bears over multiple years.

## RESULTS

We calculated the home range size for 130 bear-years ( $n=52$  male-years and 78 female-years) for 65 different individuals ( $n=33$  M, 32 F) in the 1980s and 63 bear-years (37 male-years and 26 female-years) for 32 different individuals ( $n=20$  M, 12 F) in the 2010s. While the KDE yielded larger home range estimates than the MCP, the magnitude of difference between the sampling periods was generally consistent. With both the KDE and MCP approach, average male home range size during the 2010s was approximately twice that in the 1980s, after accounting for age (Table 1, 2; Figure 2, 3;  $P=0.015$ ,  $P=0.011$ , KDE and MCP, respectively). Female home range sizes in the 2010s also were generally larger than those in the 1980s (Table 1, 2; Figures 2, 3), after controlling for age. Home ranges of females with cubs in the 2010s were 2–3 times larger ( $P=0.012$ ,  $P=0.086$ , KDE and MCP, respectively) and their core areas (50% KDE) were almost 4 times larger ( $P=0.010$ ) than in the 1980s. Females without cubs in the 2010s had home ranges that were approximately twice the size of 1980s, using either KDE ( $P=0.051$ ) or MCP ( $P=0.053$ ). Core area size was not significantly different ( $P=0.058$ ,  $P=0.117$ , KDE and MCP, respectively). Home ranges of bears increased with age. Within the first 10 years of a bear's life, 95% MCP home ranges doubled in size, on average; whereas, the core home range size increased by approximately 50% ( $P=0.001$ ). KDE ranges increased less than MCP in the first 10 years (20% for 95% KDE, 30% for 50%KDE), but still significantly ( $P=0.001$ ). There was no difference between the 2 sampling periods regarding home range expansion as they aged.

We detected 137 migrations (62% of bear-years) during the 1980s and 52 (74% of bear-years) during the 2010s. The propensity to migrate was not different from the 1980s to 2010s for males (65% vs. 75%,  $P=0.095$ ), females with cubs (61% vs 86%,  $P=0.248$ ), and females without cubs (62% vs. 71%,  $P=0.447$ ). The probability of migrating increased by 10% every 5 years of a bear's life, until about 15 years old (then remained stable,  $P=0.026$ ). The average duration of spring migrations was about 1 week shorter for males ( $P=0.008$ ), females with cubs ( $P=0.296$ ) and females without cubs ( $P=0.047$ ) in the 2010s than the 1980s (Table 3). In fall, males ( $P=0.255$ ), females with cubs ( $P=0.219$ ), and females without cubs ( $P=0.573$ ) were away from their home ranges for about 1 month in both the 1980s and 2010s (Table 4). The maximum spring migratory distance for each bear-year averaged 19 km for males, 5 km for females with cubs, and 6 km for females without cubs during the 1980s; in the 2010s, distances were similar

for males (mean = 17 km,  $P=0.713$ ), females with cubs (mean=5 km,  $P=0.260$ ), and females without cubs (Table 3; mean = 9 km,  $P=0.162$ ). In fall, migrations were farther than in spring for all demographic groups, but not different between the 2 time periods (Table 4). Males in the 1980s traveled an average distance of 38 km compared to 41 km in the 2010s ( $P=0.863$ ). The average fall migration distance for females with cubs was almost twice as large in the 2010s (27 km) than the 1980s (14 km) (although not significantly different,  $P=0.217$ ); whereas, females without cubs traveled an average of 16 km in the 1980s and 14 km in the 2010s ( $P=0.783$ )

## DISCUSSION

We found evidence to support our first hypothesis that home range size had increased since the 1980s. The home range size doubled for males and tripled for females. However, variability among individuals was high and our limited sample size of relocations may have hampered our statistical power, especially for 50% KDE and MCP. While some of our results were not statistically significant due to this variability, the consistent trend over time for both MCP and KDE estimators and both sexes suggest that home range size likely increased over the 3 decades in response to reduced food.

Bear density on the study area was significantly reduced from the 1980s to 2010s, but given the extensive overlap among ranges of bears, it seems unlikely that they expanded their home ranges just because there were fewer bears. Also, it is unlikely that competition for food is a major factor in bears, except possibly at very high densities, so a reduction in bear density did not provide more food per bear. Hence, in order to maintain body mass and reproduction, reduced foods prompted them to expand their home ranges. Male ranges also may have expanded to incorporate the larger and less densely-packed ranges of breeding females. It appeared that females with and without cubs had similar home range sizes, which suggests that mobility of cubs was not a limiting factor in home range size over the course of the active season.

Reduced food abundance did not cause bears to migrate more frequently, farther, or for longer despite the greater difficulty in locating far-flung bears by airplane in the 1980s versus GPS locations in the 2010s. We did observe a slight reduction in the duration of spring migrations for males and females with cubs in the 2010s and an apparent doubling in the fall migration distance for females with cubs in the 2010s. We believe these results may be related to the small sample size and high variability among individuals, particularly for the increase in fall migration distance for females. Two females made fall movements greater than 65 km with cubs; whereas, the remaining 5 female bear-years with cubs made movements more similar to the 1980s (i.e., 7-10 km). Garshelis and Noyce (2011) found that bears were less likely to migrate during years of poor natural foods to potentially avoid the risk of only finding poor foods elsewhere. Additionally, fall migrations may be influenced by human-related food sources: notably corn fields and hunters' baits. Preliminary results from our stable isotope analysis (a method for reconstructing annual assimilated diet) suggest that most bears in our study area had spent some time feeding on corn or corn-derived foods (such as high fructose corn syrup) in the fall. While there are no corn fields within the study area, we know that at least 30 study bears (either collared or ear-tagged) have used hunters' baits in some capacity (based on either direct reports by hunters or bears harvested at baits during 2015-2019).

Our results demonstrated the resilience of black bears to a substantial decline in natural foods, which they accomplished, in part, by altering their movements. However, other potential consequences of these movements should be noted. In Minnesota, harvest vulnerability varies by sex and age and is mediated by food availability, particularly key fall foods (Noyce and Garshelis 1997). While individuals maintained body conditions and reproduction similar to the 1980s despite the diminished foods, these bears are now traveling farther for food and

potentially supplementing their diet more with human-related foods, which may increase their exposure to bait piles and influence their harvest vulnerability. Understanding the variation among individuals and the repercussions it could have on current population models is vital for making informed management decisions.

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Table 1. Average 95% Kernel Density Estimate and Minimum Convex Polygon home range estimates (km<sup>2</sup>) with 95% CI for American black bears in the 1980s (1981-1989) and 2010s (2015-2019) in northcentral Minnesota. Estimates are from a linear mixed-effects model that accounts for age of the bear.

Demographic group	Minimum convex polygon		Kernel density estimate	
	1980s	2010s	1980s	2010s
Male	47 (34 – 64)*	89 (61 – 128)*	180 (126 – 256)*	359 (238 – 541)*
Female with cubs	9 (6 – 14)	19 (9 – 37)	24 (14 – 42)*	86 (37 – 199)*
Female without cubs	12 (9 – 16)	22 (13 – 35)	35 (25 – 49)	68 (39 – 119)

\* Significant difference between time periods ( $P < 0.05$ ).

Table 2. Average 50% Kernel Density Estimate and Minimum Convex Polygon home range estimates (km<sup>2</sup>) with 95% CI for American black bears in the 1980s (1981-1989) and 2010s (2015-2019) in northcentral Minnesota. Estimates are from a linear mixed-effects model that accounts for age of the bear.

Demographic group	Minimum convex polygon		Kernel density estimate	
	1980s	2010s	1980s	2010s
Male	7 (5 – 10)*	17 (12 – 24)*	40 (28 – 55)*	79 (54 – 117)*
Female with cubs	2 (1 – 4)	4 (2 – 8)	6 (3 – 9)*	19 (9 – 42)*
Female without cubs	3 (2 – 3)	4 (3 – 7)	9 (6 – 12)	16 (9 – 27)

\* Significant difference between time periods ( $P < 0.05$ ).

Table 3. Spring (April – June) migration summary table for American black bears in the 1980s (1981-1989) and 2010s (2015-2019) in northcentral Minnesota. Numbers in parentheses indicate 1 SE.

Demographic group	1980s				2010s			
	Mean duration (days)	Max (days)	Mean Distance (km)	Max (km)	Mean duration (days)	Max (days)	Mean distance (km)	Max (km)
Males	41 (3)*	44	19 (3)	75	23 (1)*	40	17 (2)	66
Females with cubs	25 (5)*	32	5 (1)	6	18 (2)*	24	6 (1)	9
Females without cubs	28 (4)	58	6 (1)	13	23 (1)	34	9 (2)	27

\* Significant difference between time periods ( $P < 0.05$ ).

Table 4. Fall (July-September) migration summary table for American black bears in the 1980s (1981-1989) and 2010s (2015-2019) in northcentral Minnesota. Numbers in parentheses indicate 1 SE.

Demographic group	1980s				2010s			
	Mean Duration (Days)	Max (Days)	Mean Distance (km)	Max (km)	Mean Duration (Days)	Max (Days)	Mean Distance (km)	Max (km)
Males	30 (3)	62	38 (5)	209	33 (2)	68	41 (6)	139
Females with cubs	35 (5)	71	14 (2)	47	42 (4)	57	27 (11)	67
Females without cubs	35 (3)	79	16 (3)	101	33 (3)	63	14 (4)	80

\* Significant difference between time periods ( $P < 0.05$ ).

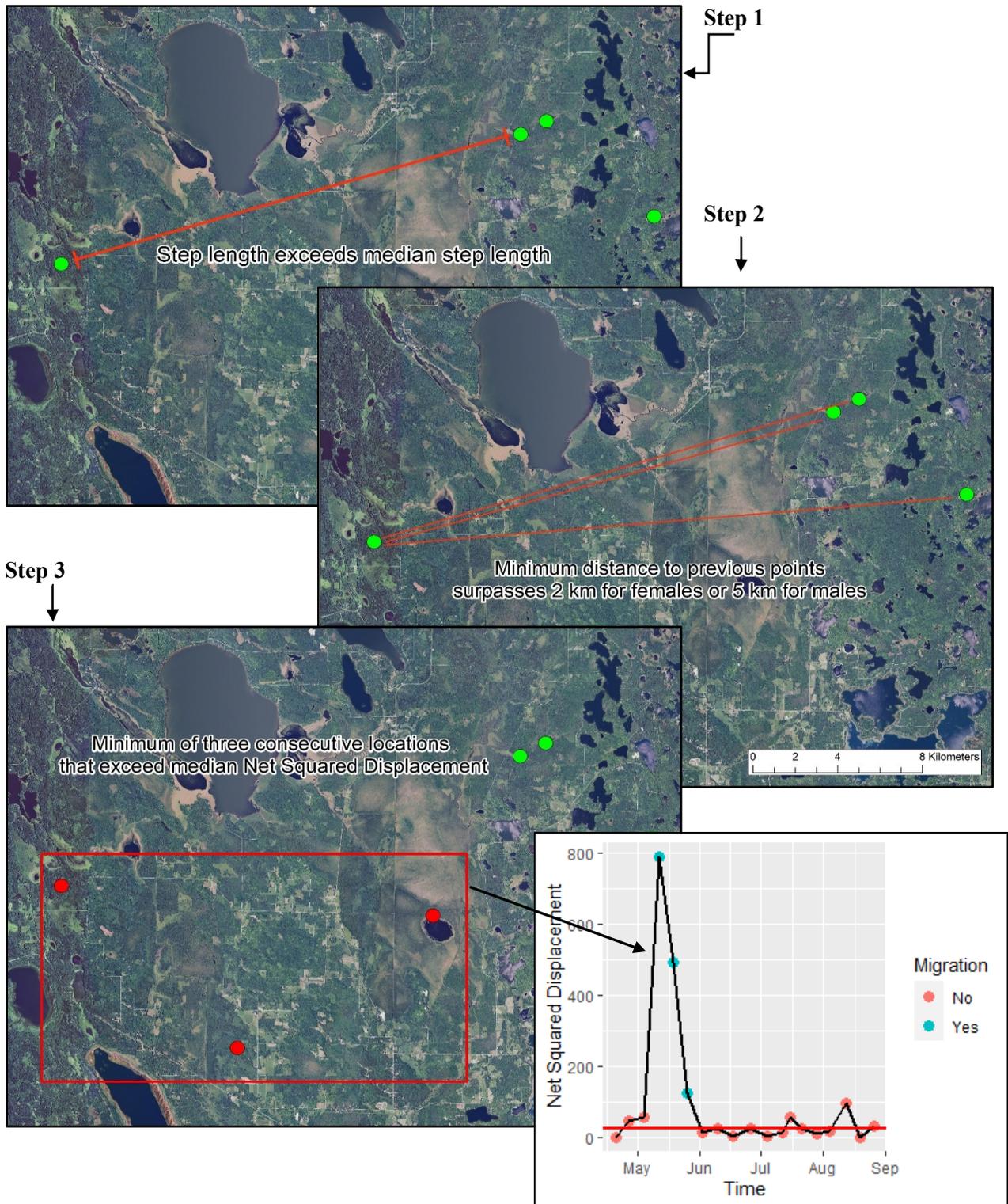


Figure 1. Flow chart depicting the 3-step process of identifying large seasonal migrations of American black bears. Migrations must contain a large abrupt movement, be far away from previous points, and must remain far away for a minimum of 3 consecutive locations.

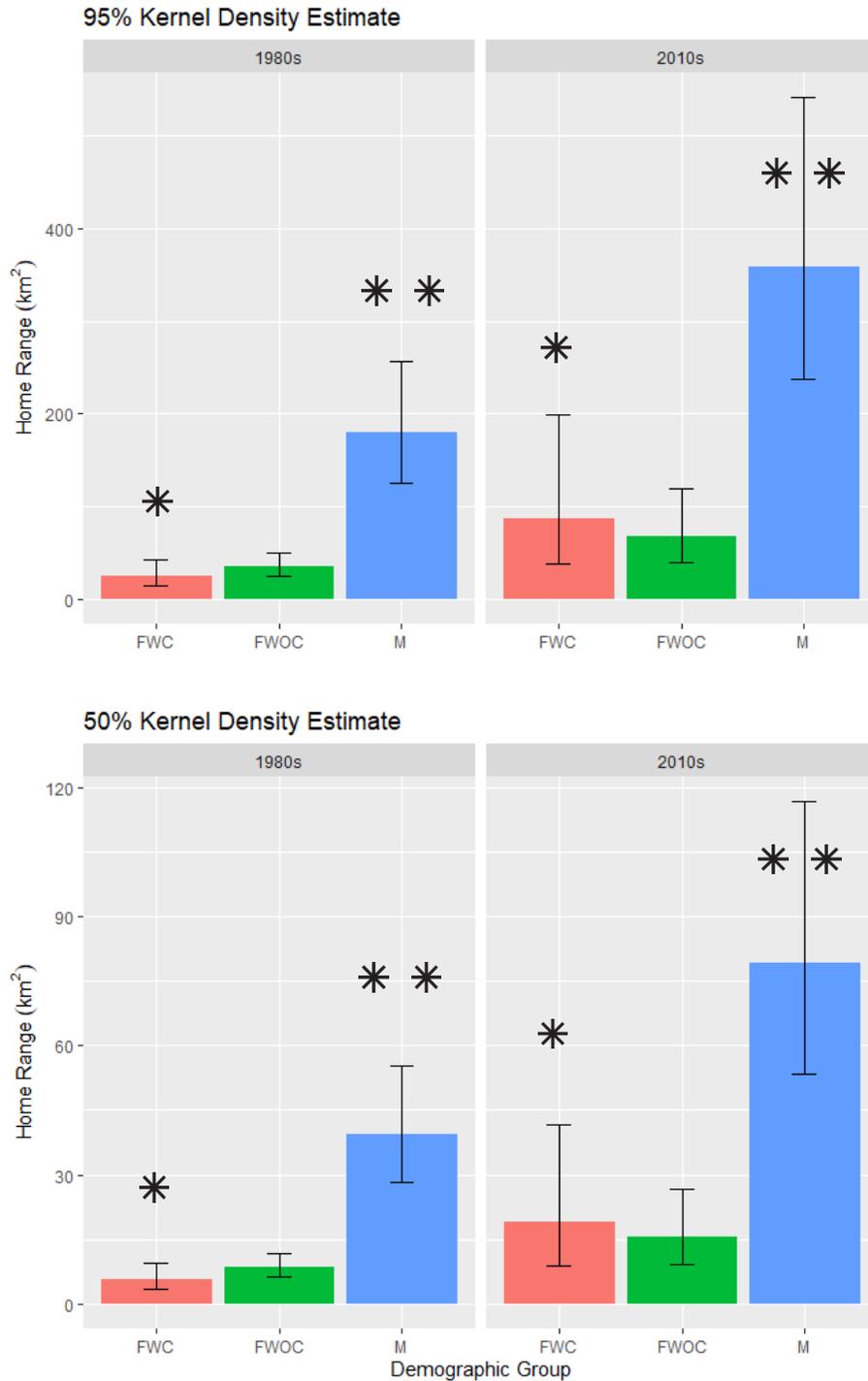


Figure 2. Bar graph of 95% and 50% ad-Hoc scaled Kernel Density Estimate home ranges for American black bear females with cubs (FWC), females without cubs (FWOC), and male (M) bears from the 1980s (1981 – 1989) and 2010s (2015 – 2019) in northcentral Minnesota. Error bars depict 95% confidence intervals. Matching asterisks indicate significant differences ( $P < 0.05$ ).

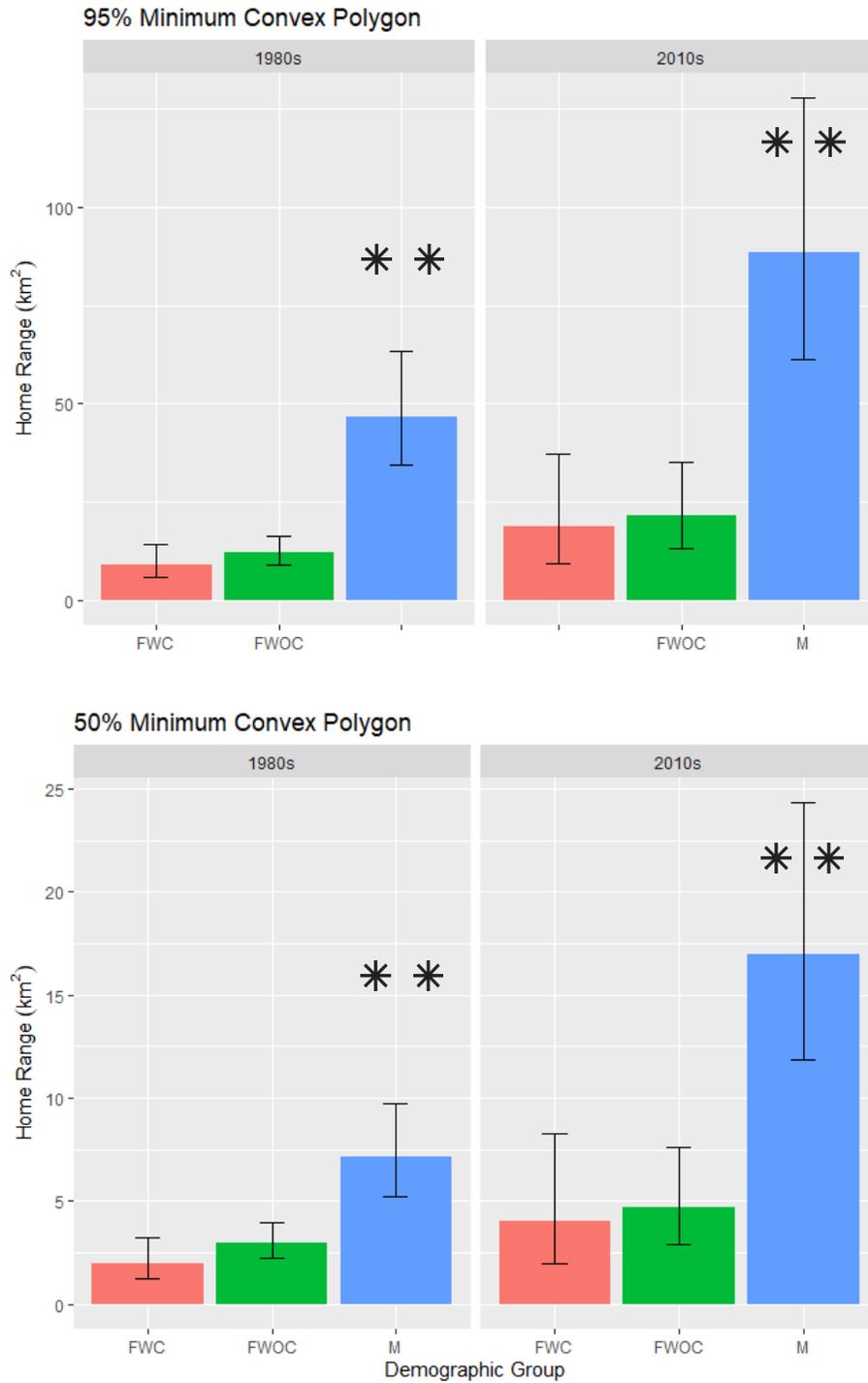


Figure 3. Bar graph of 95% and 50% Minimum Convex Polygon home ranges for American black bear females with cubs (FWC), females without cubs (FWOC), and male (M) bears from the 1980s (1981 – 1989) and 2010s (2015 – 2019) in northcentral Minnesota. Error bars depict 95% confidence intervals. Matching asterisks indicate significant differences ( $P < 0.05$ ).



## ECOLOGY AND POPULATION DYNAMICS OF BLACK BEARS IN MINNESOTA

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### SUMMARY OF FINDINGS

During April 2019–March 2020, we monitored 34 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. This is part of an ongoing study, initiated in 1981, with current efforts aimed at causes of mortality and spatial and temporal variation in cub production. During summer, we captured and collared 11 more bears in the CNF. Hunting has been the primary source of mortality in all areas; even though VNP and Camp Ripley are unhunted, bears may wander off to other areas in fall where they are vulnerable to hunters. Hunters were asked not to shoot collared bears, all of which are conspicuously marked with large colorful eartags. Two were shot in the CNF this year. Reproduction was strongly affected by food supply. Bears grew fastest and matured earliest in the NW and Camp Ripley. Age of primiparity was most delayed in VNP. Litter size, though, did not correspond with weight. Food supply in the CNF has significantly diminished since the study began there in the 1980s, but there is no indication of reduced reproduction, indicating that these bears compensated for the reduced natural foods through changes in behavior (e.g., enlarged home range size, altered habitat use, increased use of human-related foods — all the subject of a companion study).

### INTRODUCTION

Telemetry-based research on American black bears (*Ursus americanus*) was initiated by the Minnesota Department of Natural Resources (MNDNR) in 1981, and has been ongoing continuously since then. For the first 10 years, the bear study was limited to a site near the geographic center of the Minnesota bear range, in an area mainly within or abutting the Chippewa National Forest (CNF; 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. A chief goal at that time was to assess causes of mortality, specifically the relative impacts of hunting. This study began just as the MNDNR switched from an unrestricted bear hunt to a quota on bear hunting license sales in 1982, in reaction to a concern that the population was being over-hunted. We used black radiocollars and inconspicuous eartags, and requested that hunters treat study bears as they would any other bear.

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Commensurately, we studied the reproductive rate, and factors influencing that rate (Noyce and Garshelis 1994). The view among bear biologists at that time was that black bears had one of the lowest reproductive rates of any large mammal in North America. But, we found higher rates of reproduction in Minnesota than studies in western states.

Using physical mark–recapture, where collared bears were the marked population, we tracked an increase in the study population on the CNF through the 1980s, despite harvest rates often exceeding 20% (Garshelis 1991, Garshelis et al. 1988). We found no other factor that significantly affected the population size or trend. A striking finding was that bears rarely died of natural causes. For example, despite (or because of) being in winter dens for 6 months per year, winter weather conditions had no effect on their survival (Garshelis et al. 2020).

We also gained considerable new information about the ecology of bears. Notable findings included: a flexible diet, but with conspicuous effects of food supply on rates of mortality (from hunting: Noyce and Garshelis 1997) and reproduction; varied use of habitat that reflected the changing availability of foods during the course of the year and among years, as well as bears apparently striving for dietary diversity (Garshelis and Noyce 2008); extensive seasonal movements (migrations) to take advantage of richer food sources outside their normal home ranges (Noyce and Garshelis 2011, 2014); and diverse sites and structures used as den sites, which showed no relation to reproduction or survival (Garshelis et al. 2020).

To better understand the dynamics of bear populations across Minnesota, we added more study sites, including 2 sites where bear hunting was prohibited, and 1 site at the front of a recent geographic expansion, where the habitat was dominated by agriculture. We hypothesized that causes of mortality and rates of reproduction would differ among these sites.

## **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.

## **STUDY SITES**

During the 1980s, the CNF study site was heavily logged, resulting in a matrix of stands of various ages, with many small unpaved logging roads. A unique feature of this study site is that the east side is dominated by upland forests, largely aspen (*Populus tremuloides*, *P. grandidentata*), whereas a large swath of the western part of this area is a forested bog dominated by black spruce (*Picea mariana*) and tamarack (*Larix laricina*). In recent years, more roads have been paved and/or widened; also, forest cutting on federal lands has diminished, so forests have aged and the amount of aspen on the landscape has declined. Nearly all bear foods are found in the understory. Oaks (*Quercus* sp.) are not common anywhere in this area, and commercial agriculture is nonexistent. A series of population estimates for this study site indicate that it increased through the 1980s, but diminished by at least half by 2012 (Garshelis 1991, Garshelis and Noyce 2013, Gondek et al. 2016).

In 1991 our study expanded to include Camp Ripley Training Center, a National Guard facility at the southern periphery of the primary bear range (Figure 1). Bear hunting is prohibited on Camp Ripley, but bears may be hunted if they range outside. Oaks are plentiful, which accounts for bears growing much faster and to a larger mature size than in the CNF (Figure 1). The 210-km<sup>2</sup> area is long and narrow (6–10 km wide), and bordered by highways and cornfields.

In 1997 we added Voyageurs National Park (VNP), located along the northern edge of the Minnesota bear range (bordering Canada; Figure 1). The study site is a 300-km<sup>2</sup> roadless peninsula bounded by 3 large lakes. VNP had the poorest and shallowest soils, no timber cutting, and a largely coniferous forest, so provided the least food for bears. Bears in this area grew slowly and never reached the size of CNF bears (Figure 1). Hunting is prohibited, but bears are exposed to hunting if they leave the park.

In 2007, we initiated work at the northwestern edge of Minnesota's bear range (NW; Figure 1). This area is largely agricultural, although only 2% of the land area is planted with crops consumed by bears. Forested land, which comprises less than 20% of the area, is patchily distributed in small, privately-owned woodlots and state Wildlife Management Areas, which are open to hunting. The density of roads is high. A low density of bears occupied this area until 1995, when poor natural food in neighboring areas spurred an influx of bears attracted to corn and sunflowers. Since then the density of bears in this area has remained higher. Despite the patchy habitat, bears in this area grew quickly and attained adult sizes similar to Camp Ripley bears (Figure 1), due to rich natural foods and agricultural crops (Ditmer et al. 2016, 2018).

## **METHODS**

During May–August, 2019, we captured bears in the CNF with barrel traps or Aldrich foot snares, and immobilized them with a relatively new drug for use in black bears called BAM (commercial drug combination of butorphanol, azaperone, and medetomidine; ZooPharm, Fort Collins, Colorado), as per Williamson et al. (2018). 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 bears that we thought would not disperse from the study area. We used GPS-Iridium collars (Telonics Inc., Mesa, AZ) on all but yearlings in the CNF, and mainly very high frequency (VHF) collars on the other study sites. All collared bears had brightly-colored, cattle-size ear tags (7x6 cm; Dalton Ltd., UK) that would be plainly visible to hunters. 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.

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 were presumed to have died.

## **RESULTS AND DISCUSSION**

### **Radiocollaring and Monitoring**

As of April 2019, the start of the current year's work, we were monitoring 34 radiocollared bears: 22 in the CNF, 8 at Camp Ripley, 2 in VNP, and 2 in the NW (Table 1). During May–August we captured 17 bears in the CNF and collared 11 of them (6M, 5F).

The sex ratio of our sample in the CNF had been heavily skewed toward males; during 2015–2018 we captured 37 males and 10 females (and radiocollared larger males and all females). This year we captured 4 new adult females (all aged 7 or 8 years old). Presumably, these female bears were all living in the study area where we previously trapped, but avoided capture.

Moreover, we did not previously detect them on camera traps. This year we switched bait, from bacon (our traditional bait since the 1980s) to trail mix (nuts, dried fruit, small bits of chocolate). It is unclear whether this was particularly attractive to these bears. We stopped using bacon because it decomposed in just a few days and needed to be constantly changed.

### **Mortality**

Since 1981, we recorded the cause of death for 391 radiocollared bears, 76% of which died (or likely died) from legal hunting (Table 2). During the 2019 bear hunting season, 2 collared CNF bears were shot (Table 1). In all 4 study sites, legal hunting was the primary cause of mortality (Figure 2), despite (a) Camp Ripley and VNP being un hunted (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 19 years (spanning the full period of the NW study).

Vehicle collisions and killing of nuisance bears each represented 7% of the pooled mortality that we observed within these 4 study sites (Table 2). One CNF collared adult male was killed as a nuisance this year, supposedly mistaken as a raccoon (*Procyon lotor*) in the trash. No collared bears were killed on roads this year. Vehicle collisions have been most common at Camp Ripley (Figure 2), which is flanked by 2 highways with fast-moving traffic.

Natural mortality accounted for <5% of deaths of collared bears, aged 1–39 years. When we established the 4 study sites we hypothesized that natural mortality would be highest in VNP, since inside the park there is no legal hunting, no roads, and no killing of nuisance bears. This year, 1 of 2 females (6-year old sisters who we have been monitoring to assess age of first reproduction) died apparently from wolf predation while in her den. We only found a chewed collar on top of the snow in mid-March and never found the den site, so we were unable to know for sure what happened. Interestingly, though, this bear chose an open nest den the previous year whereas her sister (who produced her first litter of cubs this year, see below) denned in rock caves for the past 2 years. This is the second wolf-killed collared bear in 2 years; last year wolves killed a collared bear in the NW.

### **Reproduction**

Since 1982, within the 4 study areas, we collected data on 328 litters with 854 cubs. This year we obtained data on 14 new cub litters and 5 yearling litters. This year, like a number of other recent years, we were unable to obtain reproductive data from all of the female dens in Camp Ripley, either because they were too far inside culverts or denned in an area that was off limits to us (impact zone).

Across all years and study sites, litter size averaged 2.6 cubs. Means within study areas ranged from 2.3–2.8; Tables 3–6). In CNF and NW, 3-cub litters were most common (Figure 3). In VNP, where adult females were smaller (Figure 1), 2-cub litters were most common. In Camp Ripley, 3-year-old first-time mothers tended to have 2-cub litters; excluding these, an equal number had 2- and 3-cub litters (Figure 3). We cannot explain why Camp Ripley bears, which are generally heavier than CNF bears (Figure 1), tended to produce smaller litters. NW bears, which were similar in size to Camp Ripley bears, produced mainly 3-cub litters, but also the highest proportion of 4-cub litters (Figure 3).

Sex ratio of cubs in March dens was 50.7% male overall (Tables 3–6). Over the 39 years of monitoring in the CNF, the cumulative cub sex ratio has remained at about 52% male (with yearly variation due to small sample sizes, Table 3). The sex ratio among yearlings in winter dens was more female-skewed (48.2% male), due to a significantly higher mortality among male cubs (23.4%) than female cubs (15.3%;  $X^2=6.29$ ,  $P=0.01$ ). Cub mortality was highest in VNP (Table 6), but the 4 sites were not appreciably different.

Age of first reproduction was the reproductive parameter that varied most among study sites. Half the females in Camp Ripley and NW sites produced their first surviving litter at 3 years old, whereas only 3 of 92 (3%) CNF bears had cubs at 3, and no VNP bears produced cubs at 3 or even 4 (Figure 4). Those bears that produced surviving litters at 3 years old had yearlings at 4, so were not available to have cubs again until age 5. Thus, for Camp Ripley and NW bears, only half the bears were available to produce at 4, and 45% did so, leaving only  $\approx 5\%$  that eventually reproduced for the first time at 5. In CNF, only 33% produced at 4 and 40% produced at 5 (only a few of which had also produced at 3). By age 6, about half the bears in each site were producing cubs every year. Two bears currently in our study, both at Camp Ripley, produced cubs at 20 years old (1 this year, 1 last year). Previously, a female in VNP produced cubs at 21 and a female in CNF produced at 22, but both were lost from the study before they reached reproductive senescence. Another female in CNF produced her last litter at 25 years old, and lived until 39.

A few bears occasionally missed having cubs in a year where they were available to do so, extending the litter interval to 3 years (Table 7). This was most common in Camp Ripley, an unexpected finding given that female bears in this area tended to be large. Whereas the small size of bears in VNP resulted in smaller litters and possibly higher cub mortality, it did not cause more missed litters.

Weights of female bears is a major driver of reproduction, as demonstrated by the differences among study sites. Likewise, we found that within the CNF study site, heavier bears were more prolific (Noyce and Garshelis 1994, Garshelis and Noyce 2008). In a companion study in the CNF, Rettler (2018) found that biomass of natural foods was 70% less in the 2010s than what it had been in the 1980s, when we first began working at this site. We have a small sample of collared females in the 2010s, but data so far show no indication of diminished weights or reproduction. In fact, reproductive rates (cubs/adult females monitored), which combine all aspects of reproduction (litter size, litter interval, and age of primiparity) have been higher during the 2010s than during the 1980s and 1990s (Figure 5). To some extent this may be a peculiarity of the relatively small sample size: during 2011–2015 we monitored only the same 2 adult females, each of which produced litters of 3 or 4 cubs every other year. During 2016–2020 we observed 13 different adult females in dens, but 4 were observed for just a single year (2020) when they happened to have had cubs. If all of the currently collared CNF females were monitored another year and remained on a 2-year reproductive schedule, and the 2021 data combined with the 2016–2020 period, the reproductive rate would drop from 0.83 female cubs/adult female to 0.67, which is still above the long-term average from pre-2000 (0.56, Figure 5). Our main conclusion, therefore, is that reproduction showed no indication of a decline despite the drastically reduced abundance of natural foods in the CNF.

### **Removal of Collars**

This study was a part of a larger investigation into how bears in the CNF coped with diminished natural foods. Given that their reproduction appeared not to be negatively affected, we posited that they may have used larger home ranges (see Rettler et al. 2020), used different habitats within their home ranges, and (or) relied more on human-related foods. GPS-collared bears of both sexes were used to test these hypotheses during 2016–2019. With sufficient data now collected, and most collars soon to expire, we terminated data collection on males and non-reproductive females, and removed 19 collars from the CNF sample during this year's winter den work. Also, with sufficient data collected in the NW, we removed collars on the 2 adult females there. Reproductive monitoring will continue with 8 adult female bears in each of the CNF and Camp Ripley, and 1 at VNP (collar kept on because this was her first litter).

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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 2019–March 2020.

	CNF	Camp Ripley	VNP	NW
Collared sample April 2018	22	8	2	2
Trapped and collared	11			
Collared in den				
Killed in vehicle collision				
Killed by Minnesota hunter <sup>a</sup>	2			
Killed as nuisance	1			
Natural mortality			1 <sup>b</sup>	
Removed radiocollar	19 <sup>c</sup>			2
Dropped radiocollar	2 <sup>d</sup>			
Collared sample April 2019	9 <sup>e</sup>	8	1	0

<sup>a</sup> Hunters were asked not to shoot collared bears (although it was still legal).

<sup>b</sup> Adult female apparently killed by wolves while denning.

<sup>c</sup> Collars were removed on males and young females.

<sup>d</sup> Expandable collars on 2 yearlings expanded too much and dropped off.

<sup>e</sup> Includes 1 male whose collar was not removed because his den could not be located.

Table 2. Causes of mortality of radiocollared black bears  $\geq 1$  year old in 4 Minnesota study sites, 1981–2019. 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 <sup>a</sup>	239	13	16	14	282
Likely shot by hunter <sup>b</sup>	9	1	0	4	14
Shot as nuisance	23	2	1	3	29
Vehicle collision	14	10	1	3	28
Other human-caused death	9	1	0	0	10
Natural mortality	8 <sup>c</sup>	3	6	1	18 <sup>c</sup>
Died from unknown causes	5	2	0	3	10
Total deaths	307	32	24	28	391

<sup>a</sup> 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.

<sup>b</sup> Lost track of during the bear hunting season, or collar seemingly removed by a hunter.

<sup>c</sup> 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, 1982–2020. 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 <sup>a</sup>
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%	—
2015	0	0	—	—	—
2016	0	0	—	—	—
2017	1	3	3.0	—	0%
2018	4	12	3.0	42%	0%
2019	1	4	4.0	50%	75% <sup>b</sup>
2020	6	22	3.7	50%	—
Overall	191	513	2.7	52%	19%

<sup>a</sup> Cubs that were absent from their mother's den as yearlings were considered dead.

<sup>b</sup> 3 of 4 cubs in 1 litter did not survive.

Table 4. Black bear cubs examined in dens in northwestern Minnesota during March, 2007–2020.

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%	25%
2019	1	2	2.0	50%	0%
2020	1	4	4.0	50%	
Overall	34	96	2.8	44%	16% <sup>a</sup>

<sup>a</sup> 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, 1992–2020.

Year	Litters checked	Number of cubs	Mean cubs/litter	% Male cubs	Mortality after 1 year <sup>a</sup>
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 <sup>b</sup>	— <sup>b</sup>	—	—	—
2015	6	15	2.5	20%	10%
2016	0	0	—	—	—
2017	4	10	2.5	60%	0%
2018	2	5	2.5	20%	— <sup>c</sup>
2019	3	7	2.3	71%	0%
2020	3	6	2.0	83%	—
Overall	58	137	2.4	50%	17%

<sup>a</sup> Blanks indicate no cubs were born to collared females or collared mothers died before the subsequent den visit to assess cub survival.

<sup>b</sup> Cubs heard, litter not handled. Camera set outside den indicated that all cubs died. This litter not included in total.

<sup>c</sup> No yearling dens could be checked: all were in culverts or in impact area.

Table 6. Black bear cubs examined in dens in Voyageurs National Park, Minnesota, during March, 1999–2020. All adult collared females were killed by hunters in fall 2007, so sample sizes greatly diminished afterward.

Year	Litters checked	Number of cubs	Mean cubs/litter	% Male cubs	Mortality after 1 year <sup>a</sup>
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 <sup>b</sup>	0	—	—	—
2017	0	0	—	—	—
2018	0	0	—	—	—
2019	0	0	—	—	—
2020	1	3	3.0	33%	—
Overall	30	68	2.3	47%	25%

<sup>a</sup> 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.

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

Table 7. Intervals between litters (where at least 1 cub survived) for black bears within 4 study sites in Minnesota through March 2020 (CNF since 1981, Camp Ripley since 1991, VNP since 1997, NW since 2007). Cubs typically remain with their mother for about 17 months, so the normal reproductive interval is 2 years.

Study area	2-year reproductive intervals	≥3-year reproductive intervals	% intervals ≥3 years
CNF	115	8	7%
Camp Ripley	37	5	12%
VNP	15	1	6%
NW	20	0 <sup>a</sup>	0%

<sup>a</sup> Excluding 1 missed litter (3-year interval) that was due to the bear leaving the den after disturbance and aborting the litter.

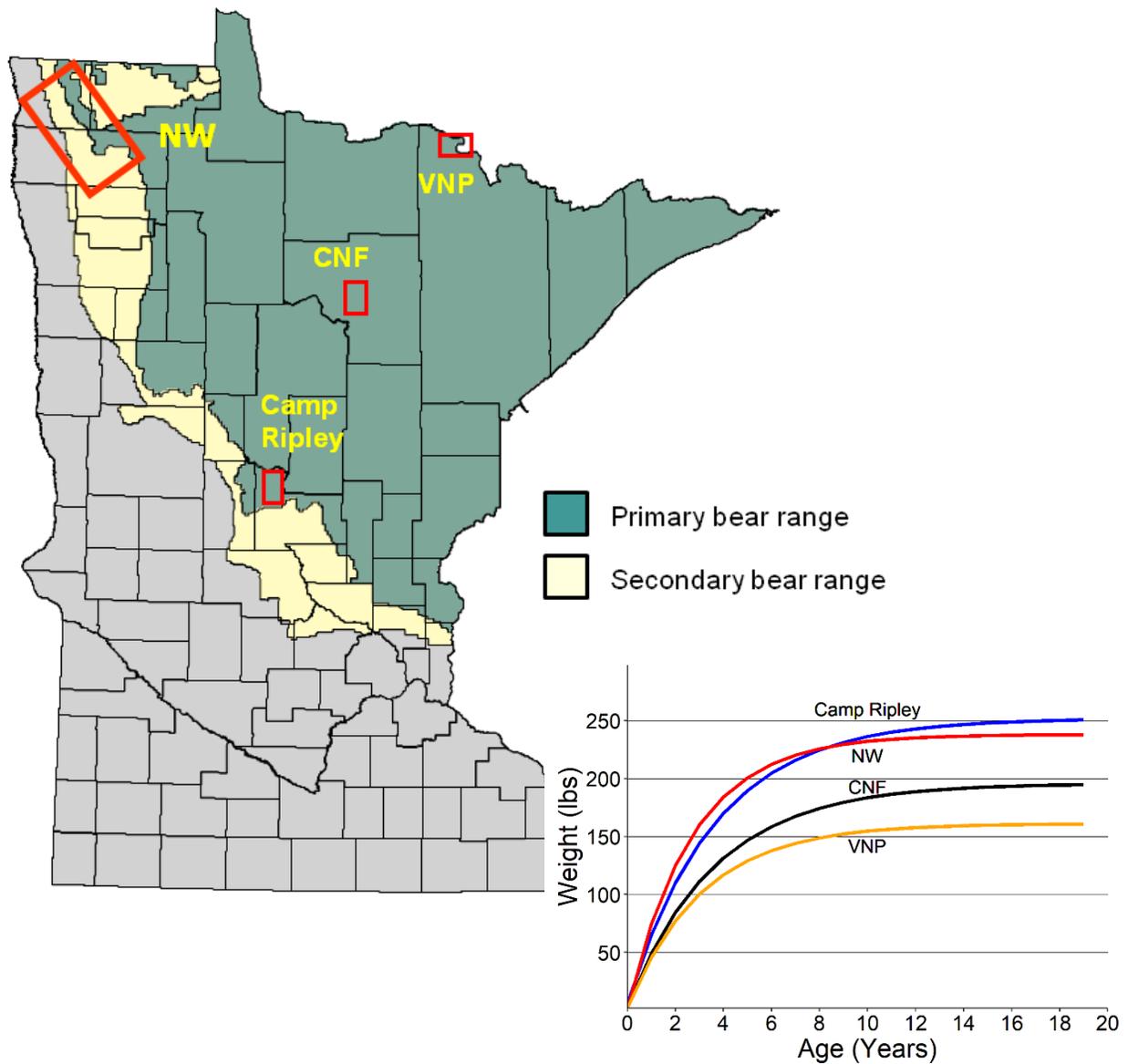


Figure 1. Location of 4 study sites within Minnesota’s bear range: CNF (Chippewa National Forest, central bear range; 1981–2020); VNP (Voyageurs National Park, northern fringe of range; 1997–2020); Camp Ripley Military Reserve (near southern edge of range; 1991–2020); NW (northwestern fringe of range; 2007–2020). Inset shows non-linear regressions of weights of female bears of each age during February–March (late denning).

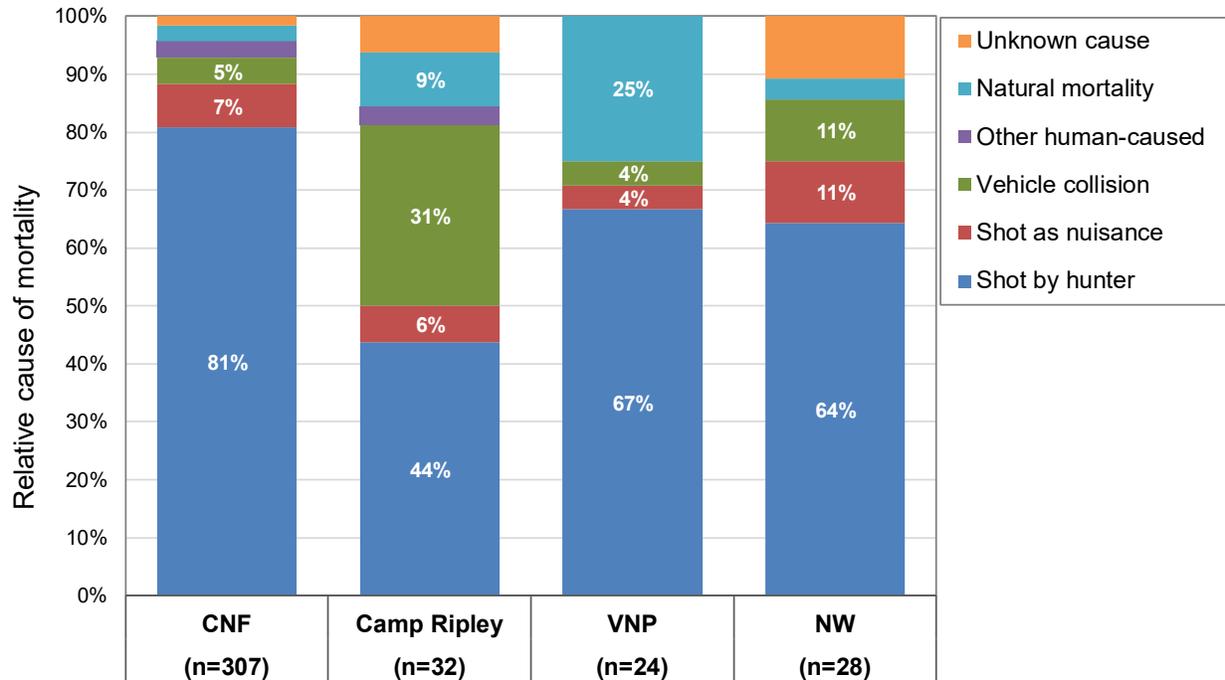


Figure 2. Proportional causes of death of radiocollared bears in each of 4 study sites in Minnesota, since the beginning of the study in each area through the winter of 2019–2020 (see map and dates for each study site in Figure 1). CNF expectedly had the highest proportion of bears killed by hunters 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 of mortality due to hunting for non-collared bears in that area would likely be considerably higher than shown here.

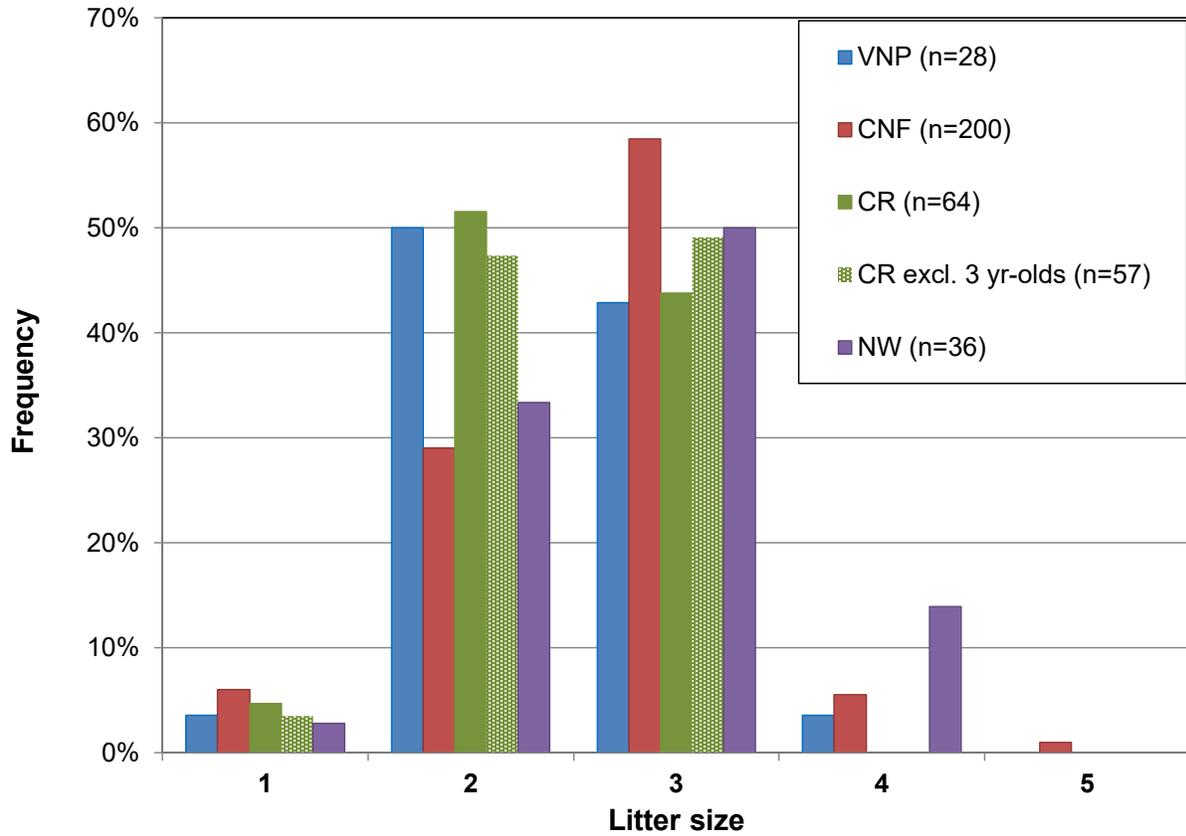


Figure 3. Frequency of cub litter sizes (examined in natal dens in March) within 4 Minnesota study sites (see Figure 1) through March 2020. Data include only litters in which at least 1 cub survived 1 year. Camp Ripley data are shown for mothers of all ages, as well as excluding 3-year-old mothers, which had smaller litters. For the other sites, elimination of 3-year-olds did not make a difference.

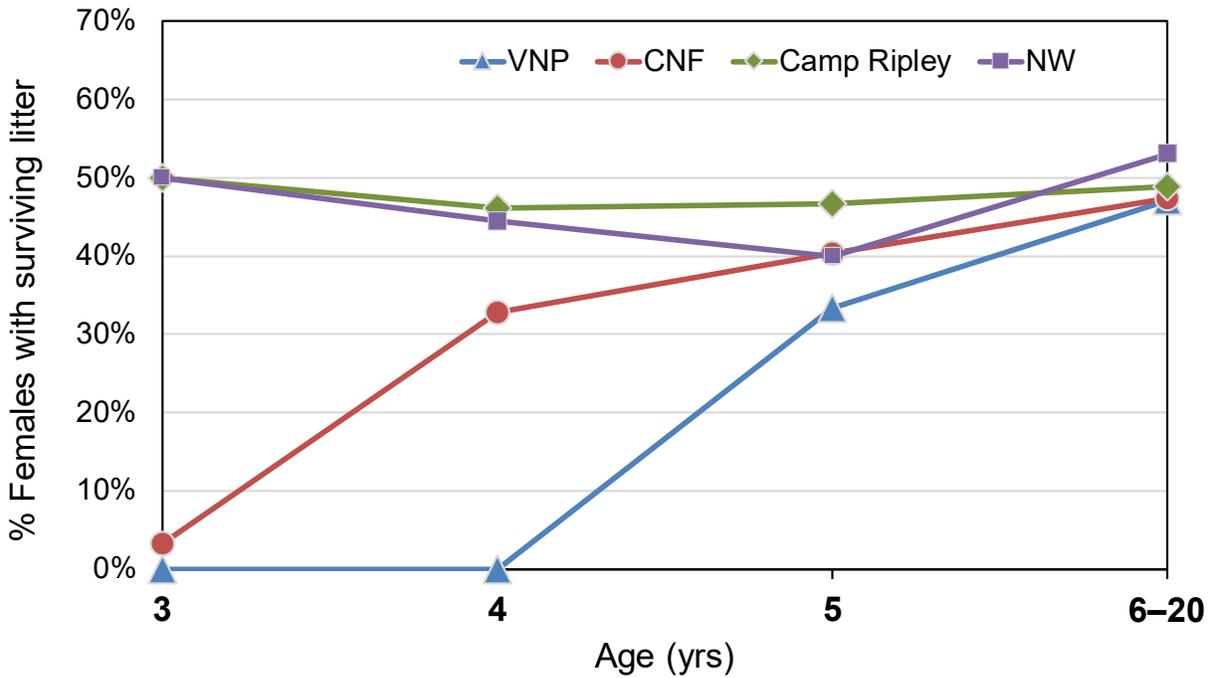


Figure 4. Percent of females producing a litter in which at least 1 cub survived until becoming a yearling (observed in the den) in each of the 4 Minnesota study sites through March 2020. Females matured earliest (half of them at 3 years old) in Camp Ripley and NW, and were most delayed in VNP (none produced cubs before age 5). Once producing cubs, most bears gave birth at 2-year intervals (Table 7), so by age 6, about half produced each year.

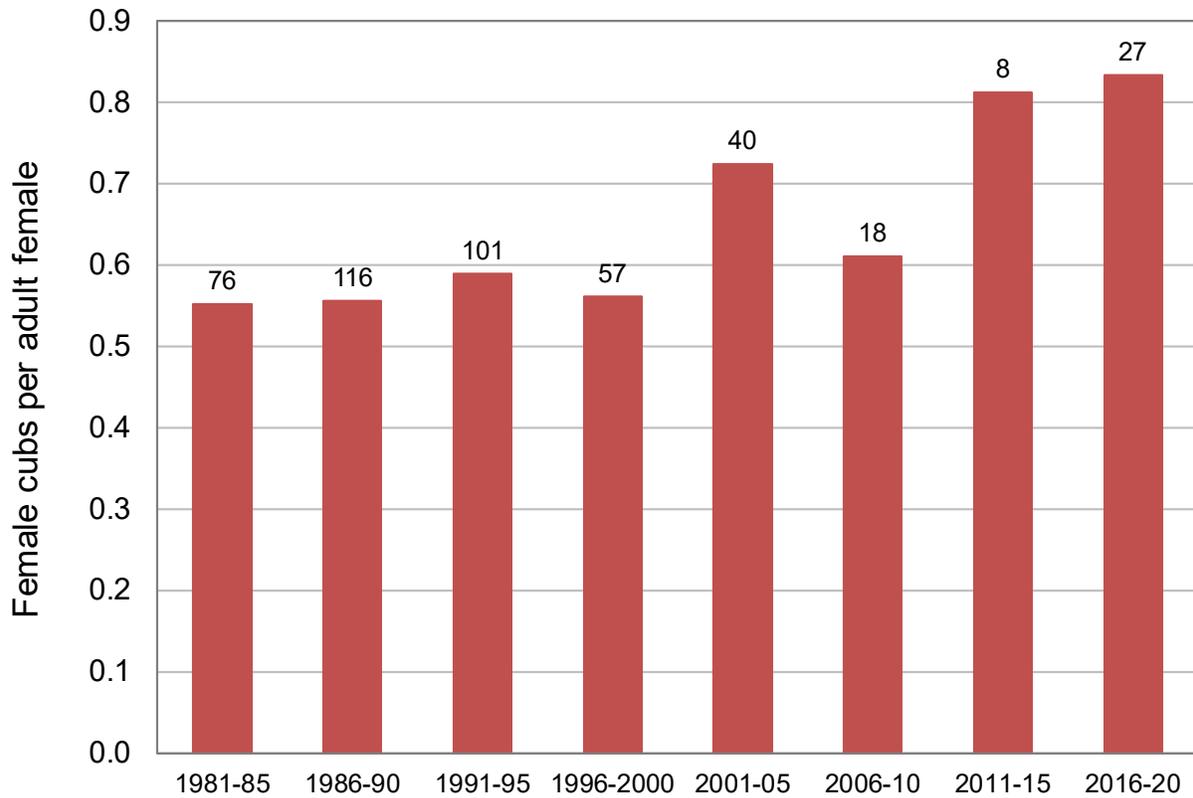


Figure 5. Reproductive rate of 4+ year-old female bears in CNF, Minnesota, study site, measured as female cubs produced (total cubs/2) divided by bear-years of monitoring (indicated by number above bar; 1 bear-year = 1 female bear observed at a den) for 5-year increments since study began. Data exclude litters in which no cubs survived. If all females produced 2.7 cubs (the long-term average at this site) every 2 years, the reproductive rate would be  $(2.7/2)/2 = 0.675$ . However, because less than half the females in the CNF produced cubs at 4 or 5 years old (Figure 4), the reproductive rate was generally  $<0.6$ . The seeming increase since 2011 may, in part, be a vagary of small sample size (see text).



## INFORMING WINTER HABITAT MANAGEMENT PRESCRIPTIONS AND POPULATION VITAL RATE ESTIMATES FOR WHITE-TAILED DEER IN NORTHERN MINNESOTA, WINTERS 2017–2018 TO 2019–2020

Glenn D. DelGiudice and Bradley D. Smith<sup>1</sup>

### SUMMARY OF FINDINGS

A recent report by the Office of the Legislative Auditor (OLA 2016) addressed the need for the Minnesota Department of Natural Resources (MNDNR) to develop a statewide white-tailed deer (*Odocoileus virginianus*) management plan to improve and maintain adequate numbers for hunting and wildlife viewing. The report acknowledged throughout that improved habitat management should be a key component of this plan. A greater understanding of winter habitat requirements of deer in northern Minnesota has been an ongoing need of wildlife managers to enhance their ability to plan, integrate and implement long-term forest and habitat management strategies with foresters. This need and the state of development of cutting-edge global positioning system (GPS) collar, remote sensing, and geographic information system (GIS) technologies prompted this study to inform a level of understanding of deer habitat requirements essential to prescribing forest manipulations that best support population goals. Herein, we present findings of ongoing analyses of data collected from 73 GPS-collared, adult ( $\geq 1.5$  yr) female deer during winters 2017–2018 to 2019–2020. Our analyses of deer winter home ranges (HR) and habitat use at the 2<sup>nd</sup> order, habitat availability at the site level and use at the HR level, strongly indicate notable individual variation in HR size and habitat use. However, on average, over varying winters 2017–2018 to 2019–2020, deer used cover groups in proportion to availability, with hardwoods, moderately dense and dense conifer stands, and forage types receiving the greatest use at both sites, with the exception of the greater availability and use of wetlands at Inguadona Lake than at Elephant Lake. Our ongoing more in-depth analyses, will examine 1) habitat selection at the 3<sup>rd</sup> order, proportional availability within deer home ranges (HRs) versus use (proportions of location-fixes) at the stand level, 2) fine-scale measurements of stands used and how they were used, and 3) how use was influenced by variations in snow depth and deer density.

### INTRODUCTION

Habitat management is recognized as the ultimate stage of progressive wildlife management (Krausman and Bleich 2013). Recently, a report by the Office of the Legislative Auditor (OLA 2016) recommended the Minnesota Department of Natural Resources (MNDNR) develop a statewide white-tailed deer (*Odocoileus virginianus*) management plan (MNDNR 2018), that included improving population estimates; also, improving habitat management was acknowledged as a necessary key component of this plan to establish and meet population

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<sup>1</sup> University of Minnesota, Department of Fisheries, Wildlife, and Conservation Biology, 2003 Upper Buford Circle, Ste. 135, St. Paul, MN 55108

goals. But the degree to which timber management is *good* deer management depends on the biologist's level of understanding of the relationship of wildlife to habitat and how well foresters can manipulate habitat to achieve population goals (Thomas 1979). A greater understanding of particularly winter habitat requirements of deer in northern Minnesota has been an ongoing need of wildlife managers to enhance their ability to plan, integrate and implement long-term forest and habitat management strategies and associated activities. The overall goal of our study is to inform that level of understanding necessary for managers to prescribe forest manipulations that best support population goals. These manipulations will consider composition, area, edge, edge: area ratio, shape, and abundance, as well as juxtaposition and interspersions or arrangement of cover types (e.g., conifer shelter, forage openings).

Phase I of this research began as a pilot study during winter 2017-2018, interfacing cutting-edge global positioning system (GPS) collars, remote sensing and geographic information system (GIS) technologies to establish the feasibility of making fine-scale measurements of habitat use and selection by deer at the *stand or cover type level* (hereafter, stand level) under varying environmental winter conditions (DeGiudice et al. 2017; Smith et al. 2019, 2020; Smith 2020). For management to benefit fully from such characterizations of cover type use, we are assessing habitat quality by examining associations with deer nutritional status and survival, another area sorely requiring additional research attention (DeGiudice et al. 2002, 2006, 2020).

Phase I's operational goal required assessing the performance of our Globalstar Recon GPS collars (Model IGW-4660-4, Telonics, Inc., Mesa, Arizona), programmed with Quick Fix Pseudorange (QFP), in different habitat types pre-deployment (stationary trials), as well as, once recovered from free-ranging deer. Details can be viewed in the aforementioned references, but to summarize, our collars consistently obtained 100% fix-success rates regardless of the cover type being used, had mean transmission rates of location-fixes to our base station during the trials and while deployed on deer of 96.7% and 88.1%, respectively, and exhibited mean spatial errors of 5.7 m and 16.1 m. Using stereo air photointerpretation of color infrared and natural color photos (1:15,840 scale) and Light Detection and Ranging (LiDAR), we successfully described the deer's winter habitat composition at the stand level down to a minimum of 0.5 ha on our Elephant Lake (1,012 total stands) and Inguadona Lake study sites. Employing the most recent version of ArcGIS (ArcGIS Pro 2.2.2, ESRI 2018), we reported preliminary estimates of each deer's winter home range (HR) and generated habitat composition layers for each site and deer HR to facilitate analyses of habitat use and selection by deer at 2<sup>nd</sup> and 3<sup>rd</sup> orders (Johnson 1980). Furthermore, using thousands of winter location-fixes, we demonstrated the ability to efficiently and accurately make fine-scale measurements to assess how deer use their habitat at the stand level (4<sup>th</sup> order) under varying environmental conditions.

Upon fulfilling our Phase 1 study goal and objectives, and recently completing our third winter of data collection, herein our goal is to highlight the beginning of our more in-depth spatial and temporal analyses of habitat availability, use, and selection on our 2 study sites, and to examine individual and cohort variability relative to each study site and among the 3 years (DeGiudice et al. 2019).

## **OBJECTIVES**

1. Present our ruleset, established for annually maintaining ecologically- and statistically-sound consistency in our analytical approach, as we progress through our spatial and temporal analyses of habitat use and selection at the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> orders.
2. Present distribution of deer captures during winters 2017–2018, 2018–2019, and 2019–2020.

3. Compare size and proportional habitat composition of the Inguadona Lake (IN) and Elephant Lake (EL) sites during winters 2017–2018, 2018–2019, and 2019–2020.
4. Compare size and proportional habitat composition of winter HRs of deer at the IN and EL sites during winters 2017–2018, 2018–2019, and 2019–2020.
5. Using a 2<sup>nd</sup> order compositional analysis approach, compare proportional habitat use (deer HR level) to proportional availability (study site level).

## STUDY AREA

The study is being conducted on 2 deer winter range sites in northern Minnesota's forest zone (Figure 1). The 46-km<sup>2</sup> IN site is located in the northcentral part of the state, 2 km south of the Chippewa National Forest, and is comprised of state, Cass County, and private land. D'Angelo and Giudice (2016) reported pre-fawning deer densities of 7–9 deer/km<sup>2</sup>, including both sedentary and seasonally migrating deer (Fieberg et al. 2008). Topography is undulant and ranges between 400 and 425 m above sea level. The area is part of the Pine Moraines region (MNDNR 2015), with uplands dominated by 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) and lowlands dominated by 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  $\geq 38$  cm during November–May. During 1981–2010, mean January temperature was  $-13^{\circ}\text{C}$  and mean annual snowfall was 110 cm (MNDNR Climatology 2018). During winters 2009–2010 to 2019–2020, mean WSI<sub>Max</sub> was 71 (95% Confidence Interval [CI] = 45–97, range = 21–160). Only 1 winter, 2013–2014, had a WSI<sub>Max</sub>  $\geq 140$ .

The 76-km<sup>2</sup> EL site is representative of the forest zone in northeastern Minnesota and includes state, federal, St. Louis County, and private land. Pre-fawning deer densities are lower than at the IN site, and actually, are below management's goal of 3–5 deer/km<sup>2</sup> since the 2 severe winters of 2010–2011 and 2013–2014 (D'Angelo and Giudice 2016). Topography is rugged with elevations ranging from 400 to 450 m above sea level. This area is part of the Northern Superior Upland region (MNDNR 2015) with lowlands of northern white cedar; black spruce; and tamarack and uplands of northern white cedar; balsam fir; red, white, and jack pine; aspen; and paper birch (MNDNR 2015). Mean January temperature was  $-15^{\circ}\text{C}$  and mean annual snowfall was 165 cm during 1981–2010 (MNDNR Climatology 2018). During winters 2009–2010 to 2019–2020, mean WSI<sub>Max</sub> was 112 (95% CI = 83–142, range = 46–212). Two winters, 2012–2013 and 2013–2014, had a WSI<sub>Max</sub>  $\geq 140$ .

Wolf (*Canis lupus*) predation is the primary cause of natural mortality of adult deer at both study sites (Nelson and Mech 1986, DelGiudice et al. 2002). Wolves were most recently (2017) estimated at 2,856, or 4 wolves/100 km<sup>2</sup> (Erb et al. 2017). Black bear (*Ursus americanus*) and wolf predation also heavily impact fawn survival (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

During winters 2017–2018 (2018), 2018–2019 (2019), and 2019–2020 (2020), we captured 20, 40, and 13 adult ( $\geq 1.5$  years) female deer. Half each were captured at the IN and EL sites during 2018 and 2019, 8 and 5 deer, respectively, during 2020 (Figure 1). All except 1 deer (captured by Clover trap [DelGiudice et al. 2001]) were captured by net-gunning from helicopter (2018: Hells Canyon Helicopters, Clarkston, Washington; 2019: Quicksilver Air, Inc.,

Fairbanks, Alaska; 2020: Helicopter Wildlife Services, Austin, Texas). Deer handling included blind-folding, hobbling, recording a rectal temperature ( $^{\circ}$  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 as warranted by any pre-existing injury or wound. New collars deployed during 2018 and 2019 were programmed to obtain 1 location-fix every 2 hours during December–June and 1 location-fix every 4 hours during July–November; however, collars deployed during 2020 obtained hourly location-fixes during December–June and 1 location-fix every 4 hours during July–November. Location data were transmitted to our base station every 10 hours (maximum 6 location-fixes per transmission). All collars included QFP programming, which enabled them to obtain QFP data when a GPS-fix was unsuccessful. These data are stored-on-board, along with activity data collected every 5 minutes using an accelerometer, then downloaded onto a computer once collars are recovered.

We developed the following ruleset to facilitate and ensure annual application of a consistent sound approach for our 2<sup>nd</sup> and 3<sup>rd</sup> order analyses of white-tailed deer winter habitat:

1. Winter location-fixes are obtained between 1 November and 30 April.
2. Location-fixes with horizontal error  $\geq 50$  m are censored.
3. Location-fixes beyond the base air photointerpretation are censored.
4. Calculate the 95% Kernel Density Estimator (KDE) HR for each GPS-collared deer.
5. Use only those location-fixes within the 95% KDE boundaries for all deer to estimate the annual 100% Minimum Convex Polygon (MCP) study site boundaries at IN and EL.
6. Potable water sources, or portions thereof, within the 100% MCP and 95% KDE HRs, are included in calculations of size and proportional habitat composition.
7. If occasionally a boundary of a deer's 95% KDE HR overlaps the 100% MCP study site boundary, the latter will be extended enough to include that portion of the KDE.

At the 2<sup>nd</sup> order, the annually expanding or contracting 100% MCP study site boundaries and resulting associated proportional habitat compositions constitute habitat available relative to use, which is the proportional habitat composition of individual deer 95% KDE HR (Aebischer et al. 1993). Subsequently, we will be conducting 3<sup>rd</sup> order compositional analyses with proportional habitat composition of deer home ranges representing availability and proportions of location-fixes within classified stands representing use; and examining 3<sup>rd</sup> order resource selection functions and fine-scale measurements of habitat use at the stand level. Third order and stand-level findings will be reported elsewhere.

We calculated 95% KDE HRs for each deer using AdehabitatHR (Worton 1989, Calenge 2006) in program R (R Core Team 2017) to compare size and proportional habitat composition among winters within deer, among deer within winters, and between deer of the 2 study sites. We calculated 100% MCP study site boundaries annually using the Minimum Bounding Geometry tool in ArcGIS Pro (Worton 1987). Compositional analyses of habitat use were conducted according to Aebischer et al. (1993).

## **RESULTS AND DISCUSSION**

An important aspect of examinations of all data (e.g., HR size, habitat composition and use) collected throughout the long-term study period is to gain an improved understanding and appreciation of the variability among deer within study sites and among winters and between study sites within winters. Ultimately, this understanding will be critical to formulating habitat management prescriptions for deer. Because deer densities are markedly lower on our EL site than at IN, we initiated our pilot study (Phase 1) with boundaries representing a larger study site at EL than at IN. This better assured our ability to capture and GPS-collar the desired number of adult females at that site, as well as at IN. As we completed each winter of the study, beginning with 10 collared females at each site, the deer's collective winter location-fixes and

overall distribution were used to subsequently define each study site's boundaries (Figure 1). At IN and EL, the 100% MCP site boundaries were derived from 4,826, 17,965, and 21,976 and 5,530, 19,583, and 24,579 location-fixes, for winters 2018, 2019, and 2020, respectively. The EL site was consistently larger than the IN site, and the size (area) of each has varied markedly among the 3 winters (Table 1 and Figure 2). This was due largely to the distribution of the additional deer captured and collared each winter, but presumably, variation of movements and habitat use of all collared deer relative to varying winter conditions had an effect. We will examine these relationships more closely as our analyses progress.

Overall, the mean and median HR sizes at IN (338 and 140 ha) and EL (287 and 133 ha) were quite comparable (Table 2). However, as expected, there also was a great deal of variation in winter HR size of adult female deer, both within sites and winters, and between sites and among winters (Table 2). The very small minimum HRs sizes were primarily attributable to deer that succumbed rather quickly in the season, most often to wolf predation, and consequently their HRs were estimated using a relatively small number of location-fixes. Noteworthy, some deer were quite mobile, and in 2020, exhibited maximum HR sizes up to 2,188 ha and 1,284 ha at IN and EL (Table 2, Figure 3). Snow conditions were quite variable between sites and among winters; therefore, assessing that potential impact will be an intricate part of our ongoing analyses.

Proportional habitat composition at each site (availability at the 2<sup>nd</sup> order of analysis) remained relatively stable among the 3 winters, despite their aforementioned changing boundaries and sizes on the landscape, associated with varying winter conditions, movements and HRs of each site's deer (Table 3). However, overall, there were some key apparent differences and similarities in available habitat (by cover group) at IN and EL (Figure 4 and Table 3). The percentage of available dense conifer cover (i.e., optimum snow shelter for deer) at EL was just over 2 times that at IN (19.4% versus 9.3%), whereas moderately dense conifer cover at IN (9.3%) was almost 3 times that EL (3.5%). Hardwood stands were similarly most abundant at IN (38.5%) and EL (37.8%), and the forage cover group, a primary winter food source for deer, similarly accounted for about 10% of each site. Importantly, the understories of these abundant hardwood stands commonly provide valuable browse species as well (DeGiudice et al. 1989, 2013b). Mixed hardwoods were proportionally more abundant at EL (10.9%) than at IN (3.9%), whereas wetlands accounted for more of the habitat at IN (19.4%) than at EL (7.3%).

Similar to habitat composition of the study sites, mean habitat composition (by cover group) of winter HRs of the GPS-collar deer (use at the 2<sup>nd</sup> order of analysis) at each site, generally reflected relative stability among winters, despite varying winter conditions and new collars being deployed on additional deer each winter (Table 4 and Figure 1). Again, as expected, there are notable differences in how individual deer range within each site (Figure 3) and in the habitat composition of their HRs (Table 4), but overall, on average, proportional habitat similarities and differences between deer of the 2 sites, interestingly reflected those we noted at the study site level above. For example, overall, dense conifer cover accounted for about 10% of deer HRs at IN, but about 2 times that (20%) at EL, similar to the 9.3% and 19.4% at the study site level, respectively. Moderately dense cover accounted for a mean 10% and 3.4% of deer HRs at IN and EL, and at the site level, 9.3% and 3.5%, respectively, and the forage cover group was an overall mean 12.3% and 13.0% of IN and EL deer HRs, similar to the 9.3% and 10.5% at the site level. Finally, hardwood stands were an overall mean 38.7% and 33.3% of HRs at IN and EL and 38.5% versus 37.8% availability at the site level. Generally, this suggests that individual deer vary quite markedly with respect to the size and shape of their HR as winter progresses, and among winters, but that on average, they are using or selecting for the habitat cover groups of particular importance in proportion to their availability.

Our compositional analyses showed little in the way of intense habitat selection for cover groups at the IN site during the 3 winters (Table 5). Just about every cover group was used significantly ( $P \leq 0.05$ ) more than “other” (residential, small agricultural plots) or open water (ice), but this was of little biological significance. And there were apparent, although non-significant, patterns of use of specific cover groups. For example, open conifer tended to be used less than dense and moderately dense conifer, forage, and hardwoods, and mixed hardwoods exhibited a consistent pattern of being used less than all 3 conifer groups, forage, and hardwood stands. At EL, similar to at IN, “other” and open water were selected for least, and open conifer also often was significantly ( $P \leq 0.05$ ) selected for less than dense conifer, forage, hardwood and mixed hardwood stands (Table 6). During winter 2019, a reasonably severe winter, dense conifer was selected more intensely than the moderately dense and open conifer groups, forage, and mixed hardwood stands. With respect to ranking the use of habitat cover groups based on our analyses, hardwoods were consistently ranked the highest at both sites, which may be attributable to the value of their understories as a food source (Table 7). Wetlands consistently ranked high at IN, then moderately dense and dense conifer (the former being more abundant), and then forage (Table 7). At EL, forage and mixed hardwoods ranked consistently high over the 3 winters, and dense conifer ranked the highest during winter 2020.

Our analyses of deer winter HRs and habitat use at the 2<sup>nd</sup> order, habitat availability at the site level and use at the HR level, strongly indicate notable individual variation in HR size and habitat use. However, on average, over varying winters 2018–2020, deer were using cover groups in proportion to availability, with hardwoods, moderately dense and dense conifer stands, and forage types receiving the greatest use at both sites, with the exception of greater availability and use of wetlands at IN than at EL. Our ongoing more in-depth analyses, will examine 1) habitat selection at the 3<sup>rd</sup> order, proportional availability within deer HRs versus use (proportions of location-fixes) at the stand level, 2) fine-scale measurements of stands used and how they were used, and 3) how use was influenced by variations in snow depth and deer density.

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Table 1. Annual size (ha) of white-tailed deer, winter range study sites at Inguadona Lake and Elephant Lake, northcentral and northeastern Minnesota, winters 2017–2018 to 2019–2020.<sup>a,b</sup>

Study site	2018	2019	2020	Overall	
				Mean	SE
Inguadona Lake	2,993	5,999	6,314	5,102	864
Elephant Lake	6,796	9,411	8,648	8,285	634

<sup>a</sup>Sizes estimated annually by 100% Minimum Convex Polygon (Worton 1987) using all location-fixes within the 95% Kernel Density Estimator winter (1 November–30 April) home ranges (Worton 1989) of all global positioning system-collared deer within each study site.

<sup>b</sup>Number of collared deer was 9, 24, and 22 at IN and 10, 26, and 19 at EL during winters 2018 to 2020, respectively.

Table 2. Annual mean ( $\pm$  SE) and median size (ha) of winter home ranges of global positioning system-collared, adult ( $\geq 1.5$  yr), female white-tailed deer at the Inguadona Lake and Elephant Lake study sites, northcentral and northeastern Minnesota, winters 2017–2018 to 2019–2020.<sup>a,b</sup>

Winter/study site	<i>N</i>	Mean size (ha)	Median	SE	Min	Max
2018						
IN	9	299	116	107	64	1,001
EL	10	160	116	49	34	566
2019						
IN	24	307	143	73	33	1,214
EL	26	236	123	58	5	1,087
2020						
IN	22	388	151	114	4	2,188
EL	19	428	241	98	75	1,284
Overall						
IN	55	338	140	58	4	2,188
EL	55	287	133	46	5	1,284

<sup>a</sup>Winter home ranges determined by the 95% Kernel Density Estimator (Worton 1989).

<sup>b</sup>The mean number of location-fixes for winter home range determinations at the Inguadona Lake and Elephant Lake sites was 536, 737, and 999 and 553, 753, and 1,293 for winters 2018, 2019, and 2020, respectively.

Table 3. Annual habitat cover group composition (% of study sites) of winter range of adult ( $\geq 1.5$  yr), female white-tailed deer at the Inguadona Lake (IN) and Elephant Lake (EL) sites, northcentral and northeastern Minnesota, winters 2017–2018 to 2019–2020.<sup>a,b</sup>

Study site/Winter	Percent of study area									Total area (ha)
	Conifer			Forage	Hardwood	Mixed hardwood	Wetland	Open water	Other	
IN										
2018	10.3	11.0	3.1	10.7	36.5	4.7	20.8	2.7	0.2	3,326
2019	9.1	8.8	3.2	8.6	39.5	3.2	19.6	5.8	2.2	6,316
2020	8.5	8.1	2.5	8.6	39.4	3.7	17.8	8.0	3.5	7,357
Overall										
Mean	9.3	9.3	2.9	9.3	38.5	3.9	19.4	5.5	2.0	5,666
SE	0.4	0.7	0.2	0.6	0.8	0.4	0.7	1.2	0.8	986
EL										
2018	21.6	4.2	3.1	8.8	36.8	13.4	6.2	5.0	0.9	6,958
2019	18.9	3.2	1.9	10.3	39.1	9.7	7.9	8.2	0.7	9,697
2020	17.7	3.0	1.7	12.5	37.6	9.5	7.8	9.5	0.7	9,207
Overall										
Mean	19.4	3.5	2.2	10.5	37.8	10.9	7.3	7.6	0.8	8,621
SE	0.9	0.3	0.4	0.9	0.5	1.0	0.5	1.1	0.0	689

<sup>a</sup>Total area of each site includes the 100% Minimum Convex Polygon (Worton 1987) and any area of each global positioning system-collared deer's 95% Kernel Density Estimated home range (Worton 1989) that extends beyond the site boundaries. This explains the difference in total areas of the 2 study sites compared to their areas reported in Table 1.

<sup>b</sup>Number of collared deer was 9, 24, and 22 at IN and 10, 26, and 19 at EL during winters 2018 to 2020, respectively.

Table 4. Mean ( $\pm$  SE) annual cover group composition of winter home ranges of global positioning system-collared adult ( $\geq 1.5$  yr), female white-tailed deer at the Inguadona Lake (IN) and Elephant Lake (EL) sites, northcentral and northeastern Minnesota, winters 2017–2018 to 2019–2020.<sup>a,b</sup>

Study site/winter	Percent of 95% KDE home range								
	Conifer			Forage	Hardwood	Mixed hardwood	Wetland	Open water	Other
	Dense	Moderate	Open						
IN									
2018									
Mean	9.1	12.5	3.0	14.8	34.5	4.3	20.7	0.8	0.1
SE	2.0	3.8	0.8	3.5	5.4	1.2	3.7	0.4	0.1
Min	1.4	1.6	0.0	0.0	0.5	0.0	1.1	0.0	0.0
Max	22.2	39.8	6.0	40.1	55.9	11.2	43.0	3.5	1.0
2019									
Mean	9.8	10.8	5.1	9.4	39.7	4.2	16.4	4.1	0.5
SE	1.9	2.6	1.2	1.4	3.7	1.6	1.9	1.2	0.4
Min	0.0	0.0	0.0	0.0	3.6	0.0	1.1	0.0	0.0
Max	35.6	60.1	20.6	26.0	72.9	39.5	34.3	25.0	9.3
2020									
Mean	10.4	6.8	3.4	12.9	41.8	3.9	12.7	6.9	0.7
SE	2.0	1.4	0.8	2.3	3.2	1.2	1.7	1.5	0.4
Min	0.0	0.0	0.0	0.0	13.7	0.0	0.0	0.0	0.0
Max	30.0	24.7	12.8	43.5	76.7	26.3	26.9	23.1	8.7
Overall									
Mean	9.8	10.0	3.8	12.3	38.7	4.1	16.6	3.9	0.5
SE	0.3	1.4	0.5	1.3	1.8	0.1	1.9	1.4	0.2
EL									
2018									
Mean	18.6	4.5	3.7	11.5	35.8	22.4	3.5	0.0	0.0
SE	5.9	1.3	1.8	3.7	5.9	6.3	1.0	0.0	0.0
Min	0.0	0.0	0.0	0.7	7.8	0.7	0.0	0.0	0.0
Max	54.0	15.4	15.4	42.0	76.9	72.9	10.4	0.0	0.0
2019									
Mean	19.5	2.8	1.5	14.9	35.8	11.4	7.7	4.3	1.9
SE	3.0	0.7	0.5	2.6	3.7	2.5	1.3	2.0	1.4
Min	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0
Max	54.0	13.4	10.3	54.0	68.8	51.3	25.8	40.6	35.9
2020									
Mean	22.0	3.0	1.4	12.4	28.3	9.0	6.5	13.5	4.1
SE	2.7	0.7	0.5	2.8	3.1	1.4	1.1	3.8	1.7
Min	7.0	0.0	0.0	0.2	1.7	1.3	0.0	0.0	0.0
Max	52.8	8.4	8.2	44.6	54.0	21.6	19.1	32.3	45.5
Overall									

Mean	20.0	3.4	2.2	13.0	33.3	14.2	5.9	5.9	2.0
SE	0.8	0.4	0.6	0.8	2.1	3.4	1.0	3.3	1.0

<sup>a</sup>Winter home ranges were determined by the 95% Kernel Density Estimator (Worton 1989).

<sup>b</sup>Number of collared deer was 9, 24, and 22 at IN and 10, 26, and 19 at EL during winters 2018 to 2020, respectively.

Table 5. Simplified ranking matrices for global positioning system-collared adult ( $\geq 1.5$  yr), female white-tailed deer, based on comparing proportions of habitat cover groups available within annual 100% Minimum Convex Polygon study site boundaries and the proportions of cover groups within each deer's winter home range at the Inguadona Lake site, northcentral Minnesota, winters 2017–2018 to 2019–2020.<sup>a,b,c</sup>

Cover group	Conifer			Forage	Hardwood	Mixed hardwood	Wetland	Other	Open water
	Dense	Moderate	Open						
2018									
Dense conifer	0	-	+	+	+	+	-	+++	+++
Mod conifer	+	0	+	+	+	+	+	+++	+++
Open conifer	-	-	0	-	-	+	-	+	+
Forage	-	-	+	0	-	+	-	+	+++
Hardwood	-	-	+	-	0	+	-	+++	+++
Mixed									
hardwood	-	-	-	-	-	0	-	+	+++
Wetland	+	-	+	+	+	+	0	+++	+++
Other	---	---	-	-	---	-	---	0	+
Open water	---	---	-	---	---	---	---	-	0
2019									
Dense conifer	0	-	+	+	-	+	-	+++	+++
Mod conifer	+	0	+	+	-	+	+	+++	+++
Open conifer	-	-	0	-	-	+	-	+++	+
Forage	-	-	+	0	-	+	-	+++	+++
Hardwood	+	+	+	+	0	+++	+	+++	+++
Mixed									
hardwood	-	-	-	-	---	0	-	+++	+
Wetland	+	-	+	+	-	+	0	+++	+++
Other	---	---	---	---	---	---	---	0	+++
Open water	---	---	-	---	---	-	---	+++	0
2020									
Dense conifer	0	+	+	-	-	+	-	+++	+
Mod conifer	-	0	-	-	---	+	-	+++	+
Open conifer	-	+	0	-	-	+	-	+++	+
Forage	+	+	+	0	-	+++	+	+++	+
Hardwood	+	+++	+	+	0	+++	+++	+++	+++
Mixed									
hardwood	-	-	-	---	---	0	-	+++	+
Wetland	+	+	+	-	---	+	0	+++	+++
Other	---	---	---	---	---	---	---	0	---
Open water	-	-	-	-	---	-	---	+++	+

<sup>a</sup>Total area of each site includes the 100% Minimum Convex Polygon (Worton 1987) and any area of each global positioning system-collared deer's 95% Kernel Density Estimated home range (Worton 1989) that extends beyond the site boundaries. This explains the difference in total areas of the 2 study sites compared to their areas reported in Table 1.

<sup>b</sup>Number of collared deer was 9, 24, and 22 at IN and 10, 26, and 19 at EL during winters 2018 to 2020, respectively.

<sup>c</sup>Triple + or - signs represent a significant ( $P \leq 0.05$ ) deviation from random (Aebischer et al. 1993). Single + or - signs indicates an apparent, but not significant deviation from random.

Table 6. Simplified ranking matrices for global positioning system-collared adult ( $\geq 1.5$  yr), female white-tailed deer, based on comparing proportions of habitat cover groups available within annual 100% Minimum Convex Polygon study site boundaries and the proportions of cover groups within each deer's winter home range at the Elephant Lake site, northeastern Minnesota, winters 2017–2018 to 2019–2020.<sup>a,b,c</sup>

Cover group	Conifer			Forage	Hardwood	Mixed hardwood	Wetland	Other	Open water
	Dense	Moderate	Open						
2018									
Dense conifer	0	-	+	-	-	-	-	+++	+++
Mod conifer	+	0	+	-	-	-	+	+++	+++
Open conifer	-	-	0	---	---	---	-	+	+++
Forage	+	+	+++	0	-	-	+	+++	+++
Hardwood	+	+	+++	+	0	-	+	+++	+++
Mixed hardwood	+	+	+++	+	+	0	+	+++	+++
Wetland	+	-	+	-	-	-	0	+++	+++
Other	---	---	-	---	---	---	---	0	+++
Open water	---	---	---	---	---	---	---	---	0
2019									
Dense conifer	0	+	+++	-	-	-	-	+++	+++
Mod conifer	-	0	+	---	---	-	---	+	+++
Open conifer	---	-	0	---	---	---	---	+	+++
Forage	+	+++	+++	0	-	+	+	+++	+++
Hardwood	+	+++	+++	+	0	+	+	+++	+++
Mixed hardwood	+	+	+++	-	-	0	-	+++	+++
Wetland	+	+++	+++	-	-	+	0	+++	+++
Other	---	-	-	---	---	+	---	0	+++
Open water	---	---	---	---	---	---	---	---	0
2020									
Dense conifer	0	+++	+++	+++	+	+++	+	+	+++
Mod conifer	---	0	+	-	-	-	+	+	+++
Open conifer	---	-	0	---	---	---	-	-	+
Forage	---	+	+++	0	-	-	+	+	+
Hardwood	-	+	+++	+	0	-	+	+	+++
Mixed hardwood	---	+	+++	+	+	0	+	+	+++
Wetland	-	+	+	-	-	-	0	+	+
Other	-	-	+	-	-	-	-	0	+++
Open water	---	-	-	-	---	---	-	---	0

<sup>a</sup>Total area of each site includes the 100% Minimum Convex Polygon (Worton 1987) and any area of each global positioning system-collared deer's 95% Kernel Density Estimated home range (Worton 1989) that extends beyond the site boundaries. This explains the difference in total areas of the 2 study sites compared to their areas reported in Table 1.

<sup>b</sup>Number of collared deer was 9, 24, and 22 at IN and 10, 26, and 19 at EL during winters 2018 to 2020, respectively.

<sup>c</sup>Triple + or - signs represent a significant ( $P \leq 0.05$ ) deviation from random (Aebischer et al. 1993). Single + or - signs indicates an apparent, but not significant deviation from random.

Table 7. Ranking of habitat cover groups used by global positioning system-collared adult ( $\geq 1.5$  yr), female white-tailed deer at the Inguadona Lake and Elephant Lake study sites, northcentral and northeastern Minnesota, winters 2017–2018 to 2019–2020.<sup>a,b</sup>

Cover group	Inguadona Lake				Elephant Lake				Overall
	2018	2019	2020	Mean	2018	2019	2020	Mean	Mean
Dense conifer	6	5	5	5.3	3	4	8	5.0	5.2
Moderate conifer	8	7	3	6.0	5	3	3	3.7	4.8
Open conifer	3	3	4	3.3	2	2	1	1.7	2.5
Forage	4	4	7	5.0	6	7	5	6.0	5.5
Hardwoods	5	8	8	7.0	7	8	6	7.0	7.0
Mixed hardwoods	2	2	2	2.0	8	5	7	6.7	4.3
Wetlands	7	6	6	6.3	4	6	4	4.7	5.5
Other <sup>c</sup>	1	0	0	0.3	1	1	2	1.3	0.8
Open water	0	1	1	0.7	0	0	0	0.0	0.3

<sup>a</sup>Each habitat cover group is ranked by the number of + signs in its respective row in Tables 5 and 6 for the Inguadona Lake and Elephant Lake study sites, respectively.

<sup>b</sup>Number of collared deer was 9, 24, and 22 at IN and 10, 26, and 19 at EL during winters 2018 to 2020, respectively.

<sup>c</sup>Residential (cabins) and small agricultural plots.

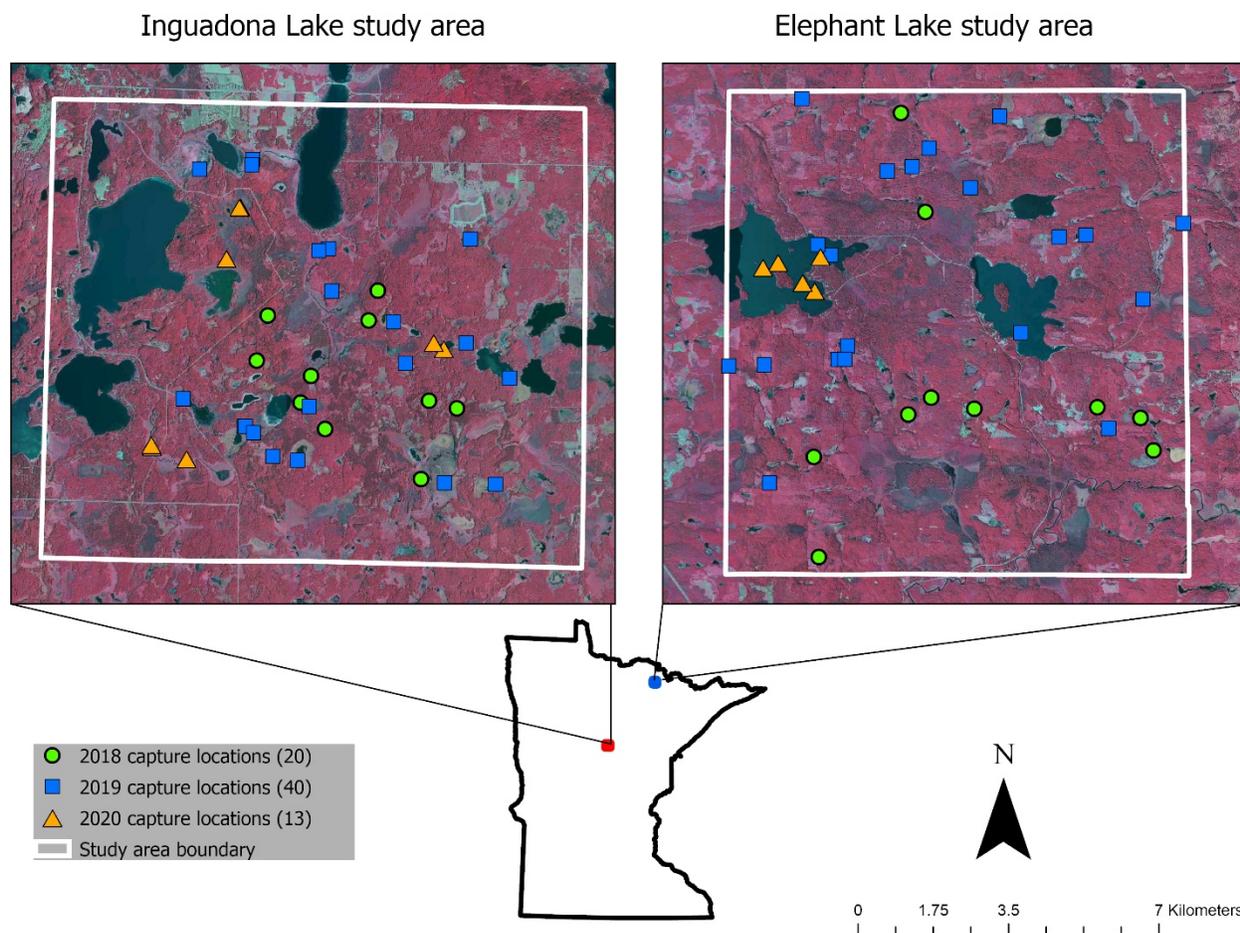
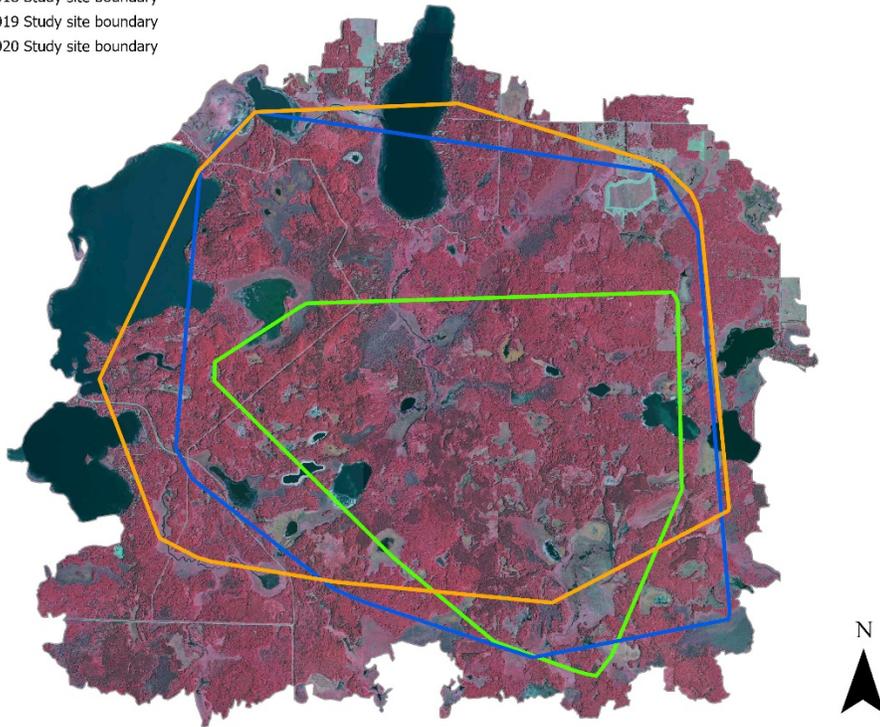


Figure 1. Helicopter net-gun capture locations of adult ( $\geq 1.5$  yr), female white-tailed deer at the Inguadona Lake (46 km<sup>2</sup>) and Elephant Lake (76 km<sup>2</sup>) study sites, northcentral and northeastern Minnesota, 10–11 March 2018, 5–8 February 2019, and 6 February 2020. One deer was captured via Clover trap at Inguadona Lake in the first winter.

2018 Study site boundary  
2019 Study site boundary  
2020 Study site boundary



2018 Study site boundary  
2019 Study site boundary  
2020 Study site boundary

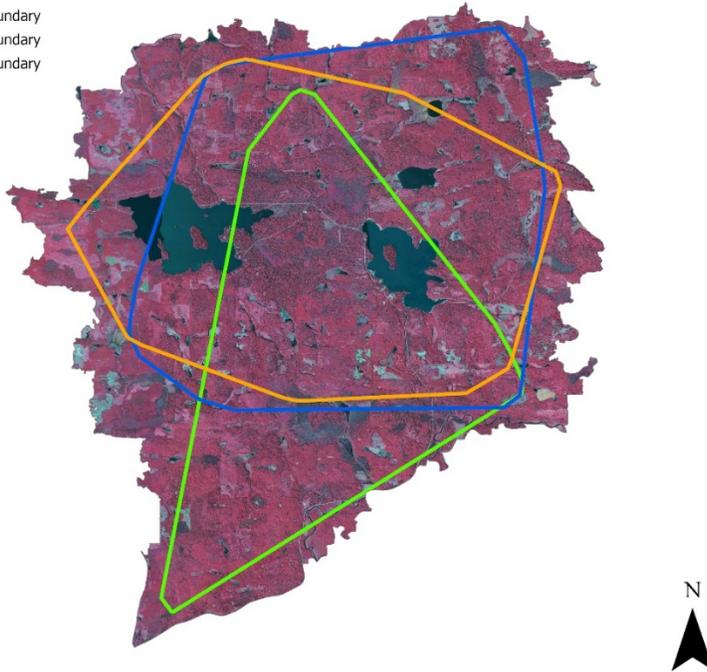
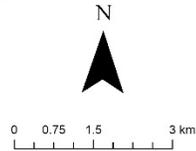
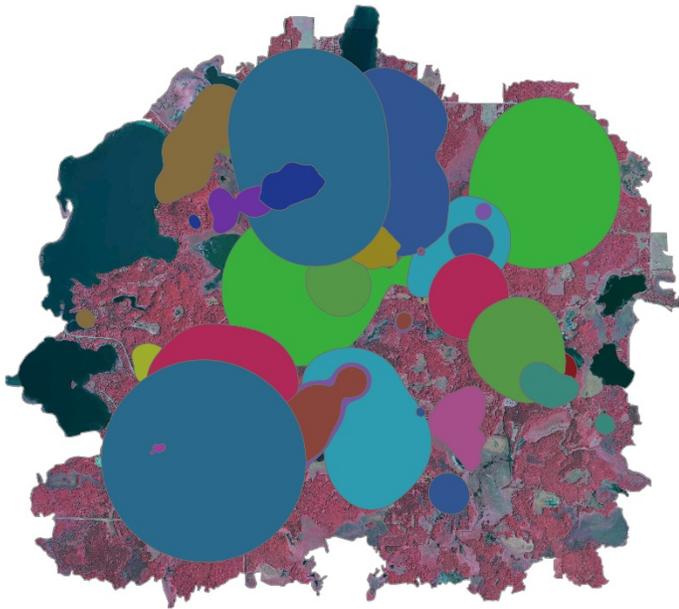


Figure 2. Annual study site boundaries based on 100% Minimum Convex Polygon of location-fixes within global positioning system-collared adult ( $\geq 1.5$  yr), female deer's home ranges at the Inguadona Lake (Top,  $n = 9, 24, 22$ ) and Elephant Lake (Bottom,  $n = 10, 26, 19$ ) study sites, northcentral and northeastern Minnesota, winters 2017–2018 to 2019–2020.

**2020 Winter home ranges**

- 697088A
- 706032A
- 706035A
- 706037A
- 706038B
- 706041A
- 706043A
- 706044A
- 706045A
- 706048B
- 706050A
- 706052B
- 706054A
- 706060A
- 706061A
- 706063A
- 706071A
- 713974A
- 713979A
- 713980A
- 713981A
- 713982A



**2020 Winter home ranges**

- 697083A
- 697089A
- 706028A
- 706029A
- 706031A
- 706033A
- 706039B
- 706042A
- 706046A
- 706053A
- 706056A
- 706058A
- 706059B
- 706064B
- 706068A
- 706072A
- 706074A
- 713973A
- 713977A

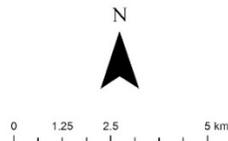
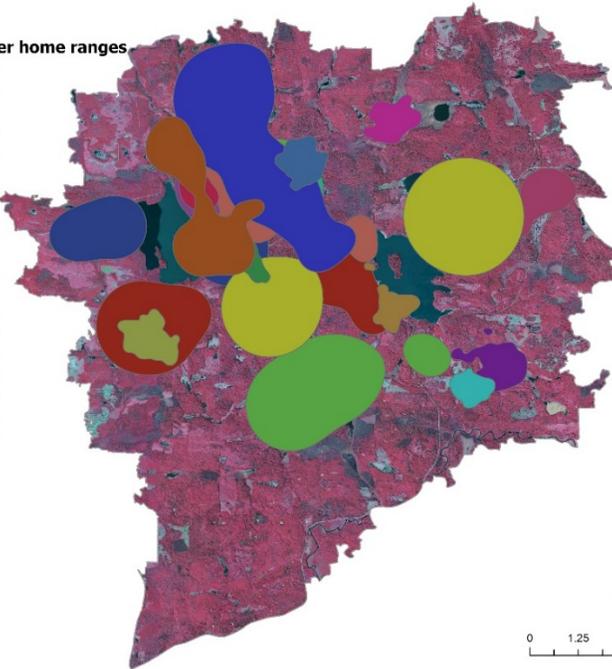


Figure 3. Winter home ranges (95% Kernel Density Estimate, Worton 1989) of adult ( $\geq 1.5$  yr), female white-tailed deer at the Inguadona Lake (top,  $n = 22$ ) and Elephant Lake (bottom,  $n = 19$ ) study sites, northcentral and northeastern Minnesota, 1 November 2019–1 May 2020.

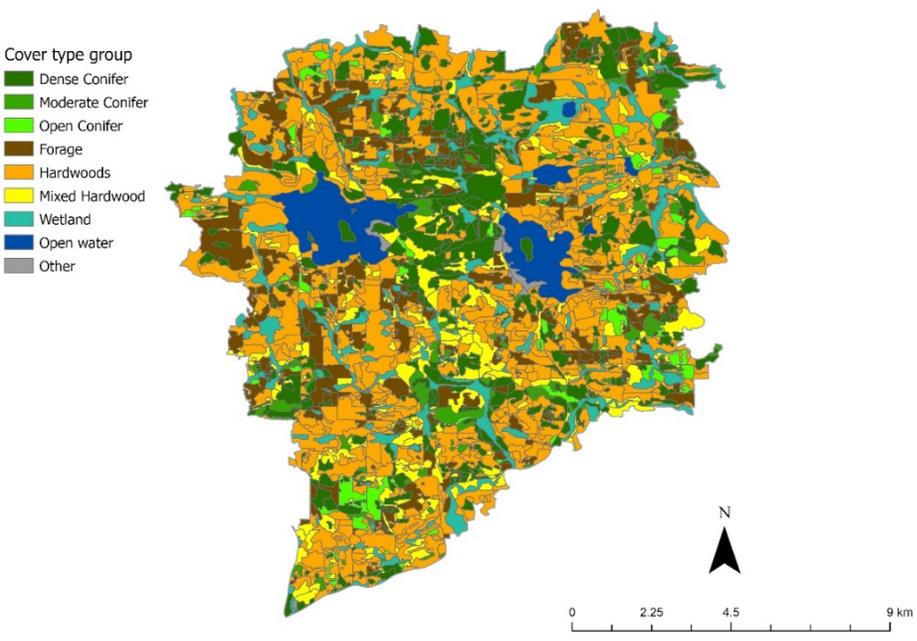
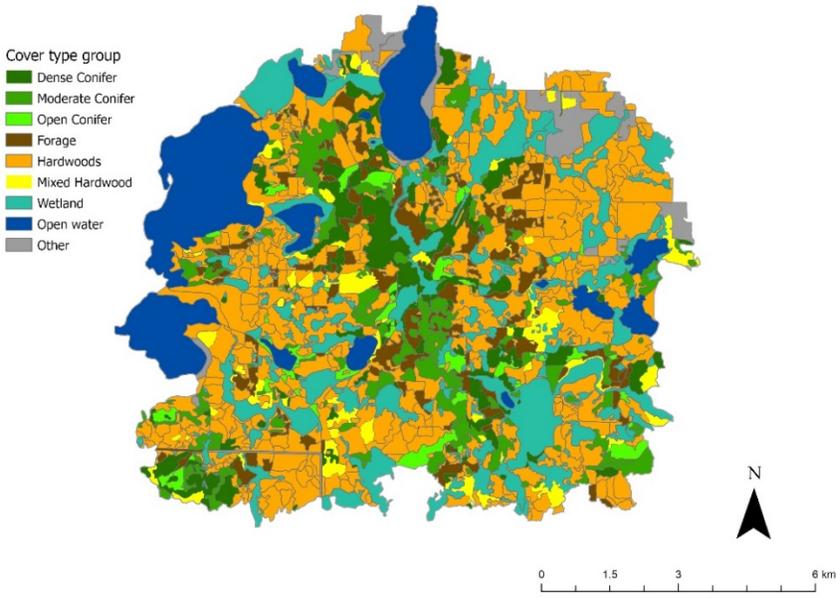


Figure 4. Habitat composition (by cover type group) of winter range of adult ( $\geq 1.5$  yr), female white-tailed deer at the Inguadona Lake (top) and Elephant Lake (bottom) study sites, northcentral and northeastern Minnesota, winter 2019–2020, accomplished by cover type (stand level) air photointerpretation and Light Detection and Ranging (LiDAR). Cover types were classified to a minimum size of 0.5 hectares, then aggregated into cover groups (see legend). Cover type codes are presented in Table 1 in Smith et al. (2019).



## SHARP-TAILED GROUSE RESPONSE TO FALL PRESCRIBED FIRE AND MOWING

Charlotte Roy, Lindsey Shartell, and John Giudice

### SUMMARY OF FINDINGS

We examined sharp-tailed grouse (*Tympanuchus phasianellus*) responses to prescribed fire and mechanical treatment (mowing) in the fall (mid-August through November) during 2015-2019 in northwestern Minnesota. We surveyed sharp-tailed grouse use at sites and measured vegetation before and after management at 16 mowing treatments and 12 prescribed burns, ranging in size from 5 to 664 ac (2–269 ha) and totaling 2,766 ac (1,118 ha). We also surveyed 22 control sites ranging in size from 6 to 460 ac (3–186 ha) and totaling 1,638 ac (663 ha). We surveyed sharp-tailed grouse use 0–28 (mean 9.1) days before (PRE) management, and 1 week (1WK), 1 month (1MO), 1 year (1YR), and 3 years (3YR) after management by conducting fecal pellet transects and documenting sharp-tailed grouse observed at the site. We detected sharp-tailed grouse pellets at 6 of 28 treatment sites and 5 of 22 control sites prior to treatment. Following treatment, we detected sharp-tailed grouse pellets in  $\geq 1$  fall survey (1WK or 1MO) at 13 treatment sites and 6 control sites. We observed sharp-tailed grouse at only 1 treatment site and 1 control site in PRE surveys, but in later fall surveys (1WK or 1MO) we observed grouse at 4 treatment and 2 control sites. Sharp-tailed grouse use of treated sites increased by more than 50% in 1YR surveys, but this was not significant in interim analysis due to high variability in site use. Our results thus far indicate that sharp-tailed grouse pellets provide a more useful indicator of site use than observations of grouse, and that sharp-tailed grouse use of treated sites was notably higher 1YR after management. Vegetation results indicate that shrub height was lower 1YR after mowing treatments and that forb cover was higher 1YR after burn treatments, however all other metrics measured did not differ 1YR after treatment. Vegetation metrics at sites occupied by sharp-tailed grouse did not differ prior to treatment, however in 1YR surveys, occupied sites had a significantly lower proportion of shrubs. Additional fieldwork is planned to complete 3YR surveys and improve statistical estimation of differences in occupancy, detection, and vegetation metrics.

### INTRODUCTION

Sharp-tailed grouse (*Tympanuchus phasianellus*) 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 sharp-tailed grouse 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 sharp-tailed grouse 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. Sharp-tailed grouse 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 grouse 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. Sharp-tailed grouse 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 sharp-tailed grouse 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 grouse 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**

1. To compare sharp-tailed grouse use prior to and following fall management within burn, mow, and control treatments.
2. 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 use than mowed sites, and both treatments having greater 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 conditions.

## **METHODS**

### **Study Areas**

Our study was focused in the northwestern sharp-tailed grouse region of Minnesota. Treated study sites were mainly on state-managed lands, however 3 sites owned and managed by The Nature Conservancy (TNC) and 3 privately-owned sites were included. In 2015, we conducted pre-treatment surveys at 16 sites planned for management and at 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 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 planned for management and 8 control sites. Seven sites were treated (2 mows and 5 prescribed burns). In 2018, we conducted pre-treatment surveys at 16 sites planned for management and 10 control sites, with 7 mows completed.

### **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 with a control site of similar size and successional condition (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  $\leq 6$  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 1 control for nearby sites treated on the same day. This also provided for a more balanced sample size among the 2 treatments and controls.

We surveyed treatment and control sites as close as possible in time (within 21 days), 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. We standardized the sampling rate 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  $\leq 0.5$  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.

We sampled transects 5 times at each site—once before treatment, targeting measurements within 2 weeks of treatment (PRE), and 4 times after treatment; at 1 week (1WK), 1 month (1MO), 1 year (1YR), and 3 years (3YR) after treatment. To date, we have conducted 3YR surveys at 4 sites treated in 2015 and at 2 sites treated in 2016, where additional interim management had not occurred, as well as 2 additional sites treated in 2016 where interim management had occurred.

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, they conducted their study on sage grouse in sagebrush, and sharp-tailed grouse habitats in Minnesota differ considerably in vegetative composition and structure.

We sampled vegetation within treatment and control sites 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 GPS coordinates to allow for relocation and 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 2017-2019, 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 highest point at which each vegetation class intercepted (touched) the pole. We also recorded whether the vegetation was dead/dormant, and combined those categories for analysis 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, we recorded the substrate type (e.g., bare ground, rock). For the purpose of this study, we considered moss and lichen a substrate type rather than vegetation.

We calculated vegetation metrics for each study site. We compared the proportion of cover in each class and mean maximum height among treatment types and between sites with and without sharp-tailed grouse use. In our preliminary analysis, we included both live and dead vegetation, using the maximum height of either type at each point. We tested for differences

among survey periods using Tukey's Honest Significant Difference, and tested for differences between sites occupied and unoccupied by sharp-tailed grouse using T-tests. We used a significance level of  $P < 0.05$ .

## RESULTS AND DISCUSSION

We detected sharp-tailed grouse pellets on transects at 6 (21%) of the 28 treatment sites and 5 (23%) of the 22 control sites prior to treatment (Table 2). Following treatment, we detected sharp-tailed grouse pellets in  $\geq 1$  fall survey (1WK or 1MO) at 13 treatment sites (46%) and 5 control sites (23%). Sharp-tailed grouse observations from transects prior to treatment exhibited similar patterns, with detections at only 1 treatment site (4%) and 1 control site (5%) in initial surveys. In later fall surveys (1WK or 1MO), however, we observed sharp-tailed grouse at 4 treatment sites (14%) and 2 control sites (9%, Table 3). In 1YR surveys, we detected pellets on transects at 7 (25%) of 28 treatment sites and 4 (18%) of 22 control sites, and we observed grouse from transects at 2 treatment sites (7%) and 1 control site (5%).

Our pellet survey results thus far suggest that our methods are capturing sharp-tailed grouse use of treatment and control sites. Naïve occupancy rates (i.e., site use) from data collected thus far are suggestive of increases in sharp-tailed grouse use of sites following management, although estimates of effect sizes are imprecise (Figure 1). Although occupancy and detection are confounded in naïve estimates for the 1WK and 1MO surveys (due to treatment effects on screening cover), 1YR and 3YR surveys should have similar detection rates to PRE surveys due to regrowth of vegetation the next growing season, especially in burn sites. Thus, the PRE vs. 1YR and 3YR comparisons should be reasonably straightforward and informative, whereas results from other time comparisons are more tenuous to interpret from naïve occupancy rates due to large differences in detection (Figure 2). The naïve probability of sharp-tailed grouse site use at treated sites increased by more than 50% in 1YR surveys at mowed and burned sites, but neither of these changes were significant in preliminary analysis due to high variability in site use (Figure 3). We also found the area of the disturbance to be a predictor of the probability of site use in interim analysis (Figure 4).

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 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 high variability among sites.

Control sites had significantly lower graminoid height ( $P < 0.01$ ) 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 surveys (Table 5). At sites that were mowed, graminoid, forb, and shrub cover ( $P = 0.01$ ,  $P < 0.01$ ,  $P < 0.01$ ) and height ( $P < 0.01$ ,  $P < 0.01$ ,  $P < 0.01$ ) were all lower in 1MO surveys, but in 1YR measurements only shrub height remained lower than PRE survey ( $P < 0.01$ ). At sites that were burned, graminoid cover, forb cover, and graminoid height ( $P < 0.01$ ,  $P < 0.01$ ,  $P < 0.01$ ) were lower in 1MO surveys, but in 1YR surveys the only significant difference was that forb cover was greater ( $P < 0.01$ , Table 5). Vegetation metrics did not differ between sites occupied and unoccupied by sharp-tailed grouse during PRE surveys, however during 1YR surveys, sites occupied by sharp-tailed grouse had lower proportions of shrubs ( $P = 0.04$ ) than unoccupied sites regardless of treatment (Table 6).

This report includes 5 fall surveys for sites managed 2015–2016, however we will conduct the 3YR survey for sites treated during 2017 and 2018 in fall 2020 and 2021. We will not include additional sites in the study. Results presented in this report are preliminary and subject to revision.

Managers throughout sharp-tailed grouse range in Minnesota have expressed a need for information to manage habitat for sharp-tailed grouse more effectively. Given the current 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 northwestern region are also interested in this information to ensure that their management actions are as effective as possible.

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Table 1. Management activities completed for sharp-tailed grouse habitat in northwestern Minnesota during fall 2015–2018 and associated control sites, in order of treatment date.

Site	Work area	Treatment	Treatment date	Treatment ac (ha)	Control ac (ha)
Roseau 2015 mow	Roseau River	Mow	28 Aug–16 Sep 15	31 (12.5)	28 (11.3)
Skull Lake 2015 burn	Karlstad	Burn	1 Sep 2015	90 (36.4)	70 (28.3)
Halma 2015 mow	Karlstad	Mow	16–23 Sep 2015	41 (16.6)	39 (15.8)
Red Lake 2015 mow	Red Lake	Mow	22 Sep 2015	12 (4.9)	22 (8.9)
Spooner 2015 mow	Baudette	Mow	28 Sep 2015	22 (8.9)	26 (10.5)
Caribou 2015 burn	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 2015 burn	Red Lake	Burn	19 Oct 2015	152 (61.5)	176 (71.2)
Prosper 2015 mow	Baudette	Mow	19–30 Oct 2015	63 (25.5)	201 (81.3)
TL 2015 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 <sup>a</sup> 2016 burn	Roseau	Burn	14 Sep 2016	71 (28.7)	22 (8.9)
Roseau 2016 mow <sup>b</sup>	Roseau	Mow	27 Sep–7 Oct 16	23 (9.3)	29 (11.7)
Espelie 2016 burn	Thief River Falls	Burn	3 Oct 2016	443 (179.3)	460 (186.2)
Halma 2017 mow	Karlstad	Mow	28 Aug–8 Sep 2017	62 (25)	61 (25)
Gates 2017 burn	Red Lake	Burn	8 Sep 2017	388 (157)	320 (129)
K 2017 burn	Roseau	Burn	13 Sep 2017	90 (36)	93 (38)
F 2017 burn	Roseau	Burn	13 Sep 2017	99 (40)	Same as K
Prosper 2017 mow	Baudette	Mow	27 Sep–26 Oct 2017	70 (28)	41 (17)
O 2017 burn	Roseau	Burn	9 Oct 2017	17 (7)	100 (40)
I 2017 burn	Roseau	Burn	9 Oct 2017	48 (19)	Same as O
TL Mow 3	Thief Lake	Mow	21 Sep – 10 Oct 2018	73 (29.5)	No control
Graceton mow	Baudette	Mow	1 – 11 Oct 2018	75 (30.4)	89 (36.0)
HQ brush mow	Roseau	Mow	4 – 5 Oct 2018	5 (2.0)	6 (2.5)
TL Mow 1	Thief Lake	Mow	24 Sep – 16 Oct 2018	53 (21.4)	45 (18.2)
TNC site 10	Karlstad	Mow	19 – 22 Oct 2018	11 (4.5)	Same as site 9
RRWMA brush mow	Roseau	Mow	17 – 25 Oct 2018	9 (3.6)	Same as HQ
TNC site 9	Karlstad	Mow	23 – 27 Oct 2018	45 (18.2)	43 (17.4)

<sup>a</sup> The Noracre burn site was burned again in spring 2017 and sprayed with herbicide in spring and summer 2017, before the 1-year post-treatment (1YR) survey.

<sup>b</sup>The Roseau brush mow of 2016 was burned in spring 2018, after the 1YR survey but before the 3YR survey.

Table 2. Sharp-tailed grouse pellet detections at treatment and control sites in northwestern Minnesota during 2015-2019. We conducted surveys before (PRE), 1 week (1WK), 1 month (1MO), 1 year (1YR), and 3 years (3YR) 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 the snow. Surveys with confirmed sharp-tailed grouse use through any source of sign are highlighted in gray. NS indicates that the 3YR survey has not yet been completed. TRT indicates that a survey was not conducted, or if it was conducted was not comparable, because of additional treatments conducted after the original treatment.

Fecal pellets Site	Treatment					Control				
	PRE	1WK	1MO	1YR	3YR	PRE	1WK	1MO	1YR	3YR
Red Lake 2015 mow	0	0	0	2	0	0	0	0	1	0
TL 2015 mow	0	0	0*	0	TRT	0	0	0*	0	TRT
Spooner 2015 mow	0	0	3	0	3	0	0	0	0	1
Roseau 2015 mow	2	1 OT	1	0	TRT	0	0	0	0	0
Halma 2015 mow	0	0	0	1 OT	1	1	1	2	0	0
TL 2015 burn	1 OT	0	1	1 OT	NS	0	0	0	0	TRT
Skull Lake 2015 burn	0	1	0	1	NS	0	0	0	0	NS
Red Lake 2015 burn	0	0	0	0	TRT	0	0	0*	0	NS
Prosper 2015 mow	0	1	0*	2	TRT	1	11	2T*	5 4 OT	TRT
Caribou 2015 burn	1	2	1 OT	0	32 16OT	-	-	-	-	-
TL 2016 burn	0	1	4 7 OT	7 1 OT	0	0	0	0	0	0
Noracre 2016 burn	0	9 3 OT	0	3T* TRT	1 TRT	0	0	0	0	0
Espelie 2016 burn	1	6	18 31 OT	1 3 OT	1 3 OT	1 OT	1 3 OT	4 5 OT	3* 2 OT	2 1 OT
Roseau 2016 mow	1 OT	0	0	0	0 TRT	0	0	0	0	NS
Halma 2017 mow	0	1 OT	1	0	NS	0	0	0	0	NS
Gates 2017 burn	0	3	0	0	TRT	0	-	-	0	NS
K 2017 burn	1 1 OT	0	7 11 OT	0	NS	-	-	-	-	-
F 2017 burn	4 1 OT	1	5 5 OT	14	NS	0	0	0	0	NS
Prosper 2017 mow	0	3T*	0	0	NS	0	0	0	0	NS
O 2017 burn	0	0	0	0	NS	-	-	-	-	-
I 2017 burn	0	0	0	1 OT	NS	3 OT	1 OT	0	6 30+OT	NS
TL mow 3	0	0	0*	0	NS	-	-	-	-	-
Graceton mow	0	0	4T* 1 OT*	0	NS	4	2	3	0	NS
HQ brush mow	0	0*	0	0	NS	0	0*	0	0	NS
TL mow 1	0	0	0*	0	NS	0	0	0	0	NS
TNC site 10 mow	0	0	2T*	0	NS	3	1 1 OT	0	2 OT	NS
RRWMA brush mow	1	0	1 OT*	0	NS	-	-	-	-	-
TNC site 9 mow	0	0	3T* 1 OT*	1	NS	-	-	-	-	-

Table 3. The number of sharp-tailed grouse observed at treatment and control sites in northwestern Minnesota during 2015-2019. We conducted surveys before (PRE), 1 week (1WK), 1 month (1MO), 1 year (1YR), and 3 years (3YR) after treatment. Sharp-tailed grouse observed while off-transect are indicated with OT, indicative of site use not captured in sampling. Surveys with confirmed sharp-tailed grouse use through observations of any birds at the site are highlighted in gray. NS indicates that the 3YR survey has not yet been completed. TRT indicates that a survey was not conducted, or if it was conducted was not comparable, because of additional management conducted after the original treatment.

Grouse observations Site	Treatment					Control				
	PRE	1WK	1MO	1YR	3YR	PRE	1WK	1MO	1YR	3YR
Red Lake 2015 mow	0	0	0	0	0	0	0	0	0	0
TL 2015 mow	0	0	0	0	TRT	0	0	0	0	TRT
Spooner 2015 mow	0	0	11	3 OT	0	0	0	0	0	0
Roseau 2015 mow	2 OT	5 OT	2 OT	0	TRT	0	0	0	0	1
Halma 2015 mow	0	0	1	0	0	0	2	0	0	0
TL 2015 burn	4	0	0	0	NS	0	0	0	0	TRT
Skull Lake 2015 burn	0	0	0	0	NS	0	0	0	0	NS
Red Lake 2015 burn	0	0	0	0	TRT	0	0	0	0	NS
Prosper 2015 mow	0	0	0	1	TRT	0	0	0	12-20	TRT
Caribou 2015 burn	0	5	13	2 2 OT	27	-	-	-	-	-
TL 2016 burn	0	0	0	0	0	0	0	0	0	0
Noracre 2016 burn	0	0	0	0	TRT	0	0	0	0	0
Espelie 2016 burn	0	1	2 OT	0	1 2OT	5 OT	1	7 OT	0	0
Roseau 2016 mow	6 OT	0	0	0	0 TRT	0	0	0	0	NS
Halma 2017 mow	0	0	0	0	NS	0	0	0	0	NS
Gates 2017 burn	0	0	0	0	NS	-0	-	-	0	NS
K 2017 burn	0	0	0	0	NS	-	-	-	-	NS
F 2017 burn	1 OT	0	0	0	NS	0	0	0	0	NS
Prosper 2017 mow	0	0	0	0	NS	0	0	0	0	NS
O 2017 burn	0	0	0	0	NS	-	-	-	-	NS
I 2017 burn	0	0	0	0	NS	0	0	0	0	NS
TL mow 3	0	0	0	0	NS	-	-	-	-	-
Graceton mow	0	0	0	0	NS	1	0	0	0	NS
HQ brush mow	0	0	0	0	NS	0	0	0	0	NS
TL mow 1	0	0	0	0	NS	0	0	0	0	NS
TNC site 10 mow	0	0	0	0	NS	0	1 OT	0	0 <sup>a</sup>	NS
RRWMA brush mow	0	0	0	0	NS	0	-	-	-	-
TNC site 9 mow	0	0	0	0 <sup>a</sup>	NS	0	-	-	-	-

<sup>a</sup> 15 sharp-tailed grouse were observed on the road between TNC site 9 and the TNC site 10 control during the survey at the control.

Table 4. Mean pre-treatment vegetation cover and height for 4 vegetation classes at control ( $n = 22$ ), mow ( $n = 16$ ), and burn ( $n = 12$ ) sites sampled for sharp-tailed grouse use in northwestern Minnesota during 2015-2019. We did not observe any significant differences ( $P < 0.05$ ).

	Control	Mow	Burn
Cover (proportion)			
Graminoid	0.94	0.94	0.98
Forb	0.31	0.41	0.22
Shrub	0.36	0.40	0.26
Tree	0.05	0.05	0.05
Height (m)			
Graminoid	0.51	0.51	0.54
Forb	0.31	0.32	0.28
Shrub	1.19	1.30	0.74
Tree	1.44	0.76	0.73

Table 5. Change in mean vegetation cover and height from pre-treatment to 1 month after (1MO, control  $n = 21$ , mow  $n = 16$ , and burn  $n = 12$ ) and 1 year after (1YR, control  $n = 22$ , mow  $n = 16$ , and burn  $n = 12$ ) at sites sampled for sharp-tailed grouse use in northwestern Minnesota during 2015-2019. Significant differences ( $P < 0.05$ ) between measurements pre- and post-treatment are indicated with an asterisk.

	Control 1MO	Control 1YR	Mow 1MO	Mow 1YR	Burn 1MO	Burn 1YR
Cover (proportion)						
Graminoid	-0.02	< 0.01	-0.22*	-0.04	-0.43*	-0.04
Forb	-0.12	0.01	-0.30*	0.01	-0.18*	0.19*
Shrub	-0.06	0.04	-0.28*	-0.03	-0.10	< -0.01
Tree	-0.02	< -0.01	-0.03	-0.04	-0.02	0.02
Height (m)						
Graminoid	-0.14*	-0.02	-0.39*	-0.07	-0.25*	-0.05
Forb	-0.04	-0.01	-0.22*	-0.05	0.07	-0.05
Shrub	-0.08	0.11	-1.10*	-0.71*	-0.02	-0.04
Tree	-0.33	0.16	-0.58	-0.65	-0.08	0.04

Table 6. Mean vegetation cover and height at sites unoccupied and occupied by sharp-tailed grouse during pre-treatment (unoccupied n = 36, occupied n = 14) and 1 year (unoccupied n = 32, occupied n = 18) surveys in northwestern Minnesota during 2015-2019. Metrics at occupied sites that differed ( $P < 0.05$ ) from unoccupied sites are indicated with an asterisk.

	Unoccupied PRE	Occupied PRE	Unoccupied 1YR	Occupied 1YR
Cover (proportion)				
Graminoid	0.95	0.95	0.94	0.91
Forb	0.35	0.25	0.39	0.34
Shrub	0.37	0.29	0.40	0.27*
Tree	0.05	0.05	0.03	0.04
Height (m)				
Graminoid	0.51	0.55	0.49	0.44
Forb	0.32	0.27	0.29	0.24
Shrub	1.17	0.98	1.02	0.76
Tree	1.00	1.19	0.85	1.04

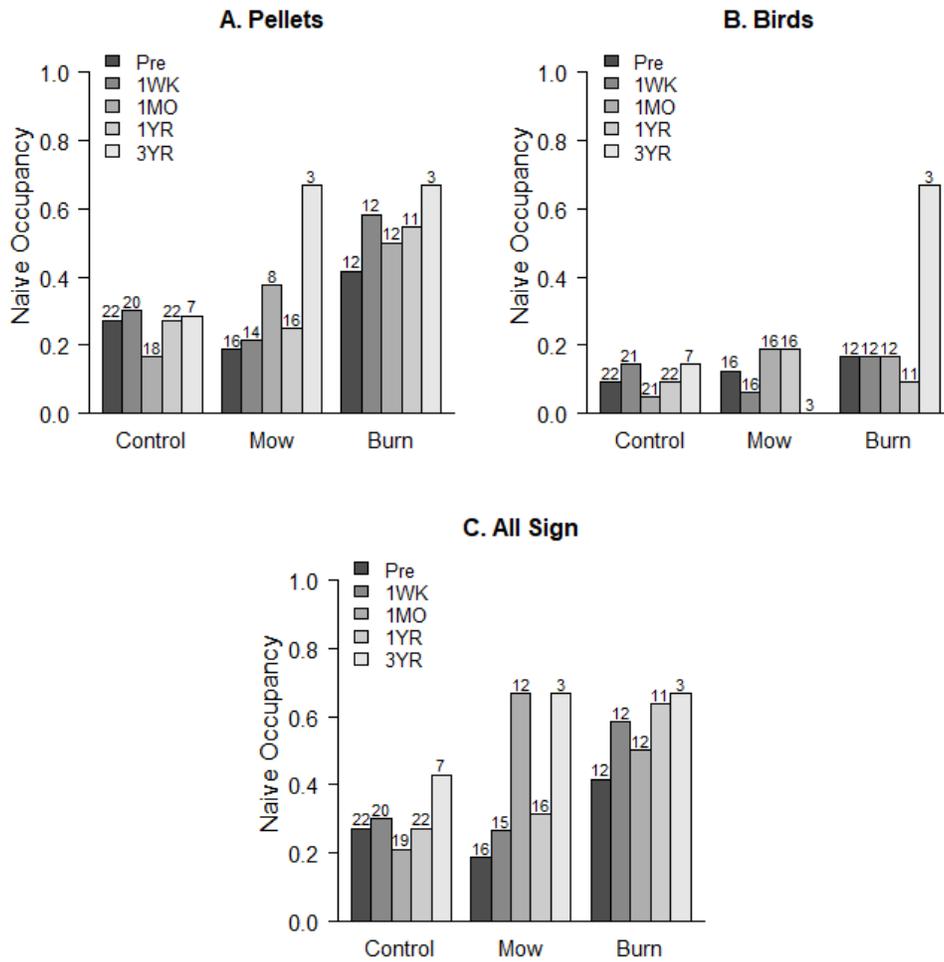


Figure 1. Naïve occupancy for sharp-tailed grouse pellets (A), sharp-tailed grouse observations (B), and all sign (includes off-transect detections, C) during surveys conducted before (PRE), 1 week (1WK), 1 month (1MO), 1 year (1YR), and 3 years (3YR) after treatment at sites sampled during 2015–2019 in northwestern Minnesota to assess the effects of prescribed burning and mowing compared to control sites. Numbers above bars denote sample sizes (number of sites).

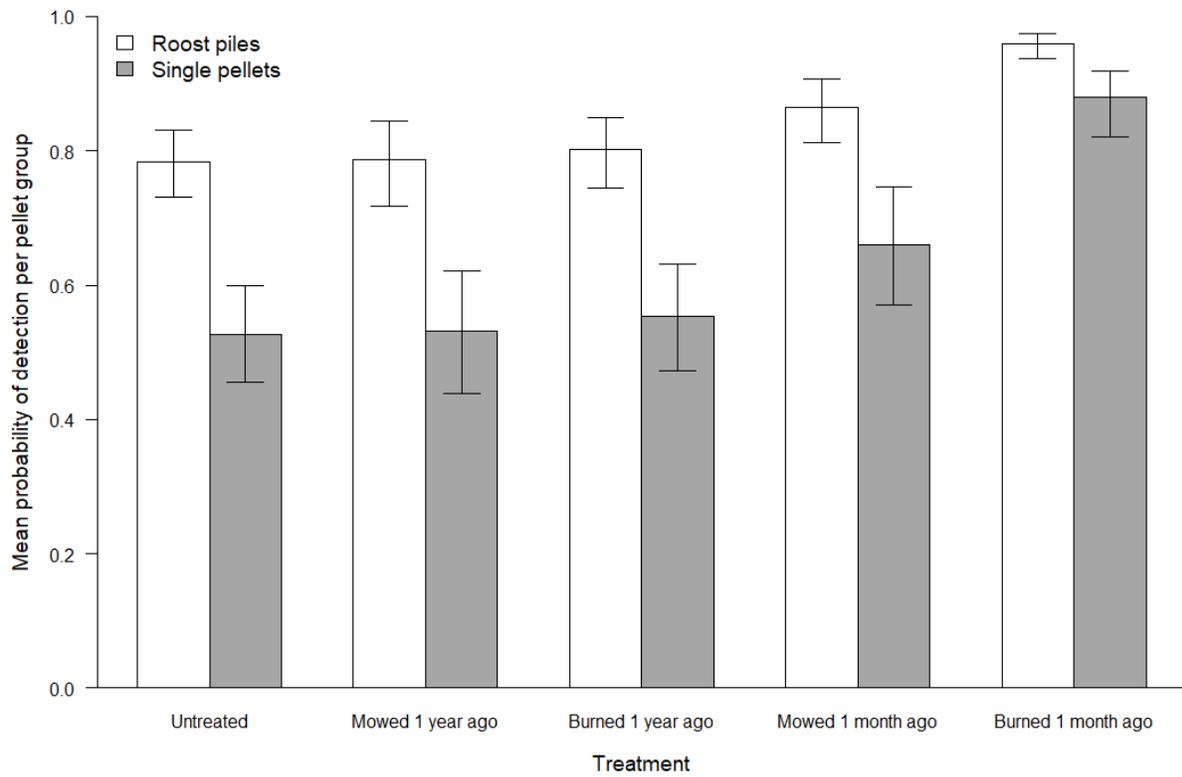


Figure 2. The probability of detecting sharp-tailed grouse fecal pellets (i.e., singles and roost piles), given that they were present at sites burned or mowed 1 month or 1 year prior and at sites not recently treated in northwestern Minnesota during 2015-2019.

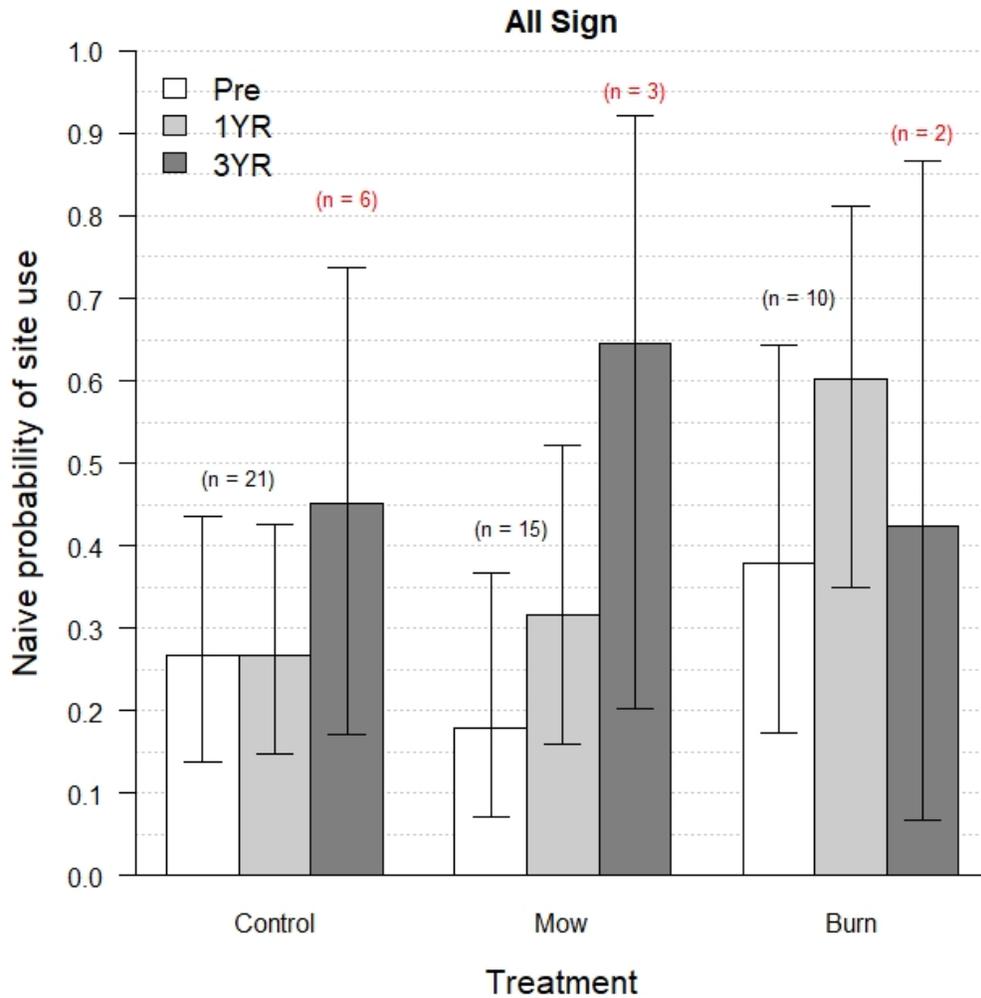


Figure 3. Mean naïve occupancy index of sharp-tailed grouse before, 1 year (1YR) and 3 years (3YR) after management (with 85% confidence intervals) at sites in northwestern Minnesota sampled during 2015–2019 based on a mixed-effect logistic regression model. We have not yet completed 3YR surveys at sites treated in 2017 and 2018.

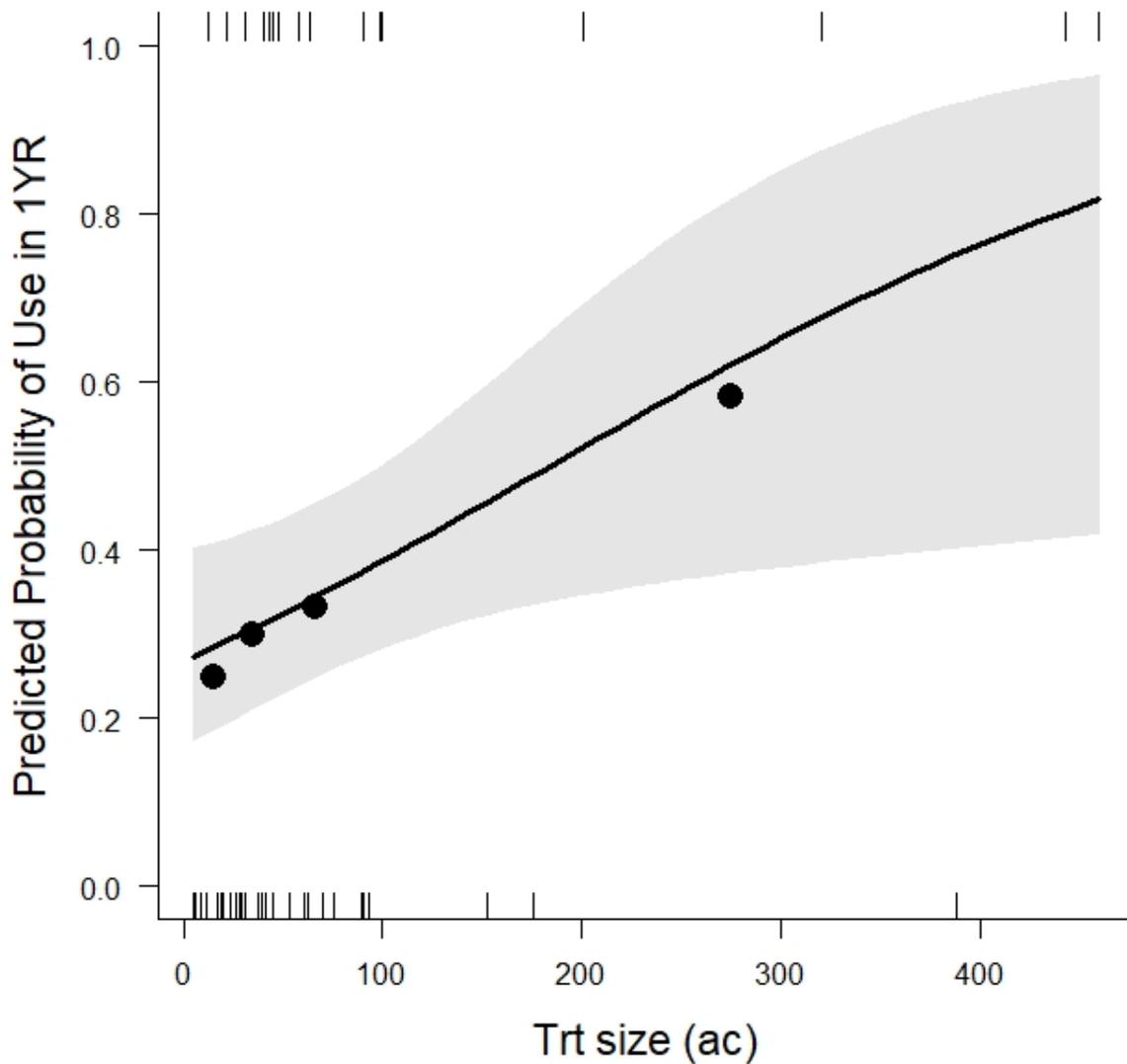


Figure 4. The relationship between the area disturbed (Trt size in acres) and the probability of sharp-tailed grouse use in northwestern Minnesota during 2015-2019. The black line is the predicted probability of use from a logistic model with 1 predictor (Trt size) when we condition on 1YR (no repeated measures) and assume no treatment effect. The gray polygon is the 85% confidence interval. Black circles are the proportion of sites, pooled across treatments by size quartiles, with evidence of site use (all sign) in 1YR. The upper rug describes the distribution of treatment sizes for sites with evidence of sharp-tailed grouse use, whereas the lower rug is the distribution of treatment sizes for sites where we failed to find evidence of site use.



## SPRUCE GROUSE RESPONSES TO TIMBER HARVEST IN MINNESOTA

Charlotte Roy and Julia Ponder<sup>1</sup>

### SUMMARY OF FINDINGS

We completed the first season of a 2-year study to examine spruce grouse (*Falci pennis canadensis*) responses to harvest of jack pine (*Pinus banksiana*) and black spruce (*Picea marina*) stands, their preferred habitat in Minnesota. Eighteen spruce grouse were marked with VHF radiotransmitters during the winter 2019-2020 capture season. Additionally, we surveyed 25 timber stands for spruce grouse and their pellets to examine use of stands before harvest. During this first winter of the study, 18 study stands were harvested, including 13 black spruce and 5 jack pine stands. We will continue to capture and track spruce grouse through fall 2021 to examine survival and movements before, during, and after timber harvest.

### INTRODUCTION

Spruce grouse (*Falci pennis canadensis*) occupy forest stands with a variety of coniferous tree species including spruce (*Picea* spp.), fir (*Abies* spp.), pine (*Pinus* spp.), hemlock (*Tsuga* spp.), and tamarack (*Larix laricina*), and habitat selection varies with the forest community in various portions of spruce grouse range (Boag and Schroeder 1992, Lycke et al. 2011, Anich et al. 2013). In the Great Lake States, spruce grouse prefer areas of black spruce (*Picea marina*) with some jack pine (*Pinus banksiana*) interspersed, but they have also been reported using balsam fir (*Abies balsamea*), tamarack, eastern hemlock (*Tsuga canadensis*), and northern white cedar (*Thuja occidentalis*; Robinson 1969, Pietz and Tester 1982, Anich et al. 2013). In Wisconsin, spruce grouse used closed canopy coniferous uplands near lowland conifer swamps, especially mature black spruce-tamarack swamps and jack pine 16–29 years old (n=55 birds, Anich et al. 2013). Similarly in Minnesota, but with a more modest sample size (n=15), spruce grouse used lowland conifers with black spruce and mixed bogs during the breeding season, and used jack pine during winter (Pietz and Tester 1982). Various successional stages are used throughout spruce grouse range, including early (Boag and Schroeder 1992), mid-successional (Ross et al. 2016), and mature forest (Anich et al. 2013). Forest structure is probably more important than stand age (Schroeder et al. 2020). Lycke et al. (2011) reported that both forests >30 years and <30 years old were used by spruce grouse in Québec. Various forest management practices in different regions likely produce favorable structure in different aged stands throughout the spruce grouse range, explaining the differences among studies.

Spruce grouse responses to timber harvest have been studied in Québec, which is more centrally located within the spruce grouse range than Minnesota (Turcotte et al. 1994, 2000; Potvin and Curtois 2006, Lycke et al. 2011). Logging in Québec during these studies had some similarities to logging practices in Minnesota (e.g., commercial thinning) and some differences (e.g., clearcutting 150–250 ha blocks with residual 60–100 m buffer strips). Following clearcuts, spruce grouse moved to other forested areas nearby (<200 m), usually overlapping with their previous home range (Turcotte et al. 1994, 2000), which is only possible when residual forest is

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nearby. Home ranges (23–41 ha) during logging were similar to those before logging (13–33 ha), but survival was higher before logging than after ( $S=0.75$  vs  $0.44$ ). Lower survival was primarily due to predation, which reduced the density of birds by 60% (Turcotte et al. 2000). In another study, spruce grouse had more extensive movements and lower survival in buffer strips and uncut forest patches after clearcutting (Girard 1999 and Strobel 1999 [in French] as cited in Potvin and Courtois 2006, Turcotte et al. 2000). Potvin and Courtois (2006) studied spruce grouse use of residual strips after clearcutting and documented that home ranges were slightly larger in residual forest strips than in contiguous forest. Survival was also lower in residual strips ( $S=0.52$  vs  $0.73$ ). Moreover, strips used by spruce grouse were located more distantly from contiguous forest (719 vs 417 m), perhaps indicating that spruce grouse abandon these strips if large residual patches of forest are nearby. In British Columbia, survival was positively related to connectivity, and areas of low connectivity acted as population sinks, but densities of spruce grouse were similar in areas of low and medium connectivity (Harrison 2001). Spruce grouse responses to commercial thinning have also been examined (Lycke et al. 2011). Thinning adversely impacted grouse based on substantially lower use of thinned stands than uncut stands in Québec, which was attributed to the loss of dense understory vegetation (Lycke et al. 2011).

Spruce grouse responses to timber harvest in Minnesota have not been investigated, but it is likely that the impact varies depending on the landscape context, availability of residual forest nearby, and logging practices. We aim to study spruce grouse responses to timber harvests of various sizes and in various landscapes to address this information gap. In Minnesota, black spruce harvest occurs in winter and jack pine harvest usually occurs in summer. Birds will be marked and monitored to capture responses to timber harvest during different periods of their life cycle. During winter, spruce grouse contrast strongly against the snow, which may make them vulnerable to predation when crossing areas that lack cover. During summer, female spruce grouse have broods that may constrain behavioral responses. By monitoring spruce grouse responses during both winter and summer, we will gain a more complete understanding of how birds immediately respond to habitat loss at different times of year, and whether loss of habitat within a substantial portion of the home range results in higher mortality relative to that in unharvested stands. We will determine if spruce grouse move to the nearest conifer forest stand, or farther, and whether there is a mortality cost of moving and occupying a new stand.

## **OBJECTIVES**

1. We will examine spruce grouse use of jack pine and black spruce stands before and after timber harvest.
2. We will examine spruce grouse responses to timber harvest by marking spruce grouse with radiotransmitters to monitor movements and survival before and after timber harvest.
3. We will determine whether responses (i.e., movements and survival) to timber harvest in winter differ from responses in summer.

## **STUDY AREA**

We have 2 focal areas for our study, one based out of Red Lake Wildlife Management Area (RLWMA) in Lake of the Woods and Roseau Counties, and one based out of Big Falls in the Littlefork DNR Forestry work area in Koochiching County (Figure 1). We identified stands scheduled for harvest by working with wildlife managers and foresters in regions where spruce grouse surveys indicated comparatively high spruce grouse populations in the state (Roy et al. 2019). Eighteen black spruce stands (11–145 ac, 4–59 ha) and 17 jack pine stands (9–43 ac, 4–17 ha) were identified for harvest near RLWMA (C. Tucker, pers. comm.). An additional 43 black spruce stands (3–221 ac, 1–89 ha) were identified in the Littlefork Forestry area (B. Feldt

and J. Rengo, pers. comm.), along with 87 black spruce stands (0.1–79 ac, 0.04–32 ha) and 10 jack pine stands (1.6–11 ac, 0.6–4.5 ha) identified by Koochiching County Land and Forestry (N. Heibel, pers. comm.). A few additional red pine (n=4) and white spruce or mixed spruce (n=13) stands were identified as planned for harvest but given a lower priority than black spruce and jack pine stands in the study. Some of the stands planned for harvest will not be harvested during the study because loggers have 3 years to complete harvest and thus may harvest some of these stands after the study concludes.

## METHODS

We used multiple methods to locate birds for capture in or near timber stands scheduled for harvest. We drove roads to find birds coming in for grit near stands close to roads. We solicited the help of volunteers with trained pointing dogs to search stands and the adjacent areas. We looked for fecal pellets in winter when pellets are obvious against the snow (Roy et al. 2020). We broadcast the cantus call, a female call, during spring to locate males and females (Fritz 1979, Boag and McKinnon 1982, Schroeder and Boag 1989, Whitcomb et al. 1996, Lycke et al. 2011, Roy et al. 2020). We captured spruce grouse primarily with noose poles (Zwickel and Bendell 1967, as used by Anich et al. 2013, Ross et al. 2016). We also tried setting mirror traps and walk-in traps baited with various lures; blueberries, grit, rose hips, and red hummingbird-food-dyed corn. Four capture seasons were planned; winter 2019-2020, spring 2020, winter 2020-2021, and spring 2021.

Once captured, we recorded capture time, capture method (noose or trap type), sex, age, any injuries during capture or other notable findings, and release time. We attached 12.0 g necklace-style VHF A-3950 transmitters from Advanced Telemetry Systems to spruce grouse (Roy et al. 2016). We also collected a feather sample for genetics, banded the bird with an aluminum band and color band, weighed birds with a pesola spring scale, and measured the tarsometarsus length with calipers.

At each capture location, we recorded GPS coordinates, stand type, the capture tree species (or tree nearest the capture location when the bird was captured on the ground), capture tree circumference, distance to the nearest tree, nearest tree species and circumference, and the number of live trees in a 3.6 m radius of the capture tree (40.7 m<sup>2</sup> plot), which is similar in size to the 1/100-ac fixed radius plot as is used in Cooperative Stand Assessment Field Procedures (MNDNR 2001). At 3.6 m from center in the 4 cardinal directions, we took densiometer readings (Fiala et al. 2006, Paletto and Rosi 2009, Baudry et al 2014) and measured shrub density in 1-m<sup>2</sup> plots. We selected these habitat measurements based on vegetation attributes that differed between stands where spruce grouse were observed and where they were not observed (Potvin and Courtois 2006), or other attributes that predicted spruce grouse presence or occupancy including stem density, shrub cover, basal area, and canopy cover (Huggard 2003, Ross et al. 2016).

We used homing techniques to obtain GPS coordinates of bird locations twice weekly. We tried to track birds more intensively (i.e., multiple times a day through triangulation) immediately before, during, and after timber harvest to characterize changes in movements to new areas. Marked birds in stands that were not harvested served as controls. At each bird location we collected the same habitat attributes as collected at capture locations.

Additionally, to locate spruce grouse for capture and to examine spruce grouse use of timber stands scheduled for harvest before we could capture birds in them, we surveyed transects in stands for spruce grouse pellets. We determined transect lengths based on timber stand area and sampled at a rate of 10 m/ac (25 m/ha). We placed transects systematically through timber harvest areas with a starting point on the harvest boundary. We set up transects to run north-south (0° or 180°) or east-west (90° or 270°) so that we could walk a compass direction from

one edge of the stand (i.e., the boundary) to the other side. The transect traversed the timber harvest area capturing both edge and interior portions. We spaced transects  $\geq 150$  m apart (Evans et al. 2007). We searched 1 m on either side of the transect for spruce grouse pellets (Evans et al. 2007, Schroeder and Vander Haegen 2014, Roy et al. 2020) and also recorded grouse observed, tracks, and ruffed grouse pellets (Huggard 2003) both on and off transect. Every 100 m along the transect, we measured habitat characteristics in a habitat plot (Huggard 2003), collecting the same data as collected at capture sites and telemetry locations to characterize habitat in the stand. Pellet surveys were conducted when it had not snowed for >3 days, unless harvest was imminent and a survey had to be completed earlier. When imminent harvest did not preclude completion of 3 surveys, we surveyed each stand  $\geq 3$  times (Huggard 2003).

## **RESULTS AND DISCUSSION**

During the first winter capture season, Nov 2019–Mar 2020, we marked 18 spruce grouse; 13 females and 5 males. At the Red Lake WMA study site, we marked 2 adult females (AF) and 5 juvenile females (JF), and at the Big Falls study site we marked 5 JF and 1 AF and 4 juvenile males (JM) and 1 adult male (AM). Males allowed us to find females that were grouped up in winter flocks for capture. Four birds (3 JF and 1 JM) were caught in mirror traps and 14 were noosed on the ground (8) or in trees (6). One additional JF died after being captured on the ground with a noose, likely related to handling stress. These 18 birds were located via triangulation 211 times (80 locations at Norris Camp, 131 locations at Big Falls). Three birds at the Big Falls site were depredated, with sign at the kill site consistent with fox depredation but inconclusive.

Spring 2020 capture efforts were interrupted due to the COVID-19 pandemic and the Stay at Home Order beginning Mar 28 by Governor Walz. Based on our earlier research (Roy et al. 2020), early April is when spruce grouse are most responsive to the cantus call in Minnesota, and responsiveness gradually drops off through the end of May. Capture and tracking efforts resumed on May 8 after the Commissioner authorized fieldwork for a limited number of staff on the study. The Commissioner authorized remaining staff to resume work on the study on May 27.

We conducted pellet surveys in 14 timber stands at the RLWMA study area and 11 stands in the Big Falls study area. In the RLWMA study area, we detected spruce grouse sign on a transect in 1 stand, and off transect in 3 additional stands. In the Big Falls study area, spruce grouse sign was detected both on and off transect in 2 stands. Nine stands were harvested during the first winter at the RLWMA study area, consisting of 5 jack pine and 4 black spruce stands. During the same period, 9 black spruce stands were harvested at the Big Falls study area. We plan to continue fieldwork through fall 2021.

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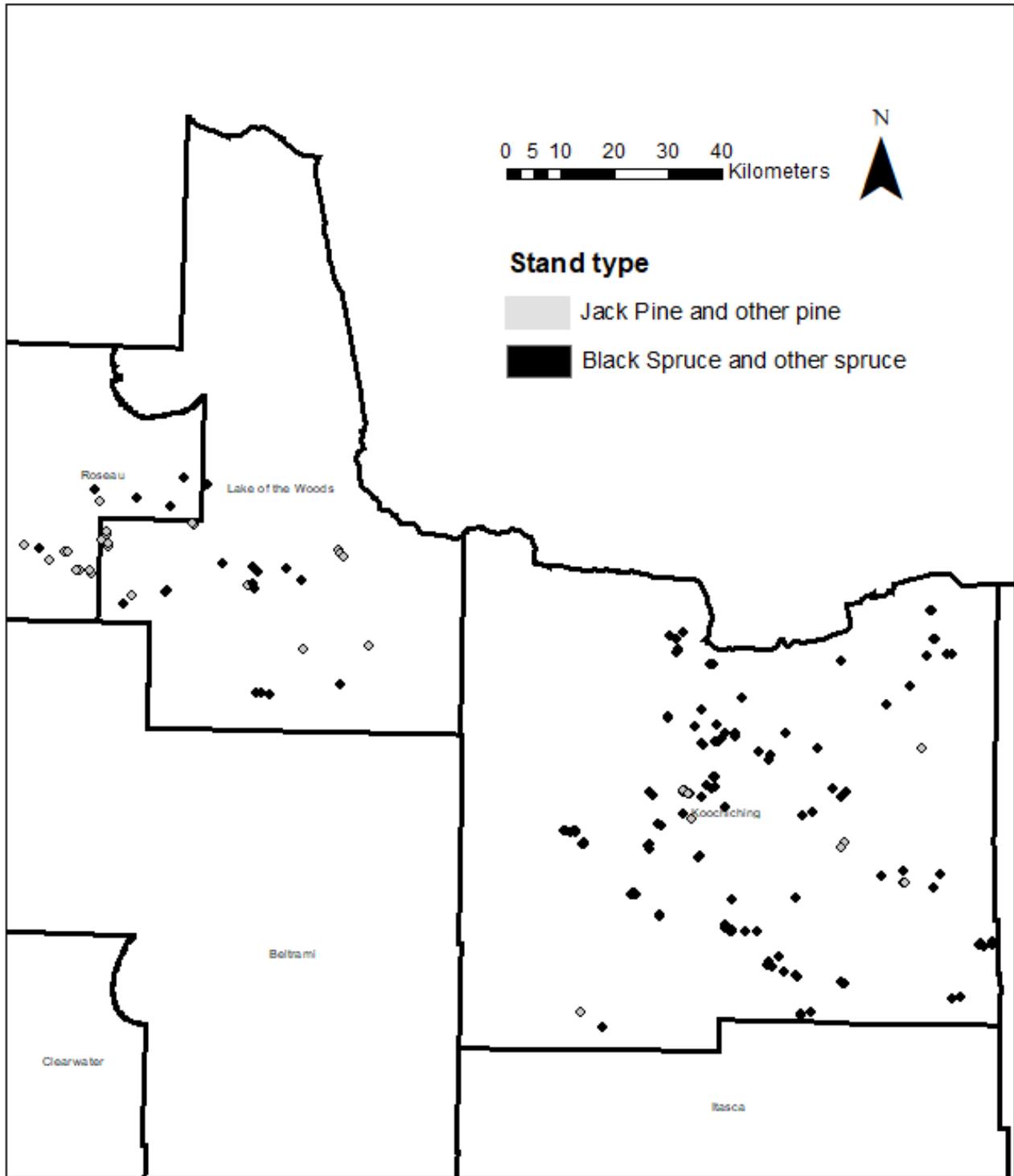


Figure 1. The spruce grouse study area in northern Minnesota with 2 focal areas, one in Lake of the Woods and adjacent Roseau County with more jack pine than black spruce stands scheduled for harvest and another centered on Big Falls in Koochiching County which had more black spruce than jack pine stands scheduled for harvest. The study is planned for winter 2019–fall 2021.



## WEST NILE VIRUS EXPOSURE AND INFECTION RATES IN HUNTER-HARVESTED RUFFED GROUSE IN MINNESOTA

Charlotte Roy, Michelle Carstensen, Kelsie LaSharr, Carolin Humpal, and Ted Dick

### SUMMARY OF FINDINGS

Minnesota participated in a collaborative, multi-state West Nile virus (WNV) study of ruffed grouse (*Bonasa umbellus*) in the Great Lakes region during 2018 and 2019. Cooperating hunters (n=117) voluntarily collected 273 samples from birds harvested during the 2018 hunting season and hunters (n=166) collected samples from 317 ruffed grouse during the 2019 season. We submitted blood on filter strips and hearts to the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia to assess both 1) viral exposure as indicated by antibodies in blood, and 2) virus in hearts indicative of infection. Laboratory results indicated that 12.5% and 12.3% of birds were positive for antibodies to WNV or a flavivirus (most likely WNV rather than St Louis encephalitis virus, based on known WNV activity during the study period) in 2018 and 2019, respectively. However, virus was not isolated from hearts in either year, indicating that exposed birds were not infected at the time of collection. These findings indicate that Minnesota ruffed grouse are exposed to WNV. Some birds either mount a successful immune response without symptoms or develop symptoms and recover. However, any birds that might have succumbed to infection over the summer and did not survive were not available for sampling during the fall, so it is difficult to know how many birds might have been lost to WNV. Hunters and other citizens also submitted carcasses from 14 birds exhibiting abnormal behavior (e.g., unable to fly when flushed) or that had reduced pectoral muscle and a prominent keel. We submitted these presumably sick grouse to the University of Minnesota for necropsy and WNV screening. Eleven were also screened for Eastern Equine Encephalitis (EEE). All of these sick grouse were negative for WNV, but 4 were determined to be infected with EEE, which marked the first time this virus was directly linked to causing morbidity or mortality in a Minnesota wildlife species. Providing high quality ruffed grouse habitat that produces birds in good condition is our best management option for buffering ruffed grouse populations from WNV and other stressors.

### INTRODUCTION

West Nile virus (WNV) is a mosquito-borne virus that infrequently causes a fatal neurological disease in people and horses, and can cause encephalitis and myocarditis in some infected birds. West Nile virus was first detected in a Ugandan woman in 1937 (Smithburn et al. 1940) and was first identified in birds, its natural reservoir host, in 1953 (World Health Organization, WHO, 2017). WNV was not considered pathogenic to birds until 1997 when a more virulent strain killed some birds in Israel. Today, avian mortality remains rare in Europe, Africa, and Asia, but mortality in birds is higher in the Americas (WHO 2017).

WNV was first detected in North America in 1999 when an outbreak of encephalitis was reported in humans in New York City (Eidson et al. 2001). Since its arrival in the U.S., WNV has become established in all of the lower 48 states and has been reported in over 300 bird species (Center for Disease Control 2017), including ruffed grouse (*Bonasa umbellus*). Although some species, like American crows (*Corvus brachyrhynchos*), blue jays (*Cyanocitta cristata*) and other

Corvids, as well as house sparrows (*Passer domesticus*) and common grackles (*Quiscalus quiscula*) readily die of WNV infection, most infected bird species survive (Komar et al. 2003). Interestingly, since the arrival of WNV to the U.S., mortality events due to WNV have never been documented in some bird species including American robins (*Turdus migratorius*), chickadees (*Poecile spp.*), and house wrens (*Troglodytes aedon*). Other species experienced an initial period of reduced survival for several years until they gained immunity to the virus, while Corvids among others, still continue to die from WNV annually (LaDeau et al. 2007, George et al. 2015).

The first documented WNV case in ruffed grouse in Minnesota occurred in 2005 (Ruffed Grouse Society, unpublished data). As many as 66 mosquito species have tested positive for WNV (Center for Disease Control 2019), but the suspected mosquito vector for ruffed grouse in Minnesota is *Culex restuans*, which feeds almost exclusively on birds and is abundant in forests (D. Neitzel, Minnesota Department of Health, personal communication). We suspect that ruffed grouse in northern Minnesota are exposed to WNV annually but do not know if the virus causes active infections that produce symptoms in juvenile or adult birds in the wild. Experimental infection of captive ruffed grouse and sage grouse (*Centrocercus urophasianus*) with WNV indicated high susceptibility of these species to the disease (Naugle et al. 2004, Clark et al. 2006, Nemeth et al. 2017). Furthermore, captive chick survival in ruffed grouse was negatively affected by the virus (Nemeth et al. 2017).

Concern for WNV in ruffed grouse in Minnesota was coincident with a publication on ruffed grouse and WNV from Pennsylvania (Stauffer et al. 2018) and heightened after the 2017 hunting season failed to meet harvest expectations. Hunters expected better-than-average hunting experiences, following a spring drumming count increase of 57% from the previous spring (Roy 2017), as has historically been the case when the 10-year cycle is nearing its peak (Amman and Ryel 1963, Stoll 1980). However, the drumming count is an index to the adult breeding population and these surveys occur before annual production, so drumming counts do not necessarily forecast the juvenile contribution to the fall population. Poor grouse production can adversely impact hunter experiences because juveniles comprise most of the fall harvest (Dorney and Kabat 1960, Dorney 1963). Despite 10-year cycles around a stable population average for decades in the core of Minnesota ruffed grouse range, some hunters have indicated that hunting experiences have been less rewarding during recent peaks in the cycle, leading to speculation that something has been affecting juvenile production.

We examined WNV exposure and infection rates in Minnesota ruffed grouse during the fall by partnering with grouse hunters to obtain samples from their harvested birds. We estimated seroprevalence as an index to WNV exposure and examined hearts for active virus infections. Infection in juvenile birds may represent recent population exposure to WNV and may directly correlate to current viral load on the landscape; whereas adult bird infections may represent either recent or maintained exposure, given the magnitude of their titer levels and presence or absence of virus.

Importantly, this study is a multi-state collaborative effort with other natural resource agencies in the Great Lakes Region, including Wisconsin and Michigan. This concerted effort will provide a more comprehensive view of the role of WNV in the region than any individual state could execute alone and demonstrates the interest of regional biologists in responding to hunter concerns.

## **OBJECTIVES**

1. Assess the feasibility of working with grouse hunters to obtain biological samples from wild ruffed grouse for disease screening and to collect relevant metadata.

2. Estimate exposure to WNV in the fall ruffed grouse population by age class (juvenile and adult).
3. Determine prevalence of active infections (those producing symptoms or where the virus is reproducing) of WNV in fall ruffed grouse populations.
4. Correlate exposure to WNV with active infection using paired samples from the same bird.

## **METHODS**

In 2018, our study area focused on a 60-mile radius around Grand Rapids, Longville, and Bemidji, MN. We chose this area in an attempt to sample along a moisture gradient from west to east, based on rainfall received the previous year, and to simplify logistics of sampling kit dissemination to a few pick-up/drop-off locations. Regional Minnesota Department of Natural Resources (MNDNR) headquarters are located in Bemidji and Grand Rapids and provided a location for distribution of sampling kits. Pineridge Grouse Camp, which is located in Longville, was committed to assisting with our sampling effort and provided a third location for distribution of kits. Numerous organized hunts were also conducted each year in the study area (e.g., Ruffed Grouse Society National Hunt, Northwoods Bird Dogs/Bowen Lake Lodge, Akeley Grouse Hunt), which further facilitated kit distribution and sample collection.

In 2019, we broadened our focal study area to include all of ruffed grouse range within Minnesota, to allow for participation by more hunters and provide a more dispersed sampling distribution within the forested part of the state. We distributed WNV kits to MNDNR Area Wildlife Offices throughout ruffed grouse range, based on anticipated demand by Area Wildlife Managers. As in 2018, we continued to make kits available at the regional offices in Grand Rapids and Bemidji and at Pineridge Grouse Camp in Longville, and worked with organized hunts. To further incentivize hunter participation, a shotgun (16Ga Stevens) and guided grouse hunt were offered as raffle prizes by the Ruffed Grouse Society and Pineridge Grouse Camp, respectively.

### **Hunter Outreach**

We shared multiple press releases with the public with the first on 21 May 2018 announcing the multi-state collaboration between Wisconsin, Michigan, and Minnesota. Additional press releases on 23 Aug 2018 and 19 Aug 2019 provided more details for hunters interested in voluntarily participating in sampling efforts. Multiple media outlets shared progress about the sampling efforts throughout the hunting season to encourage public engagement (e.g., Duluth News Tribune, Outdoor News). The first year we gave presentations at local universities (e.g., Itasca Community College, Bemidji State University), hunting camps (e.g., Pineridge Grouse Camp), regional DNR staff meetings, and distributed kits during these visits. We also attended organized hunts to distribute and collect kits (e.g., Akeley Grouse Hunt, Ruffed Grouse Society National Hunt, Northwoods Bird Dogs/Bowen Lake Lodge). We added a short paragraph about the study to the 2018 and 2019 Minnesota Hunting Regulations and provided a contact for more information. Information about the study was available on the MNDNR website via the [DNR Grouse Hunting Page](#). In fall 2019, we sent every hunter that participated in the 2018 surveillance a letter detailing the test results from their bird(s). We also had a multi-state press release on 22 Oct 2019 announcing the findings from the first season. We will mail letters with results for the fall 2019 sampling efforts to participating hunters in summer 2020.

### **Field Sample Collection**

Each WNV sampling kit contained the following: 1 Nuboto filter strip (Advantec) for blood collection, 1 snack-sized zipper-top plastic bag (e.g., Ziploc brand) for storage of the filter strip in the field, a 3-inch coin envelope for storage of filter strip once blood had dried, a 4-oz whirlpak to collect the heart, a quart-sized zippered plastic bag for collection of feathers to confirm sex

and age, and a datasheet to record hunter contact information and sampling location. In 2018, we also provided *A Grouse in the Hand* pamphlets with kits, courtesy of the Ruffed Grouse Society. We provided a protocol in each sampling kit with detailed directions on how to determine the sex and age of harvested birds based on feather characteristics. The sex of ruffed grouse can be determined through tail length and rump feather dot patterns. Likewise, juvenile (<1 year) and adult (>1 year) age classes can be determined via feather wear of primary feathers collected from the wing of harvested birds in the fall. The instructions stressed the importance of collecting the blood on the filter strip within 30 min of harvest but also indicated samples collected after 30 min still had value. We instructed hunters to thoroughly coat the filter strip with blood until uniformly red and to allow the strip to air dry following the hunt. We asked hunters to record date and time of harvest and blood collection, location of harvest (GPS coordinates or distance and direction from nearest town), county of collection, hunter-determined-age class (juvenile, adult, or unsure) and sex (male, female, or unsure), any relevant comments, and hunter contact information (address, phone, and/or email address) if communication of results was desired. We stored samples collected through organized hunts or through local hunting camps either at room temperature (feathers, Nuboto strips in 2018) or frozen (Nuboto strips in 2019, heart samples) until submitted. Otherwise, we provided hunters with mailing kits with pre-paid United Parcel Service shipping labels, along with freezer packs and thermal bubble mailers to keep samples cold during shipment the following business day. We confirmed age and sex of harvested birds before sending blood and heart samples to the Southeast Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia (Athens, GA) for diagnostic testing after the end of hunting season.

Hunters or members of the public that encountered presumably sick grouse or discovered recently deceased birds occasionally reported these cases to MNDNR because of the ongoing study. When possible, carcasses from these birds were collected by MNDNR staff and stored frozen until submission to the University of Minnesota, Veterinary Diagnostic Laboratory (St. Paul, MN). Board certified pathologists performed whole necropsies to look for histological lesions consistent with clinical infection with WNV or Eastern Equine Encephalitis (EEE). Samples of brain and heart were outsourced to Cornell University to screen for WNV and EEE by polymerase chain reaction (PCR) testing.

### **Laboratory and Data Analysis**

Nuboto strips were reconstituted at SCWDS to test for antibodies to WNV using virus neutralization. Virus isolation was used to detect WNV and EEE in hearts. PCR was used to confirm virus presence. When virus was detected, histological examination of the tissue was performed.

We calculated age-specific apparent seroprevalence of WNV antibodies from serum collected with Nuboto strips using the number of positive detections relative to the total number of blood samples collected. We calculated active infection rates using the number of PCR-positive tests of heart tissue divided by the total number of heart samples submitted. Both seroprevalence and active infection data were mapped using harvest location information.

We performed a space-time scan model in SaTScan v 9.6 (Kulldorff 2018; [SatScan](#)) to look for clustering of positive test results in space and time. We used a Bernoulli cluster-scanning model with positive and negative results to look for clusters of cases where relative risk of infection exceeded that in the surrounding area.

## **RESULTS**

During 15 Sep 2018 – 1 Jan 2019, 117 hunters collected 273 samples from ruffed grouse harvested during the hunting season, of which 213 were collected from within the 60-mile

sampling foci (Figure 1). Most of the returned kits contained all components requested, but 22 samples did not contain hearts, 40 samples were missing some or all feathers for sex or age determination, and 4 samples were missing location information.

During 14 Sep – 22 Dec 2019, 166 hunters collected samples from 317 ruffed grouse throughout the forested region in Minnesota (Figure 1). A road-killed grouse was submitted on 4 Jan 2020. Samples from one spruce grouse were also submitted, but excluded from analyses. Most submissions were complete, but 9 kits lacked hearts, 42 lacked the appropriate feathers for age determination, 10 lacked appropriate feathers for sex determination, and 5 were missing location information.

Antibodies consistent with WNV exposure, as indicated by WNV and flavivirus positive samples (i.e., most likely WNV, based on known WNV activity during the study, but possibly St. Louis encephalitis virus), were detected in 12.5% and 12.3% of samples in 2018 and 2019, respectively. Ten samples in 2018 and 3 samples in 2019 tested positive for WNV, with 24 and 36 positive for flavivirus in 2018 and 2019, respectively. None of the heart samples tested positive for WNV virus in either year. However, virus isolation resulted in 2 positive heart samples, but further testing by PCR confirmed Highland's J Virus, which is similar to Western Equine Encephalitis virus. Highland's J virus has a known distribution in the eastern USA and this was considered an incidental finding. Prevalence in Sep was similar to Oct in both years (12.9% and 11.3% in 2018, and 10.0% and 14.5% in 2019). Sample sizes were not sufficiently large in other months to calculate reliable prevalence estimates (range: 1 – 19).

Of the 14 carcasses of birds suspected to be sick and submitted for necropsy and testing due to abnormal behavior (e.g., unable to fly when flushed) or reduced pectoral muscle and a prominent keel, all birds were negative for WNV. Two of these birds were included in the tally of 317 hunter-submitted samples. Eleven were also screened for EEE, and 4 were determined to be infected with EEE.

### **Cohort Composition and WNV Prevalence**

In 2018, we corrected the hunter-determined age in 50 of 211 cases (24%), and corrected sex 15 of 245 times (6%), not including cases where hunters indicated that they were unsure, or when feathers were not provided for verification. In 2019, we corrected the hunter-determined age 53 of 186 times (28%) and corrected sex in 24 out of 229 cases (10%).

In the sample of birds for which feathers were submitted to verify sex and age, the sex of sampled birds in 2018 (n=212) was fairly evenly split between males (53%) and females (47%). In 2019, the sex ratio was more skewed in favor of males (64%) with 36% females (n=235). The age of birds in the verified sample in 2018 was 64% juveniles and 36% adults; and the age ratio in 2019 was slightly more dominated by juveniles, 71% vs 29% adults. We used these samples to examine the prevalence among sex and age cohorts.

We determined the prevalence of WNV and flavivirus antibodies among verified age and sex cohorts for both years combined because sample sizes were fairly small for some cohorts when split among years (range: 21 – 102). Adults had similar prevalence to juveniles (15.1% vs 11.6%, Chi-square = 1.05, P = 0.31), and males had similar prevalence to females (14.9% vs 9.7%, Chi-square = 2.59, P = 0.11). Adult females (10.7%, n=56) had similar antibody prevalence to juvenile females (10.1%, n=129) and juvenile males (13.3%, n=172), with adult males having the highest antibody prevalence though not statistically different (17.8%, n=90, Chi-square = 3.08, P = 0.38). However, the power to detect statistical differences was low, as 650 individuals per cohort would be necessary to detect a 5% difference in prevalence.

Our spatial analysis did not reveal any significant statistical patterns among test results. We found a marginally significant cluster of positive cases about 20 km northeast of Hibbing, MN ( $P = 0.07$ ).

## DISCUSSION

A small but consistent proportion of hunter-harvested ruffed grouse in Minnesota tested positive for WNV and flavivirus antibodies from blood samples, indicating they had been exposed to virus, but we did not isolate virus in any of the submitted heart tissues. Assuming that false negatives were not an issue, which is a reasonable assumption given the short time for seroconversion to occur, at least some of the birds that were infected with WNV over the summer survived to the fall. We cannot know how many birds might have become infected with WNV and were either asymptomatic, experienced mild disease, or died from the virus over the summer.

Adult males had the highest antibody prevalence of all cohorts, and although not statistically higher, males had 1.66x the prevalence of females, or 7 percentage points higher than females, which is likely biologically relevant. Males might have higher prevalence because they utilize different summer areas than females and their broods (Mangelinckx et al. 2018), and they might experience higher exposure rates to mosquitoes carrying WNV in these areas. Males tend to utilize areas with greater densities of woody stems and less *Rubus* ground coverage than females with broods in the summer in Maine (Mangelinckx et al. 2018). However, we do not know if similar differences in summer habitat use occur in Minnesota, or how mosquito populations and exposure rates might vary across habitats in Minnesota, so sources of variation in exposure rates are not clear. Alternatively, once exposed to WNV, females may have higher mortality rates from the virus than males due to reproductive costs leaving females in poorer condition to face immune challenges and other stressors. Female ruffed grouse with broods had lower survival (69%) than those without broods and also males (98%) in Maine (Mangelinckx et al. 2018), and these findings are consistent with male-dominated sex ratios among harvested birds in our study and others (Dorney 1963, Davis and Stoll 1973). Adult sex ratios for hunter-harvested birds usually favor males, but juvenile sex ratios are usually closer to 50:50 (Dorney 1963, Davis and Stoll 1973). However, the juvenile sample in our study was also skewed towards males, although not as skewed as the adult sample (i.e., 43% juvenile female and 38% adult female). This skew among juvenile females might suggest that females are more susceptible to mortality from WNV or other causes and were less available for harvest in the fall by hunters, but this would require additional research to confirm and is speculative at this point. Juvenile females did have lower antibody prevalence than juvenile males, although not statistically significantly lower, as was seen in adults. However, sex differences in survival to WNV were not observed in American crows, a highly susceptible species to WNV, based on gender determination by discriminant functions (Yaremych et al. 2004). Male vertebrates often have higher disease incidence because testosterone is immunosuppressive (Grossman 1985), but higher antibody prevalence in male ruffed grouse is not consistent with immune suppression.

We found that females had similar antibody prevalence as juvenile birds. We would expect exposure of females and their broods to be similar over the summer because they spend so much time together. However, adult females might be expected to have higher prevalence of antibodies than their broods if WNV antibodies are maintained for an extended period and exposure occurred before broods hatched. The similarity in prevalence between juveniles, who could only be recently exposed to WNV, and adults could indicate that antibody titers do not persist between years for adults. Further study into WNV antibody persistence would be needed to provide a better understanding of titers in adults.

Prevalence of WNV and flavivirus antibodies in Minnesota ruffed grouse was similar or lower than prevalence found in other states, including Pennsylvania, where researchers conducted statewide serosurveys for WNV from hunter-harvested birds in 2016 and 2017 and found apparent prevalence rates of 14% (n=202) and 22% (n=217), respectively (J. Brown, Pennsylvania Game Commission, unpublished data). In the Great Lakes region, Wisconsin reported a prevalence of 29% in 235 ruffed grouse samples and Michigan reported a prevalence of 13% in 213 samples in 2018. Data from our collaborating Great Lakes States during the 2019 sampling season were not available at the writing of this report. Unlike Minnesota, both Wisconsin and Michigan reported cases where WNV was detected in grouse hearts (2 and 4, respectively) in 2018. We cannot know if lower prevalence of antibodies in Minnesota is due to lower exposure rates to WNV or lower survival rates of exposed ruffed grouse, but given the lack of detections of virus in grouse hearts in Minnesota and the high abundance of forested habitat in Minnesota relative to other parts of ruffed grouse range, we suspect that lower exposure rates may be responsible for lower antibody prevalence and the absence of virus in hearts in Minnesota.

The first confirmed case of WNV in wild ruffed grouse in Minnesota was in 2005 (RGS, unpublished data). The first confirmed case in Michigan was in 2017 (Michigan Department of Natural Resources (MIDNR) 2017); 2 grouse were found dead, and 3 hunter-harvested grouse were submitted for testing because they were malnourished and acting strangely. In all 5 cases, heart lesions caused by WNV were observed (MIDNR 2017). The first time WNV was confirmed in Wisconsin ruffed grouse was also during this study, 3 birds tested positive for WNV, with 2 also being co-infected with EEE (Wisconsin Department of Natural Resources, WIDNR, unpublished data). Of the 14 suspect ruffed grouse carcasses submitted for testing in Minnesota since 2017, WNV was not detected, but 4 grouse were confirmed to have EEE.

This is the first time EEE has been confirmed in Minnesota ruffed grouse, although EEE is native to North America. This virus has been present in ruffed grouse populations in the region for a long time, with 50% of road-killed ruffed grouse having antibodies to EEE in a 1957 Wisconsin study (Karstad et al. 1960). However, the first clinical case of EEE was detected in Wisconsin in 2018 (WIDNR 2019). Evidence of EEE in any wildlife species in Minnesota was first discovered in moose and wolves, both showing exposure to the virus but without any evidence of direct morbidity or mortality (Butler et al. 2013, Carstensen et al. 2017). Primary ranges for both moose and wolves directly overlap with ruffed grouse range in Minnesota as well. These first EEE-infected ruffed grouse cases in Minnesota were reportedly exhibiting clinical signs of illness, and we likely only detected these cases because hunters were aware of the WNV study and knew how to submit carcasses of birds. Interestingly, these 4 birds all had lesions in the brain consistent with EEE infection and had no apparent heart lesions from the virus. Thus, it is possible that by relying only on heart tissue submissions for evidence of viral infection of WNV or EEE, we may be missing cases that have brain lesions, as brain samples were not part of our hunter-harvested surveillance. It is reasonable to assume that clinical cases of EEE occur each year in grouse, but go unreported.

Viruses have challenged wildlife populations for longer than wildlife managers have been managing wildlife. However, viruses become a concern when a population is naïve and newly exposed, or when other stressors impact the resistance of a population to immune challenges. For example, a recent study in Pennsylvania found a relationship between ruffed grouse population recovery in areas with poorer habitat and WNV (Stauffer et al. 2018). The interaction between invasive diseases and land-use can result in complex effects on survival of wild birds (George et al. 2015). Minnesota has a lot of forested habitat in a variety of successional stages that benefit grouse. Providing high quality habitat for grouse is our best option for buffering ruffed grouse populations against WNV because birds in good condition have stronger immune

responses. We cannot currently reduce mosquito populations at a broad-scale or eliminate viruses, but we can try to provide the best habitat possible to produce grouse populations that are robust to a variety of stressors, including viruses.

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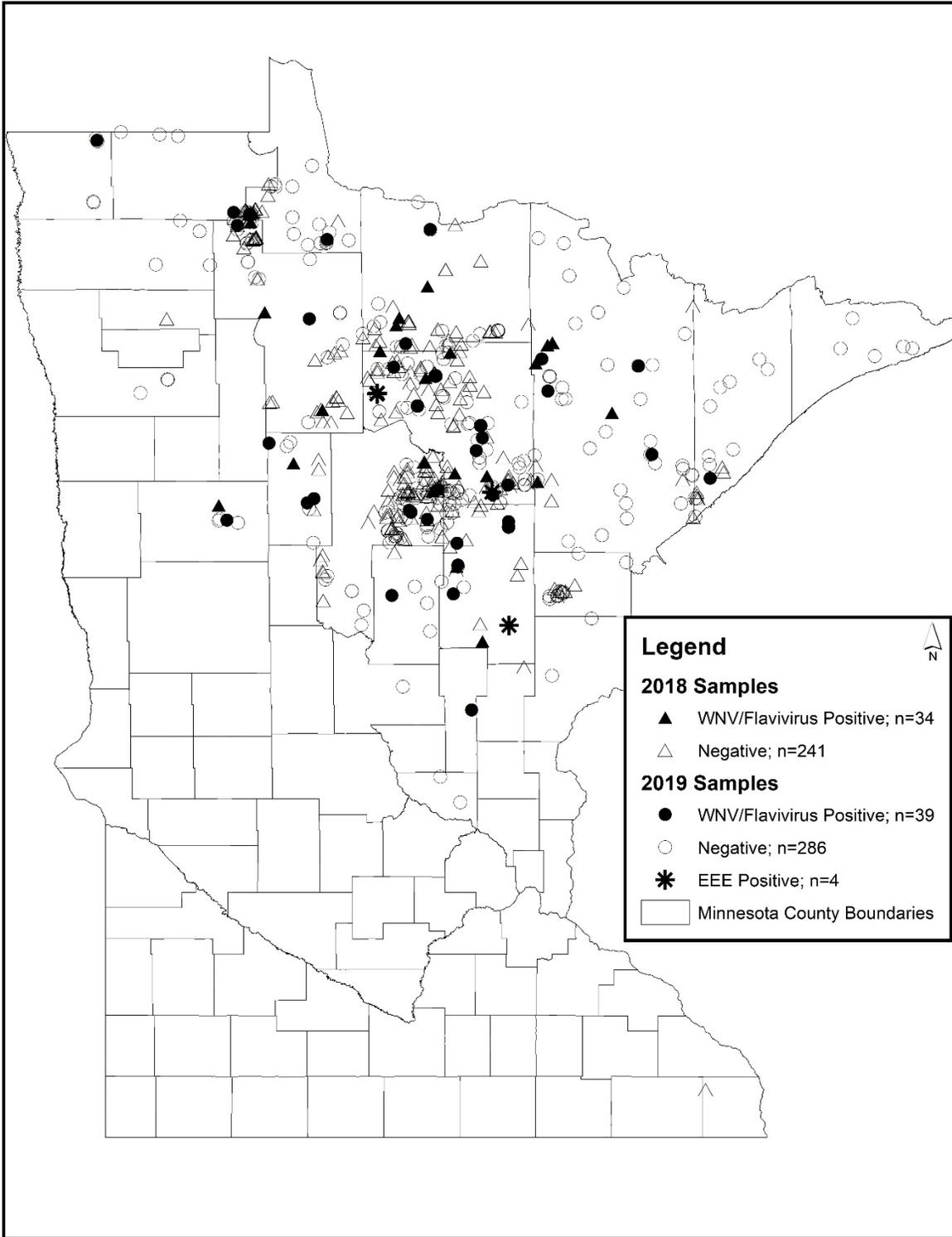


Figure 1. The distribution of hunter-harvested ruffed grouse samples and carcasses of presumably sick birds submitted for testing in Minnesota during 2018 and 2019. Samples are indicated as West Nile virus (WNV) positive/ flavivirus positive (WNV suspect), negative, or Eastern equine encephalitis (EEE) positive.



## WINTER SURVIVAL AND CAUSE-SPECIFIC MORTALITY OF WHITE-TAILED DEER IN NORTHERN MINNESOTA: WINTER 2020 UPDATE

Bradley D. Smith<sup>1</sup> and Glenn D. DelGiudice

### 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) collars, and 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 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 ( $\geq 1.5$  yr) female deer residing on study sites in northcentral (Inguadona Lake) and northeastern (Elephant Lake) Minnesota. 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. The *natural* mortality rate during the first winter (2017–2018) was high; 6 of 19 (31.6%) GPS-collared deer (3 at each site) were killed by wolves during 10 April to 31 May 2018. Overall survival had decreased to 0.68 (95% confidence interval [CI] 0.50–0.93) by the end of May. Since this was a *pilot year*, the survival estimate was limited by small sample sizes (10 collared deer per site) and represented only the late-winter season (12 March to 28 May 2018) due to delayed capture operations. However, during the second and third winters (2018–2019 and 2019–2020), with more than twice the sample size ( $n=51$  and  $n=42$ ), the *natural* mortality rate was also high (38.7% and 24.4%); 18 of 49 deer were preyed upon by wolves and 1 by bobcat (*Felis rufus*) between 1 November 2018 and 31 May 2019, and 10 of 41 deer were preyed upon by wolves between 1 November 2019 and 31 May 2020. The overall survival rate was 0.60 (95% CI=0.48–0.75) and 0.73 (95% CI=0.62–0.88) for winters 2018–2019 and 2019–2020, respectively. Wolf predation rates during the 3 winters (31.6%, 36.7%, and 24.4%) notably exceeded what we expected based on the documented relationship of the previous long-term study. Typically, adult female deer enter winter in better physical condition than fawns and adult males, and thus have the highest probability of surviving winter. Our findings at least suggest that during the 3 winters overall mortality rates at the population level, across sex and age classes, were likely higher than indicated by our adult female data. Ongoing federal protection

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of wolves in Minnesota limits the Minnesota Department of Natural Resources (MNDNR) management options and 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 may be warranted in interpreting our preliminary findings, but they highlight the need for continuation of this study to better understand whether deer-habitat-wolf predation relationships have been changing since completion of the MNDNR’s previous long-term study, a potentially significant consideration relative to implementation of the state’s recently developed deer management plan.

## INTRODUCTION

Studies that have examined the influences of extrinsic (e.g., 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 dynamics of white-tailed deer (*Odocoileus virginianus*) and other ungulate populations in northern Minnesota and elsewhere (Nelson and Mech 1986a,b; Fuller 1990; Bartmann et al. 1992; DelGiudice 1998; Gaillard et al. 2000; DelGiudice et al. 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 in northcentral Minnesota (DelGiudice et al. 2002, 2006). 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, hereafter 1996) compared to the mildest winter (1991). That study also documented that the relative risk of death of female deer 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 mortality by wolves increases sharply for adults 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). This document focused on improving population estimates, but emphasized that improved habitat management should be a key component of a new statewide deer management plan to establish and meet population goals. 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. Partially in response to the OLA report, we recently launched a study using cutting-edge global positioning system (GPS)-collars, and remote sensing and geographic information system (GIS) technologies, that will provide 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 (DelGiudice et al. 2017, 2019).

Fieldwork for this study was initiated during winter 2018. In addition to an overall objective of establishing the technical feasibility of making fine-scale measurements of habitat use by deer at the forest stand level (see Smith et al. 2019), we monitored winter survival and cause-specific mortality as a means of assessing habitat quality (DelGiudice et al. 1989a,b) and to update 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 mentioned above and in a previous research summary (Smith et al. 2019), this study includes 2 deer winter range sites, Inguadona Lake (46 km<sup>2</sup>) in northcentral and Elephant Lake (76 km<sup>2</sup>) 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/km<sup>2</sup> in the vicinity of the Inguadona Lake and Elephant Lake sites, respectively. The MNDNR calculates a winter severity index (WSI) throughout the state by accumulating 1 point for each day with an ambient temperature  $\leq -17.7^{\circ}$  C and an additional point for each day when snow depth is  $\geq 38$  cm during 1 November–31 May. Generally, winters with maximum WSI values (by 31 May)  $< 100$ , 100–180, 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 (DelGiudice et al. 2006). Maximum WSI at Inguadona Lake at the end of winter was 60, 113, and 63 during 2018, 2019, and 2020, respectively. The maximum WSI values at Elephant Lake were 130, 121, and 117. 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. (2019).

## METHODS

During 10–11 March 2018, 19 adult ( $\geq 1.5$  yr 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 2018 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. 2019). Similarly, 20 adult female deer were captured on each site by net-gunning from helicopter (Quicksilver Air, Inc., Fairbanks, Alaska) during 5–8 February 2019 and 13 more deer, 8 and 5 on the Inguadona Lake and Elephant Lake sites, respectively, were captured on 6 February 2020 (Helicopter Wildlife Services, Austin, Texas), and handled following the same protocol. All deer were fitted with a Globalstar Recon GPS collar (Model IGW-4660-4; Telonics, Inc., Mesa, Arizona). We programmed all collars deployed in 2018 and 2019 to collect 1 location-fix every 2 hours during December–June and 1 location-fix every 4 hours during July–November. Collars deployed in 2020 were set to 1 location-fix every hour during December–June and 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 and 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 DelGiudice 1985). We conservatively

assigned ultimate cause of death as “capture-related” when the mortality occurred within 7 days of capture, regardless of the proximate cause (e.g., wolf-kill; DelGiudice 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 to 0.68 (95% confidence interval [CI] 0.50–0.93), 0.60 (95% CI 0.48–0.75), and 0.73 (95% CI 0.62–0.88) during winters 2017–2018 (2018), 2018–2019 (2019), and 2019–2020 (2020), respectively, and was consistently similar at Inguadona Lake and Elephant Lake (Figure 1). The overall crude natural mortality rate (reported for consistency with the estimate from the previous study, but not to replace use of the aforementioned survival rates) was 31.6% (6 of 19 deer), 38.7% (19 of 49 deer), and 24.4% (10 of 41 deer) during winters 2018, 2019, and 2020, respectively (Figure 2). These rates exclude 1 capture-related mortality during each of the first and second winters and an unrecovered hunter-harvested deer during each of the second and third years. Wolf predation rates were 31.6% (6 of 19), 36.7% (18 of 49), and 24.4% (10 of 41) during the 3 winters (Figure 2). Wolf predation accounted for all of the natural mortality during winter 2018 and 2020, and all but 1 of the mortalities during 2019, which was a deer killed by a bobcat (*Felis rufus*). Many of the wolf-killed deer were in poor condition as indicated by a mean marrow fat content of 71.8% ( $\pm$  5.3 [SE], range = 3–91%,  $n=22$ ; Watkins et al. 1991).

Given the low to moderate maximum WSI values during winters 2018, 2019, and 2020 at Inguadona Lake (60, 113, and 63) and Elephant Lake (130, 121, and 117), reflecting mild to moderately severe conditions, 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. Deepening snow increasingly impedes deer mobility and escape (deer have a heavier weight-load-on-track than wolves) and steadily compromises their 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 and body 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 in any of the 3 years, as indicated by maximum WSI values, prompts consideration of the role of winter habitat deficiencies compromising the ability of deer to adequately fulfill their biological requirements and avoid wolf predation as the season progresses. Indeed, it is additionally noteworthy that winter survival and wolf predation rates were similarly low and high, respectively, on both sites during all 3 winters. 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 continued deer research that enhances the MNDNR’s understanding of vital estimates and associated population dynamics for improved management (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 these 3 years could have been even higher than indicated by our adult female data. 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 1989 (1,521 wolves), just as the previous deer study was initiated (winter 1991), to the present (~2,900 wolves, Erb and DonCarlos 2009, Erb et al. 2017). Accumulating evidence critically highlights the need for multi-year continuation of this 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).

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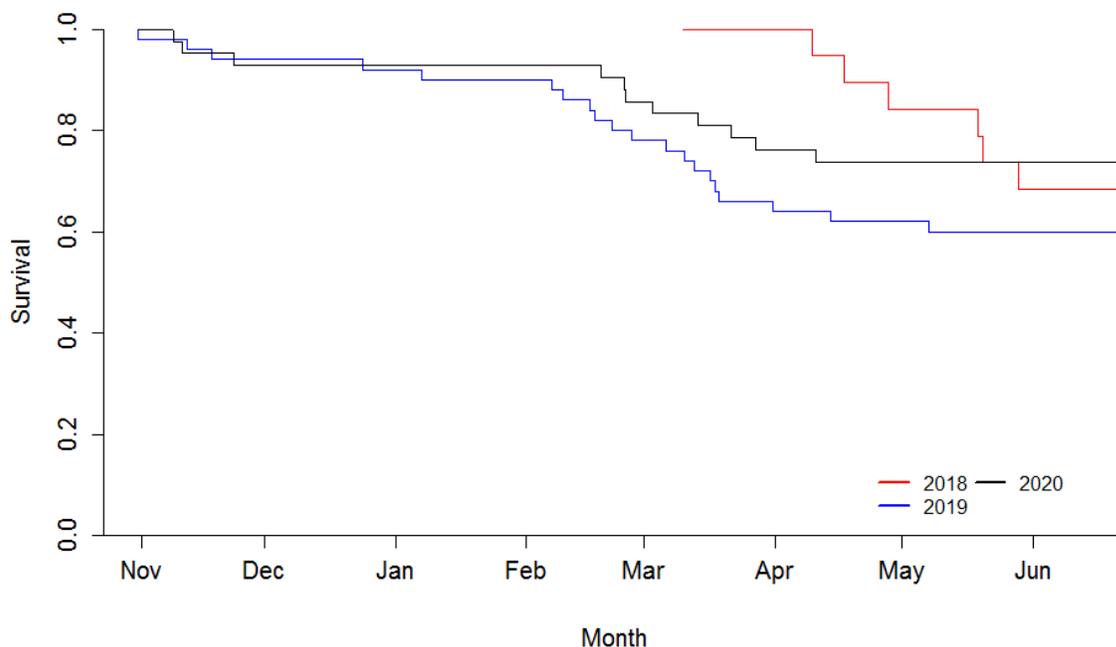


Figure 1. Kaplan-Meier survival curves of adult ( $\geq 1.5$  yr), female white-tailed deer from date-of-capture, 10–11 March ( $n=19$ ) to 31 May 2018, from 1 November 2018 to 31 May 2019 ( $n=50$ ), and from 1 November 2019 to 31 May 2020 ( $n=42$ ), at the Inguadona Lake and Elephant Lake study sites (pooled), northcentral and northeastern Minnesota. One capture-related mortality was excluded from the analysis for each of the first 2 winters.

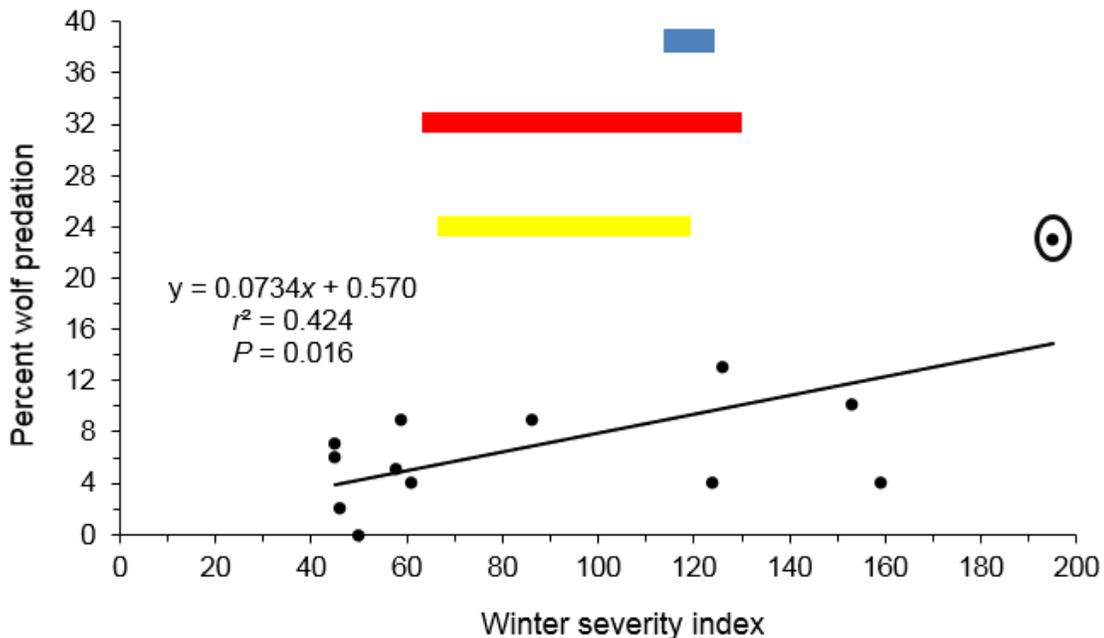
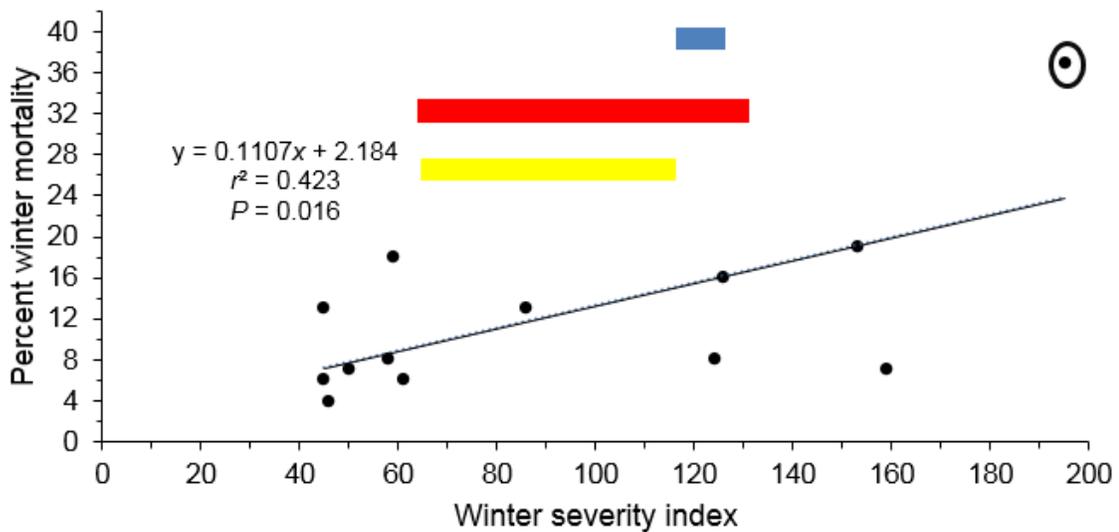


Figure 2. Comparison of pooled crude winter mortality (top) and wolf predation rates (bottom) of adult ( $\geq 1.5$  yr), female white-tailed deer at the Inguadona and Elephant Lake sites, northcentral and northeastern Minnesota, respectively, during winters 2017–2018 (red,  $n=19$  deer), 2018–2019 (blue,  $n=49$ – $50$ ), and 2019–2020 (yellow,  $n=41$ – $42$ ), to the long-term relationship of these rates for adult, female deer to the maximum winter severity index (WSI) in northcentral Minnesota, during winters 1990–1991 to 2002–2003 (DeGiudice et al. 2006). Maximum WSI values at the 2 sites spanned 60 to 130, 113 to 121, and 63 to 117 during the 3 recent winters, respectively. One capture-related mortality was excluded from each of the first 2 winters. The circled data point represents historically severe winter 1995–1996.



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## ASSESSING NEONICOTINOIDS EXPOSURE IN FREE-RANGING WHITE-TAILED DEER IN MINNESOTA

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### SUMMARY OF FINDINGS

Recent research has raised concerns about potential adverse effects of neonicotinoid exposure in white-tailed deer (*Odocoileus virginianus*) including reduced survival and productivity. To assess whether free-ranging deer in Minnesota are being exposed to neonicotinoids, we solicited deer hunters to voluntarily submit spleens from harvested animals during the 2019 deer hunting season. Interested hunters signed up to receive a sampling kit (n=1836) and were asked to submit the spleen of their harvested deer and also report the sex, age, date of harvest, and kill location. Hunters could also submit a tooth sample if they wanted their harvested deer aged. A total of 770 spleens and 517 tooth samples were submitted by hunters throughout Minnesota and 29 additional spleens were collected by agency staff from opportunistic deer (agency culling and sick deer). A subsection of each collected spleen (n=799) was submitted to the Ecdysis Foundation (Estelline, SD) for analyses of neonicotinoid exposure; results are expected to be returned by late summer 2020.

### INTRODUCTION

With the rising use and popularity of neonicotinoid pesticides in agricultural practices, there are increasing concerns over the potential adverse impacts of these pesticides on wildlife. Neonicotinoids, including imidacloprid, thiamethoxam, and clothianidin, can negatively affect bees and other pollinators through decreased winter survival (Dively et al., 2015), and reduced reproductive success (Laycock et al., 2012). However, less is known about the potential impacts on large mammals, such as white-tailed deer (*Odocoileus virginianus*). With much of Minnesota in an agricultural region, Minnesota deer are likely exposed to various levels of neonicotinoids depending on their location.

Neonicotinoid pesticides are insecticides that are applied through seed treatments, in foliar sprays, applied granularly to pastures, and injected into trees to target destructive insects. They are most commonly used in agricultural practices but also can be found in some residential applications such as gardens, turfs, and animal practices. Five compounds of neonicotinoid insecticides are currently used in the United States, they include: acetamiprid, clothianidin, dinotefuran, imidacloprid, and thiamethoxam (Environmental Protection Agency, 2019). Neonicotinoids act as a neurotoxin by binding to acetylcholine receptors. One advantage of neonicotinoids is their specificity to receptor sites on insects making them less toxic to mammals. By 2008, neonicotinoids accounted for 80% of the seed treatments, and 24% of all insecticide use globally. In North America, of the 133 million acres of corn, soybean, wheat, cotton, and sorghum treated with insecticides, over 98% were treated with neonicotinoids (Minnesota Department of Agriculture, 2016). Furthermore, Main et al. (2014) found canola, wheat, soybeans, corn, barley, field peas, dried beans, and oats were the most common crops treated with neonicotinoid seed treatments in the prairie ranges of Canada.

The most common neonicotinoids used in Minnesota are clothianidin, thiamethoxam, and imidacloprid (Minnesota Department of Agriculture, 2016). In 2016, Minnesota Governor Mark

Dayton passed an executive order stating that a verification of need by the agricultural producer is required to use neonicotinoid pesticides (Executive Order 16-07, 2016). Minnesota was the first state in the U.S. to require such reporting. However, the Minnesota Department of Agriculture (MDA) does not have the authority to regulate treated seeds. This means that the use of neonicotinoids in seed treatments is difficult to track. According to an MDA document (2016), “almost all corn seed and 20% of soybeans treated outside of Minnesota’s borders and shipped into the state for planting is not tracked”.

A recent study conducted in South Dakota was designed to identify the effects of neonicotinoids on captive white-tailed deer by adding controlled amounts of imidacloprid to their water source (Berheim et al., 2019). Berheim et al. (2019) found imidacloprid exposure in all groups of deer, including the control group. Furthermore, as imidacloprid levels in the spleen increased, fawn survival, thyroxine levels, jawbone lengths, body weights, and organ weights decreased suggesting imidacloprid could negatively impact deer population performance. Archived liver and spleen samples from free-ranging deer in North Dakota were also tested with the same procedure and results showed imidacloprid levels were 2.8 times higher in the liver and 3.5 times higher in the spleen in the free-ranging deer than the captive deer in their study (Berheim et al., 2019).

## **OBJECTIVES**

1. Assess the feasibility of working with deer hunters to obtain biological samples from free-ranging white-tailed deer for disease screening and collecting relevant metadata for those samples.
2. Estimate exposure of neonicotinoids in white-tailed deer across different regions of Minnesota.
3. If high levels ( $>0.33$  ng/g) of neonicotinoids are discovered in white-tailed deer, provide a basis to direct future research on potential impacts of neonicotinoid exposure on fawn survival and recruitment.

## **METHODS**

Our study area consisted of all deer permit areas (DPAs) in the state of Minnesota. DPAs were categorized based on estimated use intensity of agricultural neonicotinoid products (Figure 1). We determined neonicotinoid intensity by looking at 3 major crops commonly treated with neonicotinoids in Minnesota: corn, soybeans, and wheat. We calculated the individual acreage of these 3 major crops using the 2019 cropland data layer (USDA-NASS, 2019) within each DPA using the deer permit areas layer (Minnesota Department of Natural Resources, MNDNR, 2019). We then summed the acreage of each crop type to calculate a total crop acreage. Total crop acreage was then divided by the total acreage of the deer permit areas and multiplied by 100 to calculate percent coverage of these 3 combined crops. DPAs were divided into one of the 3 following categories classified by percent coverage of these row crops: heavy use ( $\geq 66\%$  crop coverage), moderate use (33-65% crop coverage), and little to no use (0-33% crop coverage). We designed these categories (i.e. bins) to capture a gradient of potential exposure across Minnesota (Figure 2).

### **Hunter Recruitment**

We used multiple methods of communication and outreach to recruit hunters and gain project interest. We contacted deer hunting groups to inform them of the study, and solicit their members to assist in sample collection. These hunting groups included the Minnesota Deer Hunters Association (MDHA), Bluffland Whitetail Association (BWA), Quality Deer Management Association (QDMA), and Backcountry Hunters and Anglers (BHA). We also collaborated with

tribal biologists (Fond du Lac Band, Grand Portage Band, and 1854 Treaty Authority Band) to encourage tribal hunters to participate in sample collections. The Minnesota chapter of The Wildlife Society (TWS), and MNDNR wildlife staff also received emails detailing study information and how they could participate and were asked to promote the project through their organizations. The project was featured in the MNDNR's monthly Deer Notes newsletters. Deer hunters who provided an email address to the electronic licensing service (ELS) database also received an email blast. This was designed to reach audiences not connected with deer hunting groups or other targeted organizations. Finally, staff participated in radio interviews and newspapers articles about the project when opportunities arose. Interested participants signed up through an online form that collected their name, email address, physical address, and which DPA they hunted most frequently.

### **Sample Collection**

Our goal was to collect 200 samples from the little to no use category, 200 samples from the moderate use category, and 400 samples from the heavy use category for 800 total samples. We began collecting samples at the start of regular firearms deer season (9 Nov 2019) and stopped collecting samples at the end of archery season (31 Dec 2019).

All hunters that agreed to participate in sample collection were mailed a small sampling kit. Kits were pre-labeled with an ID and preassigned to an individual based on the online form. Each kit contained: a detailed sampling protocol, 1 cold pack, 1 sealable sample bag (spleen), 1 ziplock bag, 1 coin envelope (tooth), and 1 return shipping box with prepaid postage and insulated insert. The hunter was instructed to collect the spleen, and record date of harvest, DPA of harvest, age class (fawn, yearling, adult), sex, and any additional comments on the data label. A link to an instructional video was included in the protocol to describe the location, texture, and color of a spleen in a freshly harvested animal. Images and written descriptions were also provided in the instructional protocol for hunters unable to access the video. Interested hunters were asked to provide a front incisor from their deer and include it with the shipment to be aged via *cementum annuli* at no cost to the hunter as an additional incentive.

Once a successful hunter collected the samples and recorded the data, they were instructed to ship their samples using the provided shipping box and prepaid label. Sample boxes arrived at the Wildlife Health Program (WHP) office in Forest Lake, MN daily, were immediately placed in a chest freezer, and were stored at -20°C. Once the hunting season ended, all samples were verified to ensure spleen tissue and 4 subsamples (2.5cm x 2.5cm) were collected after allowing spleens to thaw overnight. We archived 3 subsections and sent 1 subsection to the lab for processing. Scissors were sanitized between each spleen with 70% ethyl alcohol (EtOH) to prevent cross-contamination. Cardboard boxes were used as cutting surfaces and were replaced after each sample was processed. The subset samples were then organized by sample ID and refrozen. If the hunter submitted a tooth, staff ensured the root was intact prior to sending to the lab.

Spleen samples were then shipped to Ecdysis Foundation (Estelline, SD) where they will be tested for the presence of imidacloprid using enzyme-linked immunosorbent assay (ELISA). A subset of positive samples (up to 100) from the ELISA screening will be submitted to Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI) to differentiate between pesticide compounds using mass spectrometry. Teeth were shipped to Matson's Laboratory (Manhattan, MT) for *cementum annuli* aging process to estimate age for each specimen.

### **RESULTS**

A total of 1,836 kits were shipped to 1,756 project participants. Of the 1,836 kits shipped, 1,190 were requested in the little to no use category, 417 were requested in the moderate use

category, and 191 were requested in the heavy use category; 38 participants did not list the area they were going to hunt in. An additional group of interested hunters (n=86) continued to inquire about the project past the kits distribution deadline; they did not receive a kit but will be sent a final report once test results are returned and data is aggregated. Successful hunters returned 798 kits to MNDNR. Upon initial examination, 774 kits contained a spleen sample. Of the returned kits, 569 contained a tooth; however, 52 teeth were unsuitable for lab submission due to missing the root. Four spleen samples were omitted due to incorrect or insufficient data. In total, 770 spleen samples submitted were by participating hunters for a 42% rate of return on all disseminated kits. To reach the goal of 800 samples, an additional 28 samples were collected from the agency culling efforts in southeast Minnesota, as well as 1 sample from a sick deer that was taken by wildlife staff in the north metro area. In March 2020, 799 spleen samples and 517 tooth samples were shipped to respective labs for analysis. Currently, lab results for both spleen and tooth samples are pending and are expected to be returned late summer 2020.

Of the 799 spleens, 497 came from the little to no use of neonicotinoids category (248% of goal), 220 came from the moderate use of neonicotinoids category (110% of goal), and 82 came from the heavy use of neonicotinoids category (20% of goal) (Figure 3).

## **DISCUSSION**

Our efforts to engage the deer hunting community to participate in this project were successful. While we reached the overall sample goal of 800 spleens, we did not achieve the exact sampling distribution we had hoped for, especially in the heavy use category. We likely fell short of our 400-sample goal in the heavy use category due to the low numbers of kits (n=191) requested by hunters in that category. The vast majority of the samples provided by hunters were the correct tissue, with only a 3% error rate. Communication to project participants seemed to be effective using a broad approach; however, the ELS email list likely reached the largest group, with 93,602 emails sent. The online sign-up form allowed staff to easily track interested participants, especially those who requested multiple kits. By pre-assigning kits to hunters, there was less data entry or effort required by the hunter and MNDNR, which potentially increased data quality. The final key item to the project's success was the short video created to show hunters how to identify and remove a spleen. This video, paired with the photos and written descriptions in the instruction packet, likely played a large role in the hunters successfully collecting the proper tissue. However, more information should have been provided to hunters regarding proper tooth extraction.

All lab results are currently pending and expected by late summer. Results have the potential to be delayed further due to the COVID -19 pandemic; however, at this time it is believed the lab will still be able to follow the timeline. Once results are received from the lab, they will be analyzed and a short project summary will be written. This summary will be distributed to all project participants, whether or not they submitted a completed kit. In addition to the summary, successful participants will receive results on their individual deer including neonicotinoid levels and deer age.

## **ACKNOWLEDGMENTS**

We would like to thank all of the project participants, for without them this project would not have been possible. We would also like to thank Bluffland Whitetails, Minnesota Deer Hunters Association, Quality Deer Management, Backcountry Hunters and Anglers, and the Minnesota chapter of the Wildlife Society for promoting the project through their members. We thank Nyssa Gesch, Pete Takash, and David Schueller with the MNDNR communication team who helped create materials and promote the project. Finally a special thanks goes out to Ashley Anderson for the long hours helping subset and organize samples.

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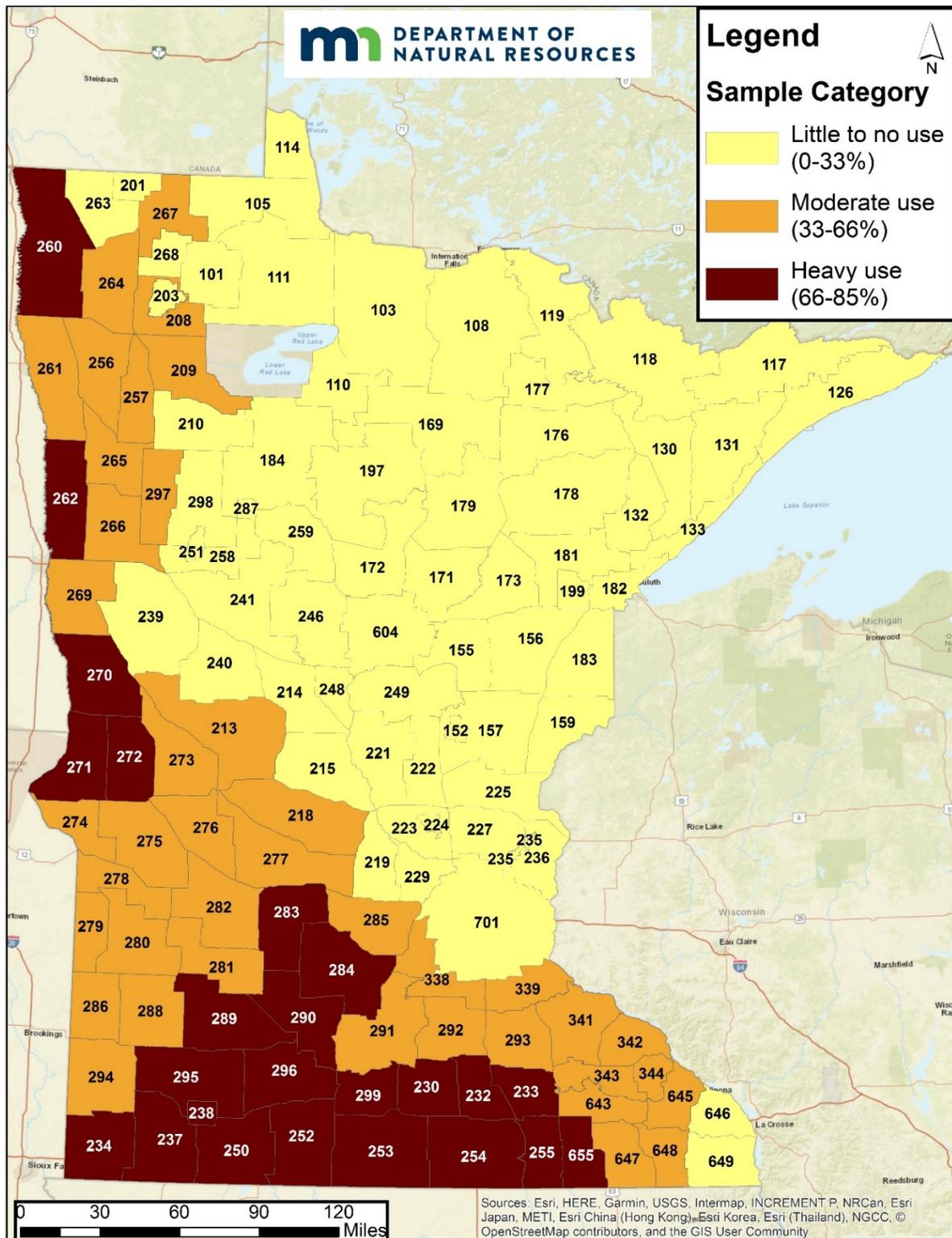


Figure 1. Minnesota Deer Permit Areas (DPA) 2019 depicting the 3 neonicotinoid use categories based on percent crop coverage, including little to no use (0-33%), moderate use (33-66%), and heavy use (66-85%).

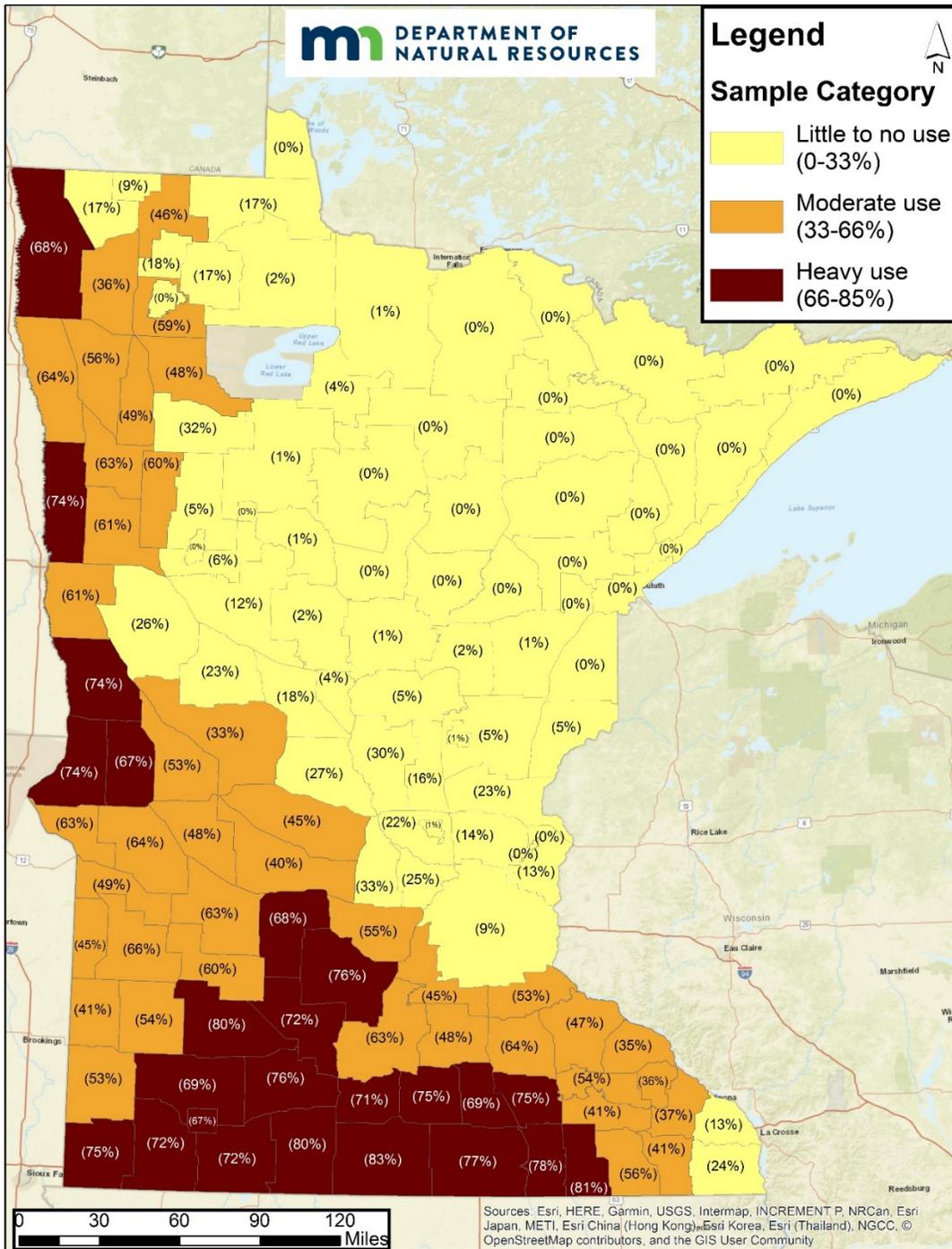


Figure 2. Minnesota Deer Permit Areas (DPA) 2019 depicting the percent crop coverage, ranging from 0 to 83 percent and binned into three usage categories.

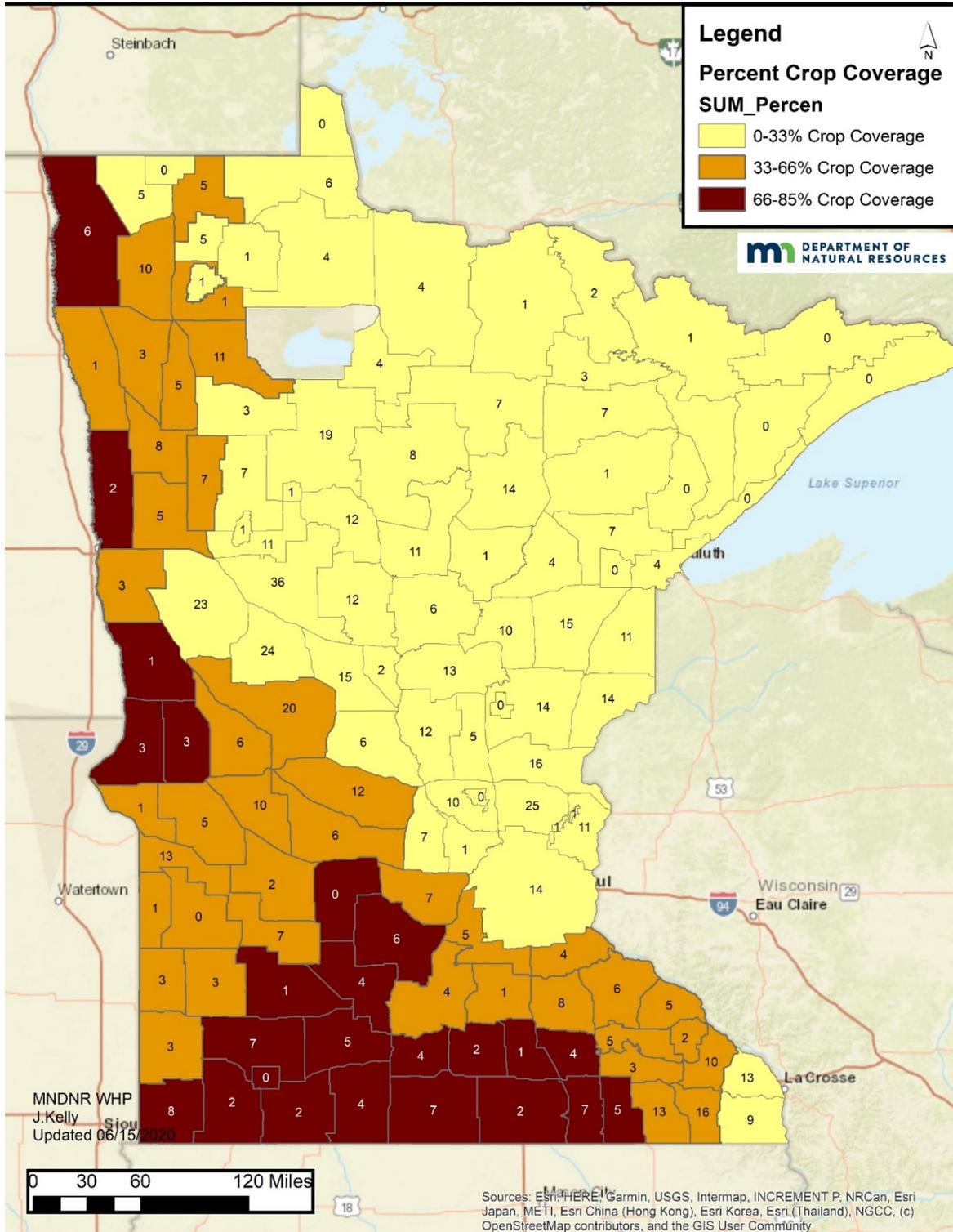


Figure 3. Spleen samples (n = 799) collected by Deer Permit Area (DPA) and crop coverage category (little to no use = 497, moderate use = 220, and heavy use = 82). Minnesota 2019.



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

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### SUMMARY OF FINDINGS

The Minnesota Department of Natural Resources (MNDNR) is in the third year of a continuing study to investigate the movement dynamics of wild white-tailed deer (*Odocoileus virginianus*) in southeastern Minnesota. This project evolved after the detection of chronic wasting disease (CWD) in fall 2016 in Fillmore County and is designed to 1) understand potential pathways of CWD spread on the landscape by movement of wild deer, 2) increase our likelihood of managing the outbreak in this and other areas of Minnesota, and 3) estimate general causes of deer mortality in southeastern Minnesota. In mid-February 2020, we captured and fitted GPS collars to 56 white-tailed deer fawns (31 females, 25 males) in a study area of approximately 2,000 square miles centered on Preston, MN. We collared a total of 226 deer since the study began in March 2018 and as of June 2020, continue to monitor 74 animals that remain available for tracking. There have been 82 known mortalities due to hunter-harvest (n=36), vehicle collision (n=12), capture-related issues (n=12), underlying health conditions (n=6), indeterminate causes (n=6), agency culling (n=4), trauma (n=4), and predation/scavenging (n=2). Average annual survival for females and males was 0.73 (CI: 0.62-0.86) and 0.54 (CI: 0.40-0.73), respectively, and the difference was marginally significant ( $Z=1.80$ ;  $P=0.072$ ). Average annual mortality due to hunter harvest was 18%, while that for other causes and vehicle collision were 11% and 6%, respectively. The low survival estimates reflect liberalized harvest regulations within the study area in order to manage CWD, coupled with the fact that yearlings and young adults are the most vulnerable group to hunter harvest. Overall, a large number of deer were censored due to collar failures; however, the failure rate has improved from 90 collar malfunctions in 2018, to 17 in 2019, and 2 in 2020. We averaged segment-derived home ranges across seasons for 127 deer (62 females, 65 males) from 2018-2019; average 50% core area size was 0.35 km<sup>2</sup> (CI: 0.28-0.42) and 0.50 km<sup>2</sup> (CI: 0.39-0.62) for females and males, respectively. Average 95% home range size was 1.75 km<sup>2</sup> (CI: 1.37-2.13) and 2.21 km<sup>2</sup> (CI: 1.72-2.70) for females and males, respectively. We found that 26% of females and 43% of males dispersed between their natal and adult home range, and this difference was marginally significant ( $\chi^2=2.73$ ,  $P=0.10$ ). Surprisingly we found that 15% of females and 6% of males underwent apparent seasonal migration between summer and winter ranges, although there was no significant difference between these proportions ( $\chi^2=1.70$ ,  $P=0.19$ ). The average distance traveled for females and male dispersers was 20.0 km (CI: 5.67-30.10) and 22.8 km (CI: 11.70-32.30), respectively. The average distance traveled for migratory females and males was 12.8 km (CI: 3.49-18.40) and 17.7 km (CI: 1.35-27.20), respectively. We observed more extreme dispersal distances by both sexes, with the maximum linear distance traveled by a female and male of 116 km and 97 km, respectively. Many animals in the study conducted temporary excursions from their home range, lasting hours to several days. We are currently in the process of analyzing these types of movements to find any patterns that might emerge. Taken together, these results underscore the high degree of deer movement by both sexes across the southeastern Minnesota landscape and potential for yearlings in particular to facilitate spatial spread of CWD. Furthermore, at least for yearling and young adult deer (2.5-3.5

years old) in this region of Minnesota, hunting appears to be the dominant cause of mortality. This underscores the importance of harvest management as a valuable tool to control and suppress CWD spread and magnitude in southeastern 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 26 states and 3 Canadian provinces in North America, as well as Finland, Norway, South Korea, and Sweden. Work has demonstrated that CWD can cause population declines in white-tailed deer and mule deer in the western US, particularly when high prevalence levels occur in a population (Edmunds et al. 2016, DeVivo et al. 2017). 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 DNR 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).

It has been nearly 4 years since the Minnesota Department of Natural Resources (MNDNR) discovered CWD during the 2016 regular hunting season in Fillmore County, Minnesota, and through 16 June 2020, 88 wild deer have tested positive in Minnesota (including one from Pine Island in 2010). Since October 2019, there have been 35 new CWD detections in wild deer, all but one located in the southeast CWD Management Zone. With the exception of an adult buck that tested positive for CWD in Dakota County in March 2020, the pattern of new detections in Minnesota suggests that spatial spread of CWD continues to radiate from the core areas around Preston, MN and a previously positive captive cervid facility in Winona County (Figure 1). The precise mechanism(s) of CWD introduction to these areas cannot be confirmed, but potential hypotheses include natural movement of infectious deer from neighboring states (e.g., Wisconsin, Iowa, or Illinois), contact between wild deer and prior CWD-positive captive cervid facilities, or contamination of the environment with infectious cervid carcass material. Given the threat of CWD in southeastern Minnesota, this study seeks to better understand natural deer movement ecology and in particular detect seasonal corridors of movement that may inform our management of CWD spatial spread.

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. The presumed main driver of spatial spread among wild deer are natural movements, which vary as a function of season, sex, age, habitat, underlying deer density, and other variables (Nixon et al. 2007, Long et al. 2008, Long et al. 2010, Lutz et al. 2015, Peterson et al. 2017). Besides our current study, there is only one published study on deer movement ecology in southeastern Minnesota. However, the Wisconsin Department of Natural Resources has an ongoing study in its fourth year designed to understand the impacts of CWD on survivorship and movements of deer in Wisconsin, which will be useful in providing a context for the implications of regional movements of wild deer and CWD spread.

Deer behavior and movements vary by sex, season, and landscape features, along with deer population demographics and social structure. Three types of movement likely facilitate disease spread across the landscape including dispersal events, recurrent seasonal movements, and temporary excursion events. The most substantial long-distance movements involve dispersal from natal to adult ranges, most likely to occur in 1-year-old deer during spring, although males may also disperse in fall. We define dispersal in this context as an asymmetric movement from the natal home range to a distinct and non-overlapping adult range. While dispersal usually

occurs once, there are cases of 2-stage dispersals when a deer makes a second asymmetric movement to a second adult home range, but this is rare. Recurrent seasonal movements can include migratory behavior and movements between summer and winter ranges. Excursions (or synonymously forays) are temporary transient movements out of an established home range that typically occur in fall, likely influenced by mating behaviors. Because deer densities and movement behavior can be altered by management actions, a better understanding of both deer density and movement activities 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 unabated spread of CWD both between and within states, and the need to find management solutions to suppress the spread of disease arising from natural deer movements.

## METHODS

### Study Area

The study area, approximately 7,250 km<sup>2</sup>, is centered on Preston, MN, in Fillmore County where the CWD cases in wild deer during 2016 appeared first (Figure 1). The study area limits are flexible and established as an approximately 30-mile buffer around Preston, MN, which includes much of the Southeast CWD Management Zone. We included extensive areas extending through Fillmore, Winona, Houston, and Olmsted Counties to capture and release Geographic Position System (GPS)-collared deer, so that our collared sample is representative of the yearling to young adult (2.5-3.5. year olds) deer population in southeastern Minnesota (Figure1).

Deer in southeastern Minnesota are managed within CWD Disease Management permit areas (DPA) in the 600-series, where the deer population is well above population objectives and disease management is the primary goal. Therefore, harvest regulations were liberalized in the study area with 3 seasons; Archery (Sept 14. - Dec 31), two 9-day firearms season, and a 16-day muzzleloader season. Harvest was unlimited for antlerless deer and limited to 1 legal buck per season (the 3-buck limit occurred in fall 2019 only). Additionally, 3-day youth hunts and early antlerless seasons took place in October of each year. Special late-season hunts (3-day) took place each year in select DPAs where CWD had been detected during the regular season. In 2019, the average deer harvest across the study area was about 5 deer per mi<sup>2</sup> compared to about 1.9 deer per mi<sup>2</sup> in 2016 prior to implementation of liberalized harvest regulations to control CWD.

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. The eastern part of the study area is composed of forested bluff lands and steep ridges and moving southwesterly, the landscape transitions to be flat and dominated by agriculture. More than 90% of the landscape is held in private ownership. There is significant heterogeneity in deer density due to both habitat variability and localized refugia (i.e., parcels with viable deer habitat where hunting is not permitted) with average deer density about 25.4 deer mi<sup>2</sup> (range 12-42).

Coyotes (*Canis latrans*) are the primary predators of deer in the region and typically prey on younger age classes. Deer also may be more vulnerable to predators in late winter due to stress and limited forage availability (VanCauteren and Hygnstrom 2011). Scent station records indicate that coyote populations have increased in the region in the last 2 decades (Erb 2019).

For our 2020 capture efforts, we secured permission to access 132,113 acres, consisting of 62,862 acres of private land (> 250 landowners) and 69,251 acres of public land. We expanded our partnerships with private landowners in 2020, adding an additional 15,000 acres of private land compared to the second year of the study. This study would not be possible without the enormous outpouring of support from private landowners in the study area. As in past years, we

did not attempt to capture deer in areas where USDA Wildlife Services had planned targeted culling efforts through March 2020 to decrease the likelihood that a collared deer would be culled by agency staff.

### **Sampling Design and Data Collection**

Given the breadth of the study area, we divided it into 10 quadrants (Figure 1) from which we established a baseline target goal of capturing 4-5 fawns ( $\approx$  7-9 months old) of each sex per quadrant in 2019. Our goal was to capture and collar 90 deer; 45 male fawns and 45 female fawns.

Deer were captured by net-gunning (Helicopter Wildlife Services, South Africa) from a MD500 helicopter. Once captured, all deer were collared, ear-tagged, auxiliary measurement taken (body temperature, age class, sex, and body condition), and an ear punch for genetic analysis. Helicopter pursuit time of animals did not exceed 5 minutes, and average handling time per animal was approximately 4 minutes.

We programmed GPS collars for males (Iridium TL330 with expandable collar, Lotek Wireless Inc, Newmarket, Canada) and females (Iridium 420, Lotek Wireless Inc, Newmarket, Canada) to collect location coordinates every day at an increased rate during spring dispersal and fall rut periods. For TL330 collars (males), we calibrated the rate of GPS location fixes to occur once every 117 minutes (approximately 12 per day) between 15 April through 15 July and 1 September through 15 December. During all other periods, we scheduled these collars to collect positional data every 3 hours and 45 minutes or approximately six locations per day. For Iridium 420 collars (females), we calibrated the rate of GPS location fixes to occur once every 75 minutes (approximately 19 per day) between 15 April through 15 July and 1 September through 15 December. During all other periods, we scheduled these collars to collect positional data every 2 hours and 45 minutes or approximately eight locations per day. We chose periodically higher transmission fix schedules in part based on timing of seasonal movements recorded from yearling males in Wisconsin. The expected battery life of I420 collars was longer than that of TL330 collars, and we balanced the fix rate schedules so that we had a reasonably high probability of receiving data from collared deer for at least two years (assuming deer survived and that all other collar functions remained viable). To ensure that we collected location data across the entire 24-hour day distribution, we included an offset from an hour (e.g., 1 hr 57 min or 3 hr 45 min) so programmed GPS fixes occurred on a staggered schedule that changed every day.

The collars included timed-release drop-off mechanisms, which after 130 weeks (2.5 years) will cause the collars to detach, be available for retrieval, and potentially be re-furbished. In addition, for male collars only, we included a line-of-sight mechanism that permits the collars to be detached remotely in line of sight to the animal (within 200m).

We programmed collars to transmit a mortality text message if inactive for 12 hours, and a field response was initiated within 48 hours, when possible. If a carcass was available, staff collected medial retropharyngeal lymph nodes for CWD testing, a muscle sample for potential genetic testing, and a front incisor tooth for age confirmation. In some cases, field necropsies were performed and tissue or organs samples were submitted for additional diagnostic testing at the University of Minnesota Veterinary Diagnostic Laboratory (VDL). If an animal died within the first two weeks following capture, whole carcasses were extracted and evaluated for capture myopathy. Outside of a 2-week window following capture, and after the window when capture myopathy is most likely to occur, staff performed field investigations to determine likely cause of death. Using all evidence available from a carcass (e.g., broken bones, bite marks, body condition) and the area surrounding a death site (e.g., evidence of struggle), staff assigned probabilities of cause of death including hunter-harvest, vehicle collision, capture-related issues,

underlying health conditions, indeterminate causes, agency culling, trauma, and predation/scavenging.

## **Data Analysis**

### *Survival analysis*

We estimated annual survival (Aalen-Johansen estimator; Therneau 2020) by sex using R software (R Core Team 2017) packages survival and mort, which correctly account for the staggered entry design of animals into the study over time and right censoring. If a collar failed prematurely or dropped off due to malfunctioning expansion device (males) or by accident (e.g., deer passing through a barbed wire fence), these animals were considered censored and lost to follow up from the date of occurrence. We also estimated cumulative incidence functions for cause-specific mortality (Heisey and Patterson 2006), which permits comparison of different causes of mortality in the presence of competing risks. We characterized mortality into three general causes for this analysis including hunter-harvest, vehicle collision, and other causes. The other causes category included animals that died due to underlying health conditions, indeterminate causes, agency culling, trauma, and predator/scavengers. We omitted from analysis any deer that died due to capture-related issues, defined as any mortality within 2 weeks of capture, as these animals may not be representative of the underlying deer population.

There was no protection afforded to collared deer and these animals could be legally harvested during hunting seasons or agency-culling efforts. We encouraged hunters to select animals for harvest based on their personal preference regardless of whether the hunter noticed a collar on the deer. On outreach materials, we asked hunters who harvested a collared deer to please contact MNDNR and return the collar.

### *Location data processing*

We define dispersal as having occurred if an individual displayed a permanent, asymmetric 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 vetted all recorded spatial locations before use in any analysis because the accuracy of a location is influenced by the number of satellites available to communicate with a collar and how a deer is juxtaposed in the landscape (i.e., influence of physical barriers); thus, any spatial location was omitted if less than three satellites were used to derive its location. These two-dimensional location coordinates resulted in highly biased altitude above sea level estimates (around 0) and high Dilution of Precision values ( $> 4$ ) indicative of inaccurate locations (generally  $> 500\text{m}$  based on controlled tests).

Locational accuracy of GPS-collars may be influenced by elevation, landform (ridgelines vs. ravines), and land cover (forests vs. open fields). For example, locational accuracy may be low when collared deer are in low elevation, forested ravines where satellite line-of-sight is inhibited. In order to better understand and correct for these potential errors, we tested collar locational accuracy in the field using 9 test collars and a Trimble GPS unit. We placed collars in 3 different land covers (forest, open fields, edges) and at relatively low (ravine) and high (ridge/hilltops) locations that were representative of the study area. For each land cover  $\times$  elevation scenario, we placed collars 6 meters apart at heights of 0.5 and 1.0 m ( $n=3$  collars each) and at ground level ( $n=3$ ). We collected collar locations every 2 hours for 2 days in each scenario. We used a Trimble Geo 7X GPS unit with a high positional accuracy within 100 cm of the true location to compare accuracy of collar locations. Across all land cover and elevation scenarios, average location error was 14 ( $\pm 15$ ) m away from the true location. Average locational error was lowest in open fields at high elevations (mean=9  $\pm$  10 m) and highest in forested ravines (mean=19  $\pm$  19 m). However, overall error did not differ significantly among cover types or

different elevations. Still, these average error rates will be taken into account in future habitat selection analyses.

Parsing location data into seasonal home ranges and dispersal events is challenging due to individual variation in movement and space use behavior among deer. Rather than parse location data into seasons based on a single calendar date for each season, we applied change point analysis to identify points of change in movement data that would signify a change in animal behavior. To identify the date(s) of spring dispersal for individual deer, we measured the Euclidean distance of each GPS location to the animal's original capture location. We used the *meanvar* function in the *changept* package in R to identify points of change in the distance of each location to the original capture location. We limited the change point analysis to five points and set the minimum bin number to 10 locations within which to calculate mean and variance. We used five change points because we found that five points effectively captured abrupt changes in distance to capture location that represented dispersal movements in the spring, as well as seasonal migrations and home range expansions during fall and winter. The first change point typically represented a spring dispersal event. We used dates of change points to identify dispersal events and parse out periods of movement from those of stationary space use.

#### *Home range analysis*

After removing locations during dispersal or travel periods, we estimated the size of the 95% kernel density isopleth, the area in which the deer spent 95% of its time (hereafter, *home range*). For ease of interpretation, we assigned each location to a season using average dates of first and last snowfall, along with average growing season dates; growing season (GS): 01 May – 31 Aug., fall (FA): 01 Sept.- 15 Nov., and winter (WN): 16 Nov.- 30 Apr. We calculated seasonal home ranges to evaluate the hypothesis that home ranges would expand in the fall, as both sexes increased movements in search of potential mates. However, for simplicity, we also calculated the annual home range size for each deer by averaging seasonal home range sizes. We hypothesized that home ranges would expand in the fall in order to seek mates. To examine potential environmental influences on home range size, we calculated the proportion of row-crop agriculture in each home range using the 2016 National Land Cover Database (USDA 2018) and the spatial join tool in ArcGIS. We also calculated the proportion of agriculture in the township in which each home range was centered. We chose the proportion of agriculture over other cover types because it was the most consistently available across the study area; forest cover was rare in many townships in the western portion of the study area. In addition, forest and agriculture were highly negatively correlated ( $r=-0.97$ ,  $p<0.001$ ) within home ranges.

We evaluated differences in the size of home ranges by sex, season, year and land cover covariates using linear mixed effects models. We specified season, sex, and year as fixed effects and the individual deer as a random effect to account for >1 seasonal home ranges per deer. We used AICc model selection to assess support for explanatory models in separate model sets. For each kernel isopleth, we evaluated a global additive model that included season, sex, year and land cover covariates, a global model that included an interaction between season and sex, and separate models of each variable alone. We compared these models to a null model that only included the random effect of the individual deer. We considered the top-ranked model and any models within two AICc units of the top model to be competitive (Burnham and Anderson 2002).

#### *Movement analysis*

We identified dispersal and other movements outside of the home range using change point analysis and then assigned those movements to one of three categories: natal dispersal,

seasonal migration, or temporary excursions. For each movement, regardless of type, we measured the straight-line distance of the movement, the overall direction of the movement (1 of 4 cardinal directions), and the dates and duration of the movement. For natal dispersal, we calculated the straight-line distance between the adult home range and natal home range centroids (Kenward et al. 2002). We performed all spatial data analysis and characterization using packages *adehabitatHR* (Calenge 2006), *sp*, and *rgdal* within R software (R Core Team 2017), and ArcMap 10.6 (Environmental Systems Research Institute, Redlands, CA, USA).

## RESULTS AND DISCUSSION

From 8-12 February 2020, we captured and outfitted 56 deer with Iridium GPS collars: 31 female fawns and 25 male fawns (Figure 1). Following initial capture and collar fitting, two male and two female fawns were able to immediately kick off their collars, resulting in a final 2020 release cohort of 52 fawns ( $n=29$  females, 23 males). Our original goal was to capture 90 fawns (equal sex ratio), and the shortfall occurred because the helicopter capture company experienced significant difficulties in the field and did not capture deer at a rate that would ensure completion of the quota within a timely manner, leaving 42 GPS collars that were not deployed.

As of June 16, 2020, we are monitoring 27 female and 19 male deer from the 2020 release cohort. There have been two collar failures of unknown cause from this cohort. A total of 90 collars from the 2018 release cohort and 17 collars from the 2019 cohort have been deactivated due to either failure or battery senescence. Two additional animals from the 2020 cohort dropped their collars by accident (likely due to moving through barbed wire fences). In total, we are actively monitoring 74 GPS-collared deer from all years of the study, including 46 females (19 from 2019, and 27 from 2020) and 28 males (9 from 2019 and 19 from 2020).

### Data Analysis

#### *Survival analysis*

To date there have been 82 known mortalities due to hunter-harvest ( $n=36$ ), vehicle collision ( $n=12$ ), capture-related issues ( $n=12$ ), underlying health conditions ( $n=6$ ), indeterminate causes ( $n=6$ ), agency culling ( $n=4$ ), trauma ( $n=4$ ), and predation/scavenging ( $n=2$ ) (Figure 2). It is important to note that five of 12 deer ascribed to an indeterminate cause of death wore GPS collars that failed after deployment, so we never received a mortality signal to follow up with carcass investigations. Instead, in these cases, either landowners or hunters happened by chance upon skeletal remains in the field preventing us from ascribing a source of mortality. We estimated average survival by year and sex (Figure 3) and grouped causes of mortality as hunter-harvest, vehicle collision, and other (all other causes) in order to estimate annual cumulative incidence curves. Average annual survival for females and males was 0.73 (CI: 0.62-0.86) and 0.54 (CI: 0.40-0.73), respectively, and the difference was marginally significant ( $Z=1.80$ ;  $P=0.07$ ). Average annual mortality due to hunter harvest was 18%, while that for other causes and vehicle collision were 11% and 6%, respectively (Figure 4). After adjusting for multiple comparisons using a Bonferroni correction, hunter harvest was significantly greater than vehicle collision ( $Z=2.37$ ;  $P=0.05$ ), but not other causes ( $Z=1.19$ ;  $P=0.71$ ). Other causes of mortality were not significantly different from vehicle collision ( $Z=1.28$ ;  $P=0.60$ ).

We captured deer when they were older fawns, at least 7-9 months old, therefore, our survival estimates are conditional on animals surviving through this age. Furthermore, our study only reflects two years of survival data, and the upper limit on age classes reflected is around 3.5 years. Deer typically experience the highest risk of mortality after birth through the first year of life (DelGiudice et. al. 2006), so our estimates do not capture the period when deer are most vulnerable. Several neonate studies in the upper Midwest have shown that fawn mortality during

the first few months of life for white-tailed deer was driven by predators or natural causes (Brinkman et al. 2004, Carstensen et al. 2009, Grovenburg et al. 2011, Warbington et al. 2017). Research in Minnesota has shown that winter severity index and wolf predation can be significant sources of mortality for deer, particularly in the northern forest region (DelGiudice et al. 2006). However, in our study area, sources of mortality are more aligned with patterns observed in southwestern Minnesota and other parts of the Midwest, where hunter harvest and deer-vehicle collisions are the primary causes in adult deer (Brinkman et al. 2004, VerCauteren and Hygnstrom 2011, Krebs 2014). Our survival estimates appear to be low and, taken at face value, would yield an average life expectancy of about 4.5 years and 2.5 years for females and males, respectively. However, these estimates potentially reflect several processes at work. Our sample purposely focuses on a narrow range of age classes, primarily yearling deer, because we sought to investigate dispersal patterns. It is well known that yearling and 2.5 year-old males are the most vulnerable age-sex class to hunter harvest (Roseberry and Klimstra 1974, McCullough 1979, Nixon et al. 1994). To facilitate CWD management efforts, in 2017 antler point restrictions (APR) were removed from Deer Permit Area (DPA) 603 and unlimited antlerless tags were available for a nominal fee, which represented a small subset of our study area. In 2019, MNDNR dissolved DPA 603 into a broader CWD Management Zone, and removed APR and liberalized harvest throughout our entire study area. The possible effect of this liberalized harvest regulation change may be reflected in lower male survival during 2019 compared with 2018 (Figure 3). Potentially adding to this effect, it is possible that hunters selectively harvested collared deer at a higher rate (Jacques et al. 2011), thus biasing our annual survival estimates low.

### *Home range analysis*

We have amassed over 450,000 records of deer location data from 23 March 2018 through 17 June 2020. Of 148 deer monitored in 2018 and 2019, 127 survived long enough to collect sufficient GPS location data to estimate areas of activity for at least one season (62 females and 65 males). Average home range size was 1.75 km<sup>2</sup> (CI: 1.37-2.13) and 2.21 km<sup>2</sup> (CI: 1.72-2.70) for females and males, respectively. These estimates fall in line with our expectations and home range sizes for other deer in the Midwest (Walter et al. 2009).

We evaluated 8 models that included season, sex, year, and the proportion of row-crop agriculture to explain variation in home range size. The full model received 87% of model support, followed by the full model with an interaction between season and sex (Table 1). The top-ranked model indicated that deer home ranges were significantly smaller during the growing season [ $\beta_{GS} = -1.75 \pm 0.34$ ; CI: -2.43-(-1.08)]; Figure 4) and winter [ $\beta_{WN} = -1.16 \pm 0.37$ ; CI: -1.89-(-0.43)] compared to fall (Figure 5). In addition to seasonal differences, home range size increased with increasing proportions of row-crop agriculture within home ranges ( $\beta_{crop} = 2.46 \pm 0.68$ ; 95% CI: 1.13-3.78; Figure 5). Males had somewhat larger home ranges, but this difference was not significant ( $\beta_{male} = 0.48 \pm 0.25$ ; CI: -0.02-0.98). The proportion of row-crop agriculture within the township did not appear to influence home range size ( $\beta_{crop\_township} = 0.57 \pm 0.65$ ; CI: -0.70-1.85).

Our estimates of home range size are well within estimates for white-tailed deer in other regions of the Midwest and eastern U.S. (Walter et al. 2009, Magle et al. 2015, Walter et al. 2018). Home range size is influenced by several environmental factors, including forest connectivity and landscape composition (Magle et al. 2015, Walter et al. 2018). In forested landscapes, home ranges tend to increase with increasing homogeneity of land cover and lower forest edge density (Walters et al. 2018). In southcentral Wisconsin, a mixed agricultural-forested region similar to our study area, Magle et al. (2015) also found that home ranges increased with

increasing proportion of agricultural land cover with the home range. In southeastern Minnesota, the predominance of row-crop agriculture, especially in the western portion of the study area, naturally results in larger home ranges, as deer must travel farther to meet food and cover needs found in forests and open habitats.

Home range dynamics can influence transmission and spread of CWD at the local scale within a population. Local spread may be influenced by social group dynamics, home range overlap, and relatedness, all of which can affect the rate of direct and indirect transmission of CWD prions among deer (Magle et al. 2013, Schaubert et al. 2015, Tosa et al. 2017, Koen et al. 2017). Risk may change seasonally as well, as deer expand or contract their home ranges during the fall rut and fawning seasons, respectively (Koen et al. 2017). At the larger population level, there is some evidence that landscape connectivity can increase the social network of deer, thus providing a conduit for longer distance spread of CWD (Norbert et al. 20, Koen et al. 2017). In particular, Koen et al. (2017) found that the social connectivity among deer increased with agricultural lands and connectivity of edge. In our study, the relationship between greater proportions of agricultural land and larger home ranges may result in a larger and more connected social network, but more research on home range overlap may be needed to fully understand the relationship between home range size and social network connectivity among deer in Minnesota.

#### *Movement analysis*

For all deer available in the study with sufficient data we found that 26% (n=16/61) of females and 43% (n=23/54) of males dispersed between their natal and adult home range, yet this difference was marginally significant ( $\chi^2=2.73$ ,  $P=0.10$ ). We also determined that 15% (n=9/61) of females and 6% (n=3/54) of males underwent apparent seasonal migration between summer and winter ranges, although there was no significant difference between these proportions ( $\chi^2=1.70$ ,  $P=0.19$ ). The average dispersal distance traveled for females and male was 20.0 km (CI: 5.67-30.10) and 22.8 km (CI: 11.70-32.30), respectively. The average one-way distance traveled for migratory females and males was 12.8 km (CI: 3.49-18.40) and 17.7 km (CI: 1.35-27.20), respectively. The pattern in dispersal distance traveled was consistent between years, and suggests that females are just as likely to disperse from their natal range and travel approximately the same average distance as males. The number of animals performing apparent migratory movements was too small to detect general patterns, and it is not clear if these movements were driven by some evolutionary or proximate resource cause. We observed more extreme dispersal distances by both sexes, with the maximum linear distance traveled by a female and male of 116 km and 97 km, respectively. While these extreme distances are outliers, deer have been reported to travel similar long distances in other agricultural landscapes (Kernahan et al. 1994, Nixon et al. 2007). In other work done in agricultural landscapes of Illinois, average dispersal distances were approximately 30 km and males and females dispersed at approximately equal rates (Nixon et al. 2007). Many animals in the study conducted temporary excursions from their home range, lasting hours to several days. It is not clear yet whether the timing of these excursions are clustered during particular seasons, but it is likely that these type of movements are important in the process of intragroup disease transmission at a local spatial scale. We are currently in the process of analyzing these movements to find any patterns that might emerge.

The variation in dispersal behavior that we observed in southeastern Minnesota reflects the transition in landscape features across the study area. Our study occurred in a region of southeastern Minnesota that transitions from the eastern forested bluff and ridge country along

the Mississippi into the row-crop agriculture in the western part of the study area. As found in southwestern Minnesota where a significant proportion of deer perform seasonal migrations initiated by severe weather (Brinkman et al 2005), we observed seasonal migrations for some animals in the southwestern part of our study area. These deer spent the summer months in northern Iowa and returned to Minnesota in winter. A study conducted in the 1980s around Whitewater Wildlife Management Area in southeastern Minnesota using radio telemetry showed that some deer migrated between Minnesota and Wisconsin (Simon 1986). We have not observed such interstate movements in our study to date and most deer have not exhibited seasonal migration patterns.

While male dispersal typically is regarded as the primary force driving potential disease spread (CWD) on the landscape (Gear et al 2006, Oyer et al. 2007), evidence suggests that high underlying deer density (Lutz et al. 2015) can drive females to disperse. Approximately half of all collared deer dispersed in the study. Given the relatively high rate and extent of female dispersal and high pre-fawn deer densities in the farmland-forest transition zone of our study area at around 42 deer/mi<sup>2</sup> (E. Michel, pers. comm.), we hypothesize that density-induced dispersal may be occurring in southeastern Minnesota. This highly productive landscape favors high deer survival and fecundity, given extensive food resources, winter cover, and relatively mild winters. Additional study of deer representative of southeastern Minnesota will further inform our understanding of dispersal and movement activities as it relates to potential spread of CWD prions on the Minnesota landscape.

Effective communication about this study to project participants is essential for our success. We have provided outreach materials both for landowners that have provided us with permission to use their properties for deer capture and for the general public. We continue to inform participating landowners twice per year with deer movement updates and maps of the collared deer in the study, and provide a summary of study findings and expectations for future work. Similarly, we continue to update a dedicated website 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 almost two dozen popular press articles covering this study in various media outlets. Overall, we strive 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**

We have approximately 45 Lotek GPS collars available to deploy in winter 2021, and plan to contract again with a wildlife helicopter capture company to conduct capture and collaring efforts. We will aim to collect equal sample sizes between sexes and focus more effort in Houston County on the border with Wisconsin. This would fill a major gap in the spatial coverage of our samples thus far, and perhaps reveal important movements of deer between Minnesota and Wisconsin.

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Table 1. Factors associated with size of 95% home ranges among deer (n=127) monitored with GPS collars 2018-2020 in southeast Minnesota. Home range sizes were fitted to linear mixed effects models with explanatory variables as fixed effects and the deer id as a random effect.

Model	df	AICc	$\Delta$ AICc	$w_i$
HR~ Season + Sex + Year + p(crop) <sup>a</sup> + p(crop_township) <sup>b</sup>	9	2449.79	0.00	0.87
HR~ Season*Sex + Year + p(crop) + p(crop_township)	11	2453.64	3.85	0.13
HR~ p(crop_township)	4	2484.73	34.94	0.00
HR~ p(crop)	4	2550.19	100.40	0.00
HR~ Season	5	2551.18	101.39	0.00
HR~ Season*Sex	8	2552.95	103.16	0.00
HR~ Sex	4	2566.83	117.04	0.00
HR~ (.)*	3	2570.06	120.27	0.00

<sup>a</sup>Proportion of row-crop agriculture within the home range.

<sup>b</sup>Proportion of row-crop agriculture within the township.

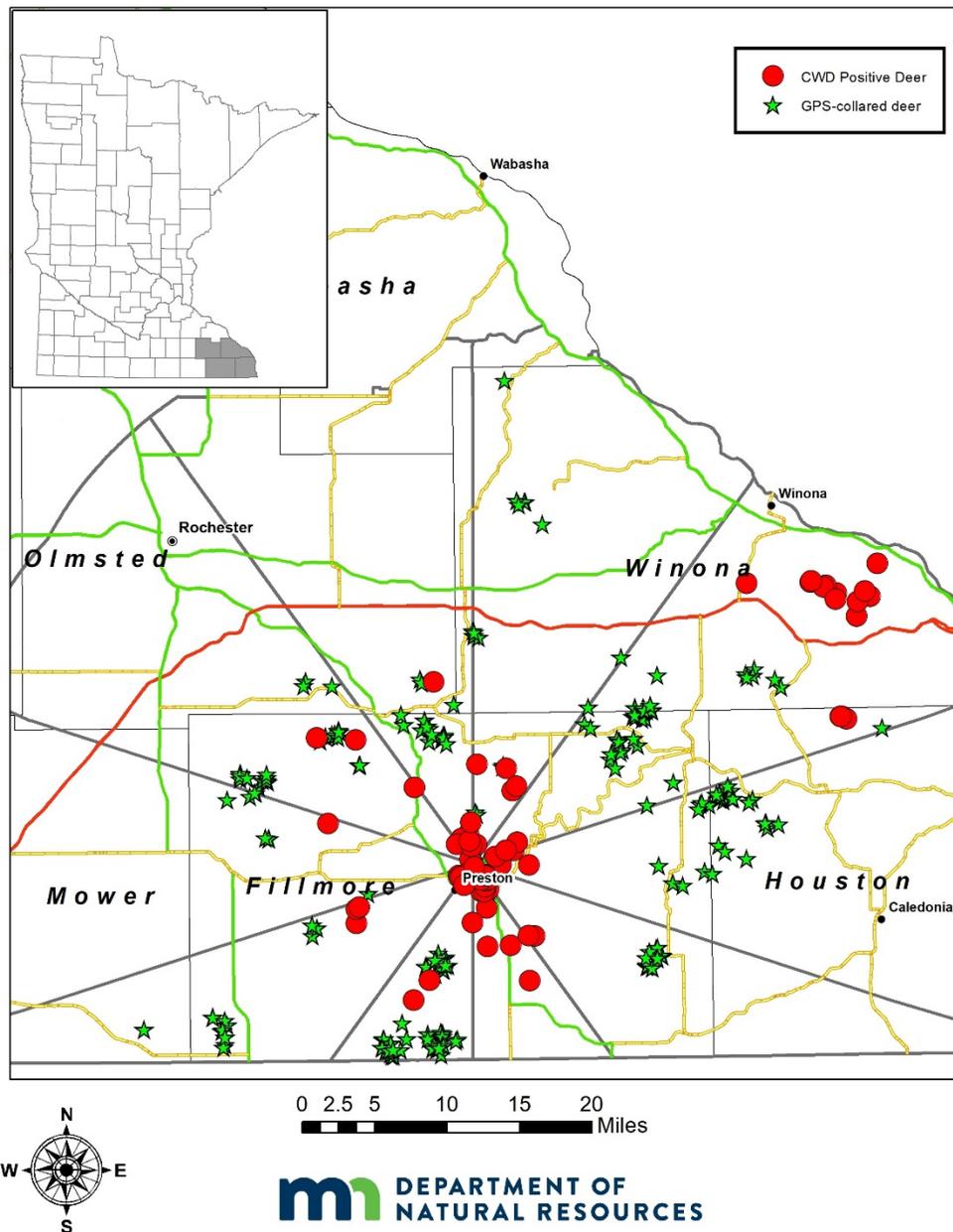


Figure 1. Spatial distribution of all deer captured and GPS-collared in southeastern Minnesota during March 2018 (n=109), February 2019 (n=64), and February 2020 (n=52). We captured deer on private and public lands, with a goal of capturing equal sex ratios across each quadrant. Green stars represent the locations where white-tailed deer were captured, fitted with GPS collars, and released. Also presented is the spatial distribution of wild white-tailed deer confirmed with CWD infection in southeastern Minnesota as of 07/26/20. There have been 85 wild white-tailed deer confirmed positive with CWD in southeastern Minnesota since fall 2016.

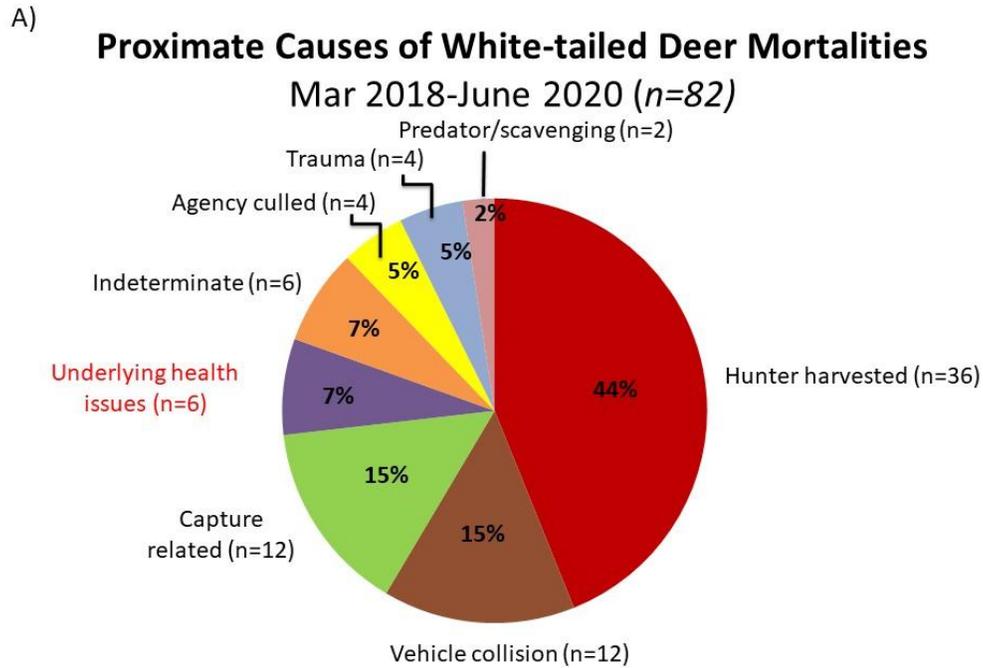


Figure 2. A) Causes of known mortality for 82 collared yearling and adult deer from March 2018 through June 2020 in southeastern Minnesota. Hunter harvest represents the primary source of mortality. B) Within the category of underlying health condition, we identified more detailed causes of mortality.

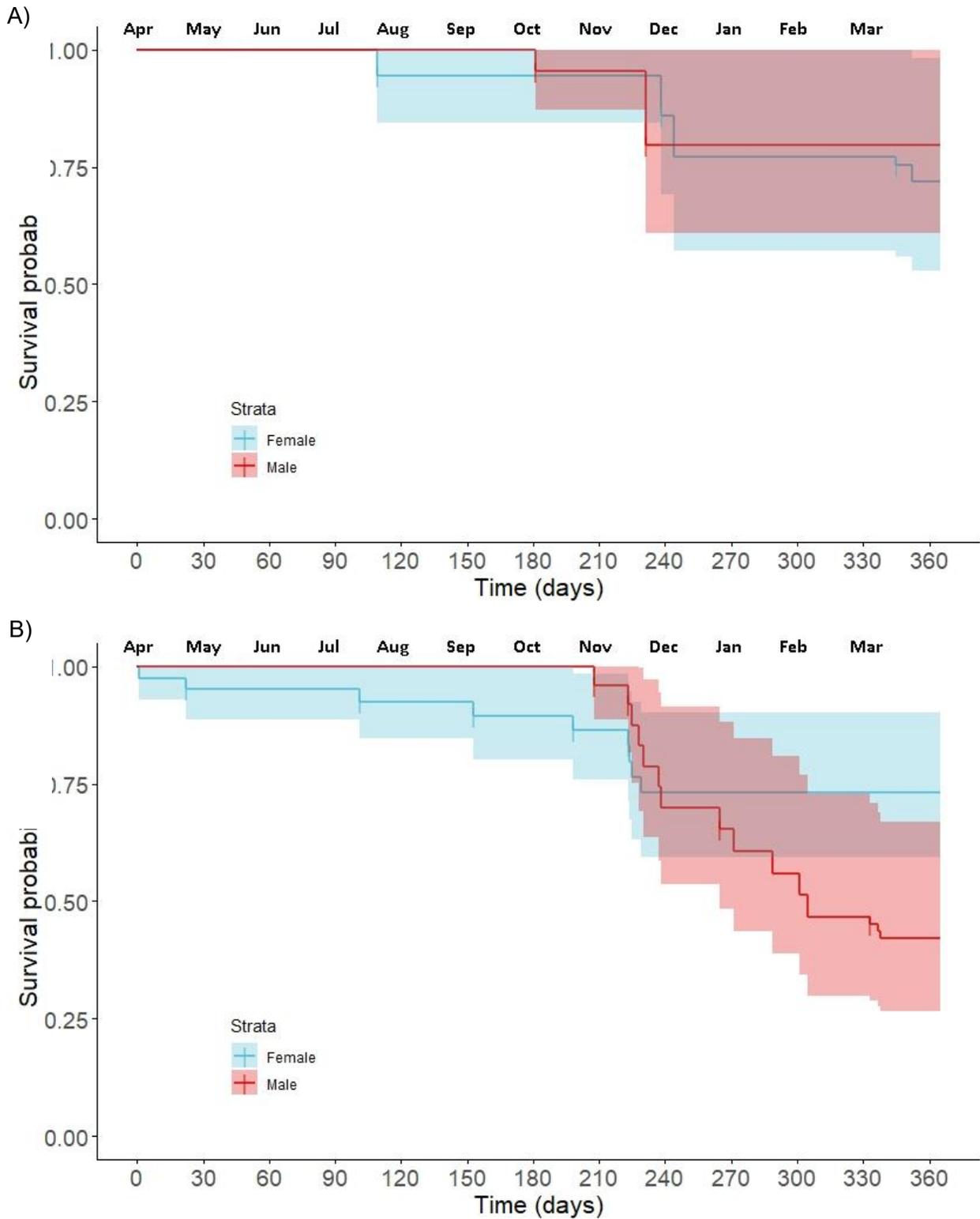


Figure 3. A) Estimated survival (and 95% CI) by sex for collared white-tailed deer in southeastern MN from 1 April 2018 through 31 March 2019. B) Estimated survival (and 95% CI) by sex for collared white-tailed deer in southeastern MN from 1 April 2019 through 31 March 2020.

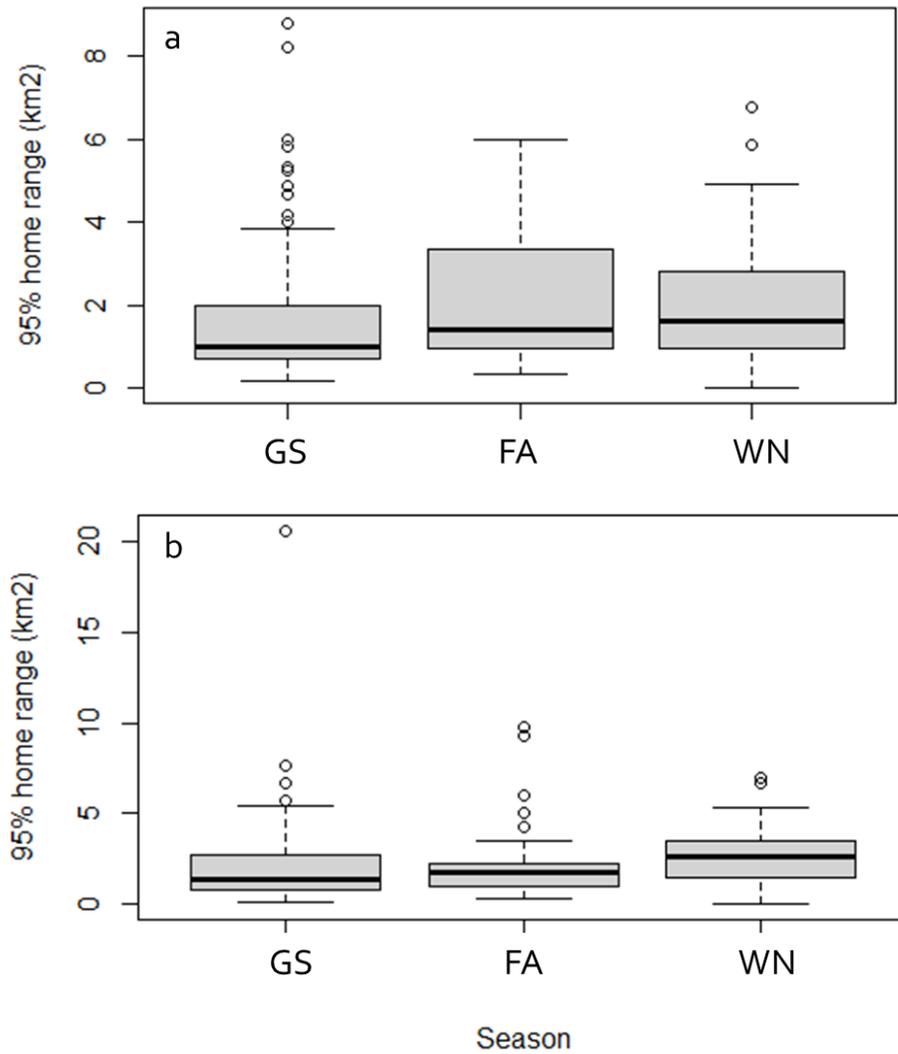


Figure 4. Distribution of 95% home range sizes (km<sup>2</sup>) by season for female (a) and male (b) white-tailed deer collared and monitored in southeastern Minnesota, 2018-2020. GS=Growing Season; 01 May–31 Aug. of each year; FA=fall; 01 Sept. – 15 Nov., WN=Winter; 16 Nov.–30 Apr of each year. Season dates were set using first and last snowfall dates and frost-free dates.

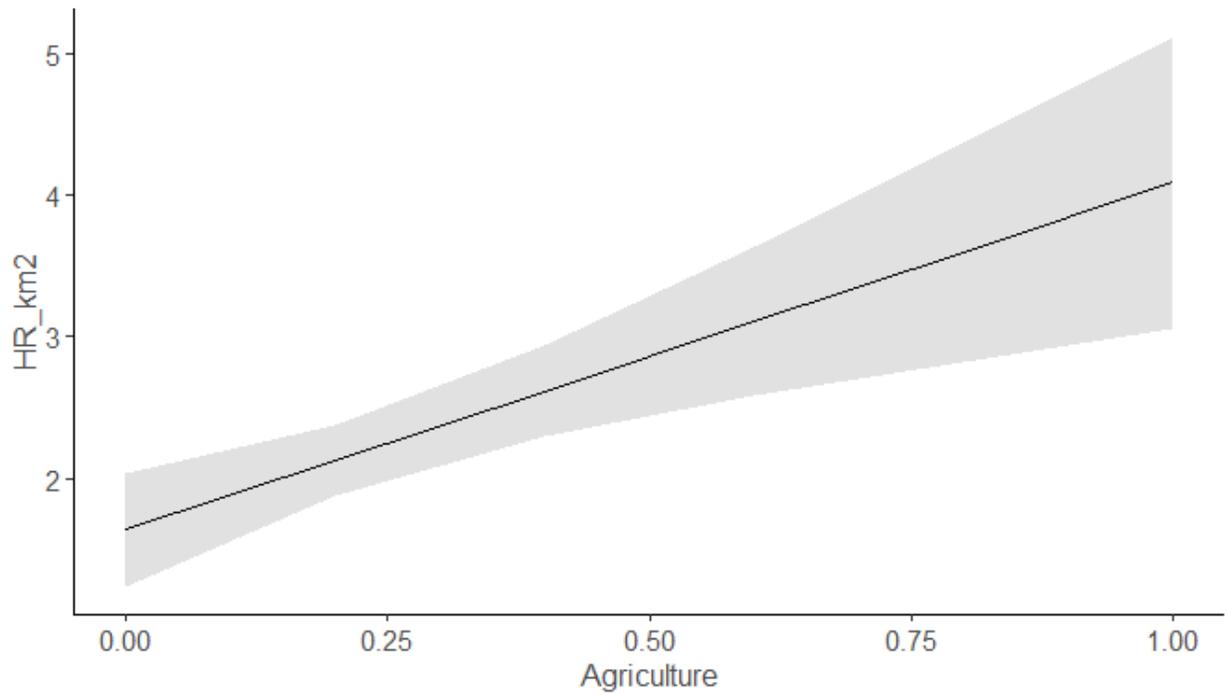


Figure 5. Model-derived estimates of home range sizes with increasing proportion of row-crop agriculture within home ranges on home range size for white-tailed deer (n=127) collared and monitored in southeastern Minnesota, 2018-2020.



## **SURVEILLANCE AND MANAGEMENT OF CHRONIC WASTING DISEASE IN MINNESOTA IN FALL 2019 AND WINTER 2020**

Kelsie LaSharr, Michelle Carstensen, Margaret Dexter, Patrick Hagen, Erik Hildebrand

### **SUMMARY OF FINDINGS**

During fall 2019, mandatory surveillance for chronic wasting disease (CWD) in hunter-harvested white-tailed deer (*Odocoileus virginianus*) occurred in 3 surveillance areas in the state. In the central surveillance area, the third consecutive year of testing found no wild deer infected with CWD surrounding the formerly positive Meeker county cervid farm; 544 samples were collected. In the north central surveillance area, a positive wild deer was found February 2019 <0.5 miles from an actively operating CWD-positive cervid farm. This led to the creation of a new CWD Management Zone, designated as 604, to enforce carcass movement restrictions and mandatory testing requirements; 3,961 deer were sampled during the fall with no additional detections found. In the southeast surveillance area, the spatial spread and increased number of CWD detections meant 2 new boundaries were created to help enforce a larger area of carcass movement restrictions and testing requirements. The southeast CWD Management Zone was enlarged and a novel CWD Control Zone was established as a buffer around the management zone. During the fall, 11,454 deer were tested in the southeast CWD Management Zone; 23 new hunter-harvested positives were detected. In the southeast CWD Control Zone, 1,159 deer were tested and no positives were found. CWD surveillance and management continued for the southeast into the winter through the use of special late hunts, landowner shooting permits, and agency culling, with 3/567, 0/10, and 7/463 positive deer detected, respectively. Statewide opportunistic sampling of symptomatic deer resulted in 3 out of 413 positive: 2 deer in the southeast where disease was already present and 1 deer in Dakota county, over 100 miles from the nearest CWD positive deer. In total, 18,571 deer were tested between 1 July 2019 and 30 June 2020, with 36 new positives. As of June 2020, there have been 88 CWD-positive wild deer in 6 Minnesota counties: Crow Wing, Dakota, Fillmore, Houston, Olmsted, and Winona.

### **INTRODUCTION**

Chronic Wasting Disease (CWD) belongs to a family of infectious diseases known as transmissible spongiform encephalopathies, or prion diseases. Members of the cervid family are susceptible and infection always results in death, with no vaccine or treatment available (Williams 2005). While a link between CWD and neurological illnesses in humans has not been established (MaWhinney et al. 2006, Sandberg et al. 2010), the Center for Disease Control recommends testing deer or elk (*Cervus canadensis*) from CWD positive areas and abstain from eating known CWD positive meat ([cdc.gov/prions/cwd/prevention.html](http://cdc.gov/prions/cwd/prevention.html)).

Minnesota Department of Natural Resources (MNDNR) began CWD surveillance in 2002 following the state's first detection of CWD on a captive cervid farm. Following an initial 3-year statewide surveillance (n=28,000 deer tested) with no positive wild deer found, MNDNR adopted a risk-based response strategy to conduct intense, concentrated surveillance in high-risk CWD areas (2010 CWD Response Plan). CWD risks were defined as positive captive

cervid farms or positive wild deer found along neighboring state borders; likewise, MNDNR makes a concerted effort to test any deer displaying CWD symptoms across the state. Due to the continued detections of CWD in southeast Minnesota, an updated response plan was released in July 2019 that addressed the management of persistent and endemic infections in wild cervids ([Surveillance and Management Plan for Chronic Wasting Disease in Free-ranging Cervids in Minnesota](#)). Based on the low CWD prevalence (<5%), but increasing spatial extent of cases, MNDNR is dealing with a persistent infection of CWD in southeast Minnesota.

Since 2016, MNDNR has actively managed an ongoing outbreak of CWD in wild deer in the southeast; it was first discovered in Fillmore county and cases have now also been detected in Houston, Olmsted, and Winona counties. A CWD-positive deer farm in Winona county was confirmed in 2017 and less than a year later, positive wild deer were found within one mile of that farm. Elsewhere in the state, precautionary surveillance has occurred since 2017 in Crow Wing (north central) and Meeker (central) county areas following the discovery of 2 CWD-positive cervid farms that shared infected animals. In February 2019, a positive wild deer was found dead <0.5 miles from the Crow Wing county farm that remained in operation. A full necropsy was completed by the University of Minnesota's Veterinary Diagnostic Laboratory and the animal was determined to have died of CWD. Shortly after, the Crow Wing county cervid farm culled their remaining animals. Throughout Minnesota's CWD surveillance history, 3 of the 5 wild deer outbreaks have occurred within a few miles of a known positive cervid farm.

As of April 2020, the Minnesota Board of Animal Health (BAH) manages 295 farmed cervid herds totaling 8,771 animals, primarily white-tailed deer (*Odocoileus virginianus*) and elk. In Minnesota, farmed cervids are classified as livestock and are subject to certain regulations including mandatory CWD testing of all deceased adult animals (<https://www.bah.state.mn.us/deer-elk/#chronic-wasting-disease>). Since 2002, CWD has been detected on 10 captive cervid farms with the most recent detections in early 2020 on 2 farms in Douglas and Pine counties that shared infected animals.

During the 2019 Minnesota legislative session, \$2.7 million was appropriated to support activities to monitor and manage CWD. This included a \$1,595,000 appropriation from the State's General Fund, which was the first time funds were ever provided to DNR to support wildlife disease management activities. Additionally, \$1,125,000 was appropriated from the DNR's Game and Fish fund and \$50,000 from its wild cervid health account; both of these funds are generated from hunting license dollars. This financial commitment from Minnesota's legislature underscores the importance of CWD response in Minnesota and the high value that is placed on maintaining a healthy deer herd.

## **METHODS**

### **Statewide CWD Efforts**

During fall 2019, MNDNR conducted hunter-harvested surveillance in 3 areas of the state (central, north central, and southeast) in response to positive captive cervid farms and outbreaks in wild deer. Through implementation of the surveillance and management plan for chronic wasting disease in free-ranging cervids in Minnesota, ([CWD response plan](#)), management and surveillance boundaries were changed based on the type of risk and severity of the outbreak in wild deer. In all surveillance areas, MNDNR staff and students from 5 universities collected medial retropharyngeal lymph nodes for CWD testing, while additional samples (muscle sample and front incisor) were collected in areas with confirmed cases in wild deer. For all samples collected, hunter contact information, harvest location, and age/sex of the deer were recorded. For the first time, all sample data were recorded digitally using tablets equipped with cellphone data packages and ArcGIS Survey123 (Environmental Systems Research Institute, Inc., Redlands, CA). Lymph node samples were sent to Colorado State

University for testing using enzyme-linked immunosorbent assay (ELISA) and all suspect cases were confirmed with immunohistochemistry (IHC) staining. Test results were received within 3-4 business days during archery season and as long as 14 days during the peak of firearms season. On the MNDNR's CWD website, test results were made available in real-time and tallied by surveillance area. Hunters were able to access their individual test results using their hunting license number. MNDNR staffed 31 stations during the firearms season from 9am – 7pm to collect samples and educate the public about the disease. MNDNR recruited and paid 28 taxidermists to collect samples in all surveillance areas. Taxidermists were compensated for their efforts (\$5 / head, \$10 / lymph node only sample in surveillance areas, \$15 / lymph node, muscle, and tooth in management areas). A new building was leased in Rushford on a 3-year agreement as a base for CWD operations in the southeast.

Newly designed self-service sampling stations were available to hunters throughout all of archery season (15 Sept through 31 Dec 2019) in the southeast and north central surveillance areas. Self-service sampling stations were constructed using waterproof, 30 gallon HDPE plastic barrels to permit hunters to drop-off their deer head when MNDNR staff were not present at stations. A 4'x4' map with Township, Range, and Section (TRS) identifiers was attached to a piece of plywood, covered with Plexiglas, and secured to the sampling station. Red paper tags with detachable receipts were located in an attached toolbox and provided space for the hunter to write down their name, phone number, license number, and harvest location information (TRS). The hunter affixed the main red tag to the deer's ear using provided zip ties, retained the perforated receipt for future identification purpose, and placed the deer head in a garbage bag to make sure the information stayed with the correct deer head. MNDNR staff collected the bagged heads 3 times per week and took them back to a central processing facility to remove the samples and record the hunter's information. Two emergency 90-day technicians were hired to support the workload, including one in the southeast and one in the north central.

Fall 2019 surveillance included an inaugural Adopt-a-Dumpster program in areas with carcass movement restrictions, as mandated by the state legislature. This dumpster program provided hunters a convenient place to quarter their deer and dispose of carcass remains in the north central and southeast surveillance areas. Dumpsters, tables, and quartering tripods were available in select locations starting with archery season and the number of locations was expanded during the firearms season (Figure 1). In the north central surveillance area, Crow Wing County Landfill took additional steps to mitigate potential disease spread and worked with MNDNR and Minnesota Pollution Control Agency (MPCA) to utilize an incinerator for all deer waste for the CWD Management Zone in that region.

Communication through the MNDNR website, social media, and local interviews helped define CWD rules and fall plans. The CWD page on the MNDNR website became the primary method for distributing the most up-to-date information. Sampling station locations, dates, and hours of operation were listed in the printed and online versions of the 2019 Hunting and Trapping Regulations booklet. Two new videos were created to give a general overview of CWD in Minnesota and demonstrate how to use a self-service sampling station. Both new videos, along with videos from 2018 (how to cape and quarter a deer), were posted on the CWD website. Social media targeted hunting and non-hunting audiences alike with important messages such as feeding and attractant bans. Pamphlets were developed to hand out to hunters at sampling stations, while posters and general information brochures were created for state and county fairs. MNDNR information officers worked with local wildlife staff in CWD areas to set up interviews for TV, radio, and newspaper articles. Throughout the year, a CWD email account and phone number are available for CWD-specific questions.

## **CWD Efforts by Surveillance Area**

For the central surveillance area, fall 2019 marked the third consecutive year of precautionary surveillance around a depopulated CWD-positive cervid farm in Meeker county. Mandatory testing was only required for deer >1 year old harvested within 15 miles of the cervid farm during opening weekend of the firearms season (9-10 Nov 2019) at 4 staffed sampling stations. There were no carcass movement restrictions or self-service sampling stations in place for this surveillance area. The central surveillance area included portions of Deer Permit Areas (DPAs) 277 and 283 east of State Highway 4, DPA 219 south of State Highway 55, and DPA 285 north of State Highway 7 (Figure 2). Deer feeding was prohibited in Kandiyohi, McLeod, Meeker, Stearns, Renville (north of Hwy 212) and Wright counties during the fall, but will be lifted beginning 1 July 2020.

In the north central surveillance area, a newly created CWD Management Zone, DPA 604, was established as a 15mi buffer around the CWD-positive cervid farm and the CWD-positive wild deer. This new Management Zone was created to enforce carcass movement restrictions for all deer and mandatory testing of all deer greater than 1 year old, including hunter-harvested deer, depredation deer, and car-killed deer. DPA 604 was created from DPAs 242 and 247, as well as portions of DPAs 246, 171, and 155 (Figure 2). Sampling stations were established at 6 locations, self-service during archery season and staffed by MNDNR employees during the 16-day firearms season (9-24 Nov 2019). Hunting opportunities were liberalized through unlimited disease management tags for antlerless deer, available for \$2.50. Deer feeding and attractants were prohibited in Aitkin, Cass, Crow Wing, Hubbard, Mille Lacs, Morrison, Todd, and Wadena counties.

For the southeast surveillance area, where disease appears to be persisting on the landscape, a new approach was taken for the first time in Minnesota's history. The original CWD Management Zone, DPA 603, that was created in late fall 2016 after the disease was first detected in Fillmore county encompassed a 15mi buffer around the original discoveries near Preston, was dissolved back to the original DPA boundaries of 347 and 348. All of the DPAs within 15mi of a known positive were renamed into a 600-series delimiter, identifying it as a CWD Management Zone DPA. The following DPAs 345, 346, 347, 348, 349 and portions of 255 and 343 were renamed as 645, 646, 647, 648, 649, 655 and 643 (Figure 2). This created a much larger CWD Management Zone and allowed MNDNR to enforce carcass movement restrictions for all deer and mandatory testing of all deer greater than 1 year old. Deer carcasses could move between southeast DPAs in the CWD Management Zone, but could not move outside of the management zone. Per the CWD Response Plan, liberalized hunting opportunities were established: unlimited disease management tags for antlerless deer were available for \$2.50, cross-tagging of bucks was allowed, and antler point restrictions (APR) were removed. Additionally, hunters were able to take up to 3 bucks within the Southeast CWD Management Zone; 1 each during archery, firearms, and muzzleloader seasons. Within the Southeast CWD Management Zone, 18 sampling stations were established; self-service sampling stations were available during the archery season and MNDNR employees and students staffed the sampling stations during the two 9-day firearms seasons. As an added precaution to prevent and detect disease spread, a CWD Control Zone was created using DPAs 255, 343, and 344 (Figure 2) and acted as a buffer, allowing whole deer carcasses from the Control Zone to move into the Management Zone or other Control Zone DPAs, but were otherwise restricted from moving outside those areas. In the CWD Control Zone, testing was only required for all deer greater than 1 year old during the opening firearm weekends for 3A and 3B seasons (9-10 and 23-24 Nov 2019) at 4 sampling stations. Outside of mandatory testing weekends, hunters were able to provide voluntary samples at self-service sampling stations throughout all of archery and firearms seasons. Deer feeding and attractants were

prohibited in Dodge, Fillmore, Freeborn, Goodhue, Houston, Mower, Olmsted, Steele, Wabasha, and Winona counties.

Due to the number of positives found in the fall in the southeast, a pair of special late hunts (20-22 and 27-29 Dec 2019) were held to give hunters more opportunity to take deer. The special late hunts occurred in DPAs with recently confirmed positive deer (643, 646, 647, and 648) and retained the same carcass movement restrictions and mandatory testing requirements that were in place during the fall hunting season. Unlimited disease management tags were available for \$2.50 to be used on either-sex deer, additionally hunters could harvest deer with any unused 2019 tags, provided they used the correct method of take (e.g., a deer tagged with an archery tag must be taken by archery equipment). In early December, MNDNR began the process of working with United States Department of Agriculture's Wildlife Services (USDA-WS) to begin preparing for agency culling. Landowners within 3 miles of known CWD-positives in Fillmore, Houston, and Winona counties were contacted by MNDNR to secure permission for USDA-WS to access their property. Concurrently, landowner shooting permits were offered by phone to landowners in those targeted areas; these permits expired prior to USDA-WS culling. USDA-WS began baiting in mid-January 2020 and culling began on 3 February 2020. Non-eviscerated deer were transferred to the Rushford building, where heads were removed for sampling, and the remaining intact carcasses were delivered to a contracted meat processor for packaging. Two emergency technicians were hired to help with equipment management, daily carcass deliveries to the meat processor, and sampling needs. MNDNR partnered with the University of Minnesota to collect additional tissue samples to help validate new CWD testing technology in development at the College of Veterinary Medicine. From each deer, MNDNR staff collected 3 pairs of cranial lymph nodes, tonsils, brainstem, muscle samples, and a front incisor. After results were returned as not detected, the packaged venison was retrieved from the meat processor and given back to the cooperating landowners or distributed to the public through the Share the Harvest Program. Two local deer groups, Minnesota Deer Hunters Association and Bluffland Whitetails Association, assisted in the distribution of venison. Agency culling and sampling occurred Monday through Friday until 20 March 2020, when national concerns due to COVID-19 brought the project to a close. All carcasses obtained through USDA-WS culling went to a lined landfill, while CWD-positive venison was disposed of through alkaline-digestion at the University of Minnesota's Veterinary Diagnostic Lab.

## RESULTS

From 1 July 2019 to 30 June 2020, 18,571 deer were tested and 36 new positive cases were discovered (Table 1). In the fall, MNDNR tested a total of 17,118 deer, of which 3,760 (22%) came from self-service sampling stations. No new detections of CWD were discovered in central or north central surveillance areas, but 23 additional positives were found in the southeast management zone during the fall (Figure 3). For MNDNR staffing, 207 wildlife and some fisheries staff worked 25,743 hours, with students from 5 universities filling in 198 weekend shifts.

The taxidermist program outperformed MNDNR's expectations with 1,428 samples submitted, including 4 CWD-positives, and costing a total of \$21,240. Hunter compliance rates for mandatory sampling were very high, with all 600-series DPAs reporting between 93-112% for adult deer. Compliance rates exceeding 100% are due to hunters reporting differing DPAs upon registration and CWD sampling for their harvested deer. An additional 1,040 deer, including 10 CWD positive cases, were harvested during winter management efforts from special late hunts (3/567), landowner shooting permits (0/10) and agency culling (7/463) (Figure 4).

During the agency culling effort, 63 landowners allowed USDA-WS to take deer off their property, in addition to access to state-owned lands, which totaled 15,247 acres (private =

11,603 acres and state land = 3,644 acres). Given the harvest locations of CWD-positive deer discovered during fall, 3 focal areas were selected for agency culling work. While deer removal efforts were successful in the Winona (n = 213, including 2 positives) and Preston/Chatfield/Lanesboro (n = 250, including 5 positives) areas, no landowners from the Houston area would allow USDA-WS access to their property (Figure 4).

MNDNR tested 413 opportunistically acquired deer statewide across 44 DPAs. Of those deer, 59 were found dead, 46 were reported sick, 102 were vehicle killed, and the rest fell into miscellaneous categories. Three of these deer tested positive for CWD. Two were from a known area of infection in the southeast management zone but the third was a symptomatic deer reported by a member of the community in Dakota county during spring 2020 (over 100 miles from the nearest known wild or captive positive).

The first year of the Adopt-a-Dumpster program was very successful, with more than 200 tons of deer parts collected and disposed of in lined landfills or incinerated, costing MNDNR and their partners \$186,000. Very few issues came up during the first year of the dumpster program; notably, dumpsters were not used as dumping grounds for non-target waste items, which was a concern from several parties. Whenever possible, MNDNR attempts to recover the carcass remains and venison from CWD-positive deer and deliver the material to the University of Minnesota for alkaline digestion. From July 2019 through May 2020, \$2.7 million was spent on CWD management and surveillance in Minnesota.

More than 445,000 people visited one of the CWD webpages between June 2019 and April 2020. The CWD test results page was the most-visited of all of the pages, with 122,619 page-views; 5.54% of that traffic went directly to the test results where a new interactive map was posted. Likely, many others visited the page to see the map and current results as the test results dashboard had 12,284 total searches in that same timeframe. Users were heavily engaged with posts about the Dakota County positive news release (top Facebook post for user engagement, 1,189 users) and the dumpster location information (top Twitter post for user engagement, 232 users).

## **DISCUSSION**

During the 2019 hunting season, the changes implemented through the CWD Response Plan resulted in nearly a disease census of sampled deer over 1 year of age in our CWD Management Zones. This was an unprecedented level of testing for MNDNR and provided fine-scale information about disease presence and absence. The high compliance rates, >93%, when compared to previous years (2017 compliance rates: 92% in north central, 90% in central, and 68% in southeast surveillance areas) demonstrates that the public was aware of CWD testing requirements and most hunters cooperated, which would not have been achievable without a strong communication effort.

In 2019, a new regulation was established in the southeast management zone which allowed hunters to take up to 3 antlered males (1 per season), whereas previously the bag limit was 1 antlered male per hunter, regardless of season. This regulation was intended to remove selection against taking smaller bucks, as hunters could now take multiple throughout the year, as well as provide more opportunities for hunters to harvest deer of both sexes. Throughout all southeast management zone DPAs, 6,319 hunters took 1 buck, 302 hunters took 2 bucks, and 17 hunters took 3 bucks. A very small subset (n=5) took 4 bucks, which was legal under the liberal regulations of the special late hunt which allowed for the unlimited take of either sex.

Following 3 consecutive years (2017-2019) of no CWD detections from 3,621 samples collected in the central zone surveillance area, future sampling will be discontinued in accordance with the CWD Response Plan. The north central surveillance will continue to monitor for the disease

in wild deer for at least 2 more years following the 2019 discovery of the positive wild deer found dead near the positive deer farm. However, surveillance efforts in this area from 2017-2019 have generated nearly 13,000 samples from hunter-harvested deer without detecting any positives, which provides evidence to support that CWD is not established in the local wild deer population.

In the southeast management and control zones, the infection appears to be persisting in the Preston-Lanesboro area with 56% of positive cases in an 8 x 8 mi<sup>2</sup> core area but is beginning to show some spread. Although CWD prevalence over the last 4 years has remained low ( $\leq 1\%$ ) in the original DPA 603 boundaries, it is beginning a slight upward trend since 2017 (Figure 5). A new area of disease that has shown increased cases is in Winona county, immediately surrounding a formerly CWD-infected deer farm that had a 100% infection rate. We have detected 13 new cases in Winona county as well as a cluster of 4 deer on the same parcel of land in northern Houston County. At this point, we are uncertain if these areas of disease are related or have independent sources for origination of disease.

### **Future Surveillance Plans**

CWD surveillance will be expanded with the discovery of 2 new CWD-positive captive cervid farms in Douglas and Pine counties, as well as the recent discovery of a CWD-infected wild deer in Dakota county. These 3 new areas of concern will be included in surveillance plan for fall 2020, in addition to our continued efforts to monitor disease in the southeast east and north central areas (Figure 6).

### **ACKNOWLEDGMENTS**

We would like to thank all MNDNR Wildlife, Fisheries, and Enforcement staff who assisted in the surveillance this past year. We thank Ashley Anderson, Joanne Crawford, and Janetta Kelly who helped pull off another successful CWD season. Bryan Lueth and Minnesota Pollution Control Agency were invaluable in the dumpster planning process. We especially thank the Ready Reserve wildlife staff who assisted in the planning process and area staff who helped with self-service samplings station head processing in the fall. A special thanks goes to the Big Game Program Staff, Leslie McInenly, Barb Keller, and Todd Froberg for their continued assistance in CWD surveillance and management. We thank Bluffland Whitetails Association and Minnesota Deer Hunters Association for their assistance with the Share the Harvest Program. We thank our students who provided enthusiasm and help. MNIT-GIS staff provided all of our GIS, mapping, tablet, and website needs: Bruce Anderson, Rob David, Julie Hines, Chris Scharenbroich, and Bob Wright. Our communication efforts were assisted by Denny Behr, Deb Beyer, Kristi Coughlon, Nyssa Gesh, Harland Hiemstra, Dan Ruitter, David Schueller, Shamiah Specht, Pete Takash, and Cheri Zepplin. Purchasing equipment, supplies, and other financial matters were facilitated by Lindsey Peterson, Deanna Gieseke, Steph Gilson, Jodi Wendorf, and Sally Dybdal. The MNDNR Info Center helped with their continued triage of questions and concerns from the public. Finally, we thank USDA-WS for their long hours and success from targeted culling efforts – especially John Hart, Pete Sahr, and Tim White for taking leadership roles during these phases.

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Table 1. Minnesota wild white-tailed deer samples submitted for chronic wasting disease (CWD) testing by operational phase between 1 July 2019 and 30 June 2020, including subsets of each sampling group obtained through self-service sampling stations and taxidermists.

Operational phase	Number of deer sampled	Positive results	Subset specifically from self-service sampling stations	Subset specifically from taxidermists
North Central Fall Hunter Harvested	3,966	0	976	97
Southeast Management Zone Fall Hunter Harvested	11,479	23	2,655	1,187
Southeast Control Zone Fall Hunter Harvested	1,160	0	141	88
Central Fall Hunter Harvested	544	0	Not applicable	5
CWD Special Late Hunts	568	3	47	21
Southeast Landowner Shooting Permits	10	0	10	0
Southeast Agency Culling	463	7	Not applicable	Not applicable
Opportunistic Statewide	358	3	40	30
Totals	18,548	36	3,867 (5 positive)	1,428 (4 positive)

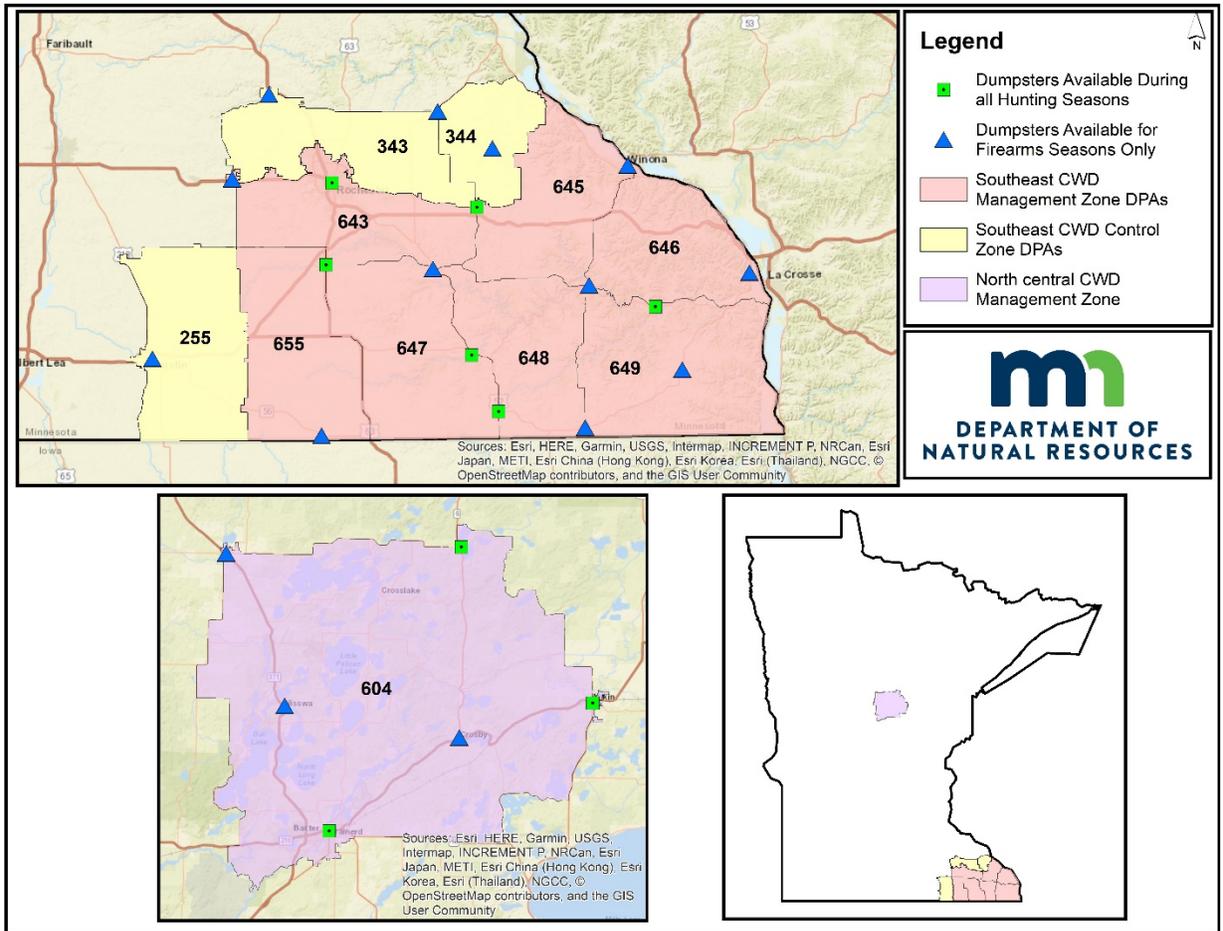


Figure 1. Placement of dumpsters for hunters to dispose of deer remains during chronic wasting disease (CWD) surveillance throughout the north central and southeast CWD Management and Control Zones in Minnesota during fall 2020.

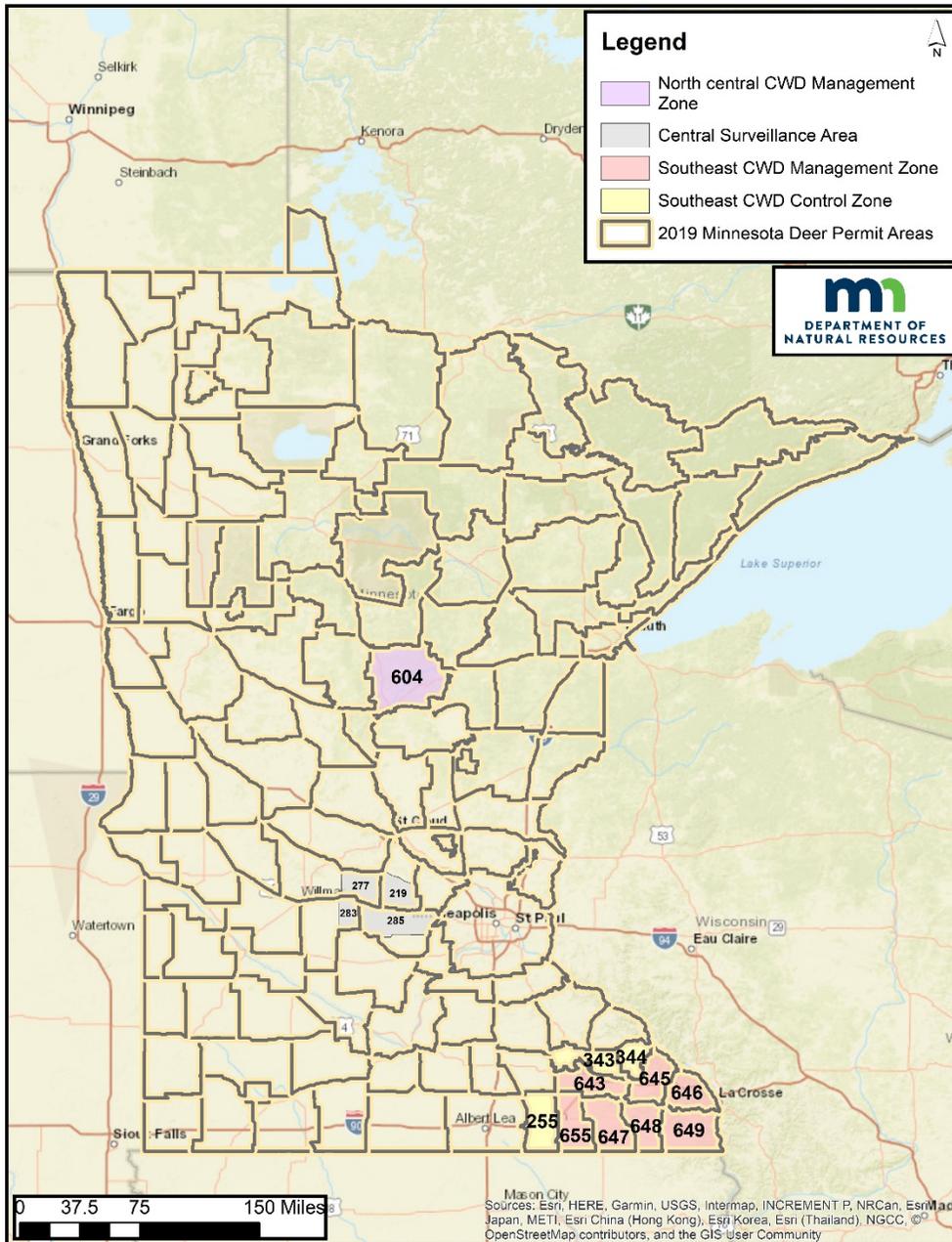


Figure 2. Fall 2019 boundaries for chronic wasting disease (CWD) surveillance in Minnesota, including the north central CWD Management Zone (including Deer Permit Area (DPA) 604), the central surveillance area (including portions of DPAs 277 and 283 east of State Highway 4, DPA 219 south of State Highway 55, and DPA 285 north of State Highway 7), the southeast CWD Control Zone (DPAs 343, 344, and 255), and the southeast CWD Management Zone (DPAs 643, 645, 646, 647, 648, 649, and 655).

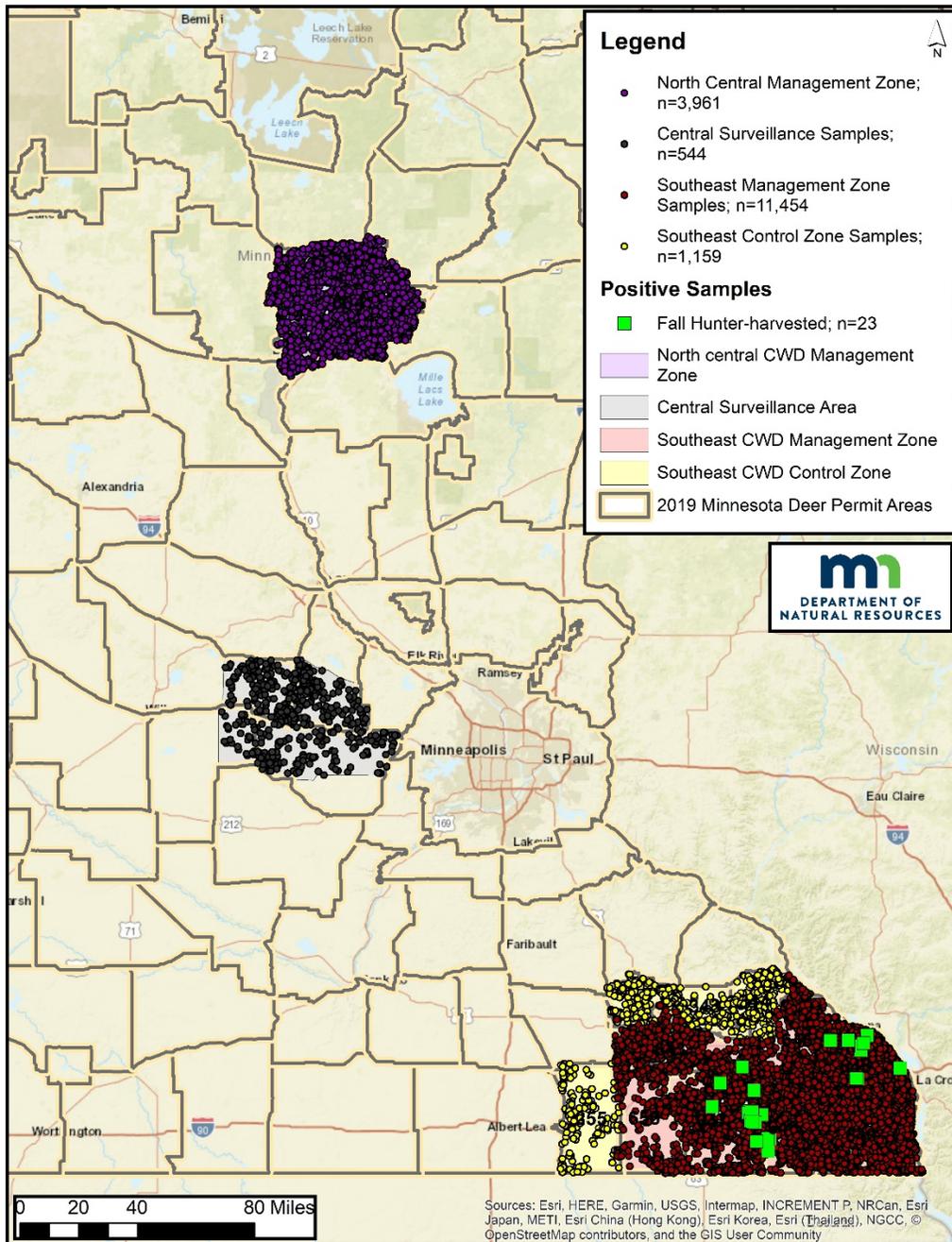


Figure 3. Locations of hunter-harvested deer (n = 17,118) sampled for chronic wasting disease (CWD) from north central, central, and southeast surveillance areas during fall 2020 in Minnesota. No CWD-positive deer were detected from deer sampled in the north central CWD Management Zone (n = 3,961), the central surveillance area (n = 544), or the southeast Control Zone (n = 1,159); however 23 new positives were detected in the southeast Management Zone (n = 11,454).



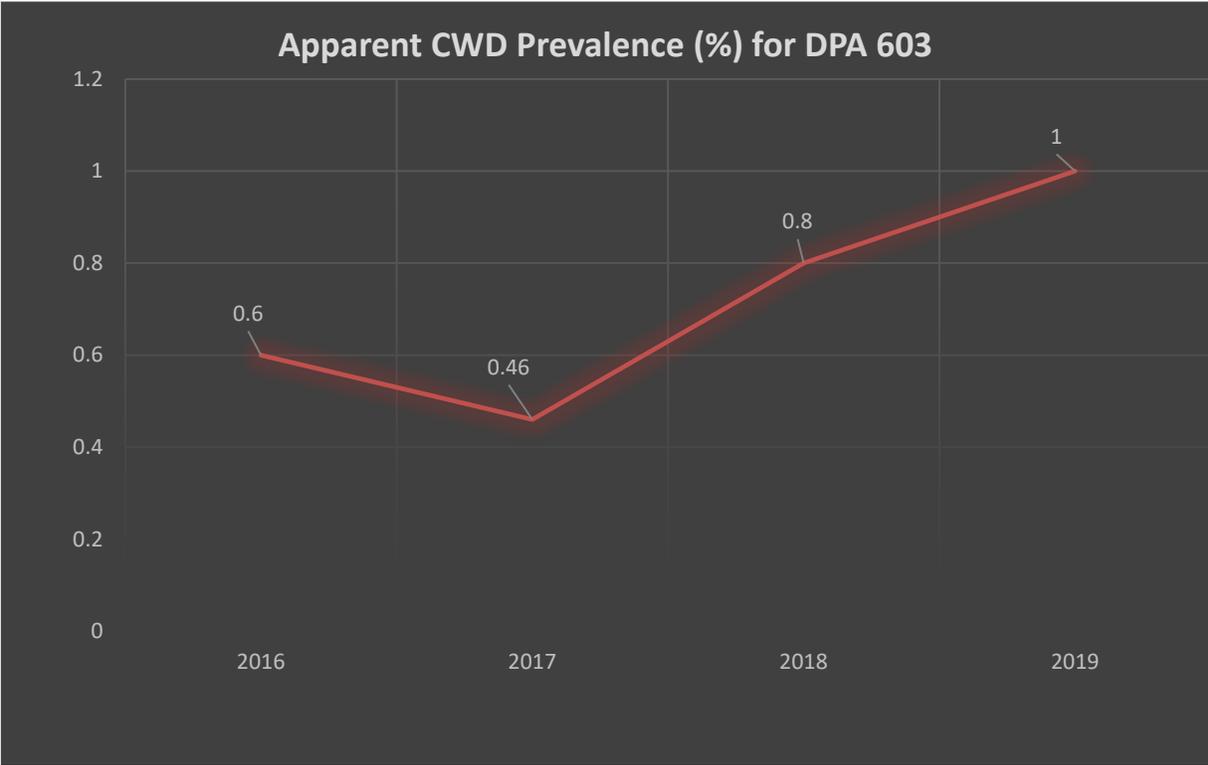


Figure 5. Changes in apparent prevalence of chronic wasting disease (CWD) in deer permit area 603 from 2016-2019, based on hunter harvested deer in Fillmore County, Minnesota.

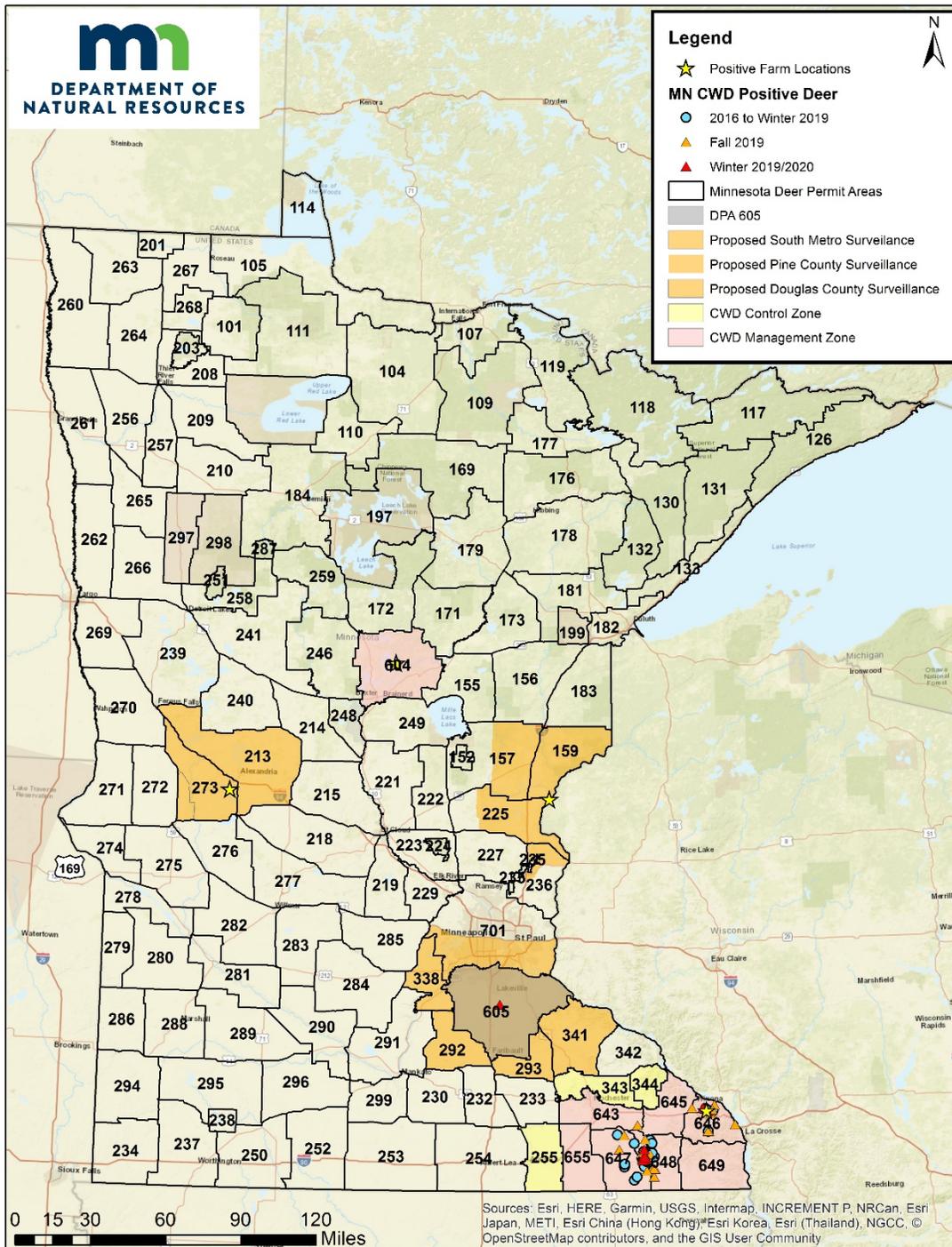


Figure 6. Proposed areas for chronic wasting disease surveillance of hunter-harvested deer planned for fall 2020 in Minnesota.

## CEREBRAL HYDATID CYST (*ECHINOCOCCUS GRANULOSUS*) IN A MOOSE (*ALCES ALCES*)<sup>1</sup>

Arno Wünschmann<sup>2</sup>, Anibal G. Armién<sup>2</sup>, and Michelle Carstensen<sup>3</sup>

### HISTORY

On January 30, 2013, a free-ranging, 11-year old, female moose (*Alces alces*) was captured, equipped with a satellite-linked Global Positioning System (GPS) collar by the Minnesota Department of Natural Resources (MDNR) and released. The moose was part of a study to examine the causes of mortality in Minnesota's declining moose population. A mortality signal was emitted from the GPS collar on December 07, 2013. The carcass was found intact, without signs of predation or scavenging, and was extracted from the field and underwent necropsy to the Minnesota Veterinary Diagnostic Laboratory (MVDL) 2 days after the mortality signal was first received.

### GROSS FINDINGS

The animal weighed 439kg and had moderate internal fat stores although measurable subcutaneous fat stores were absent in the rump region and near the base of the tail. An approximately 7 cm by 5 cm by 3 cm unilocular cyst replaced large portions of the frontal and parietal lobe of the right cerebral hemisphere. The cyst was slightly raised over the meningeal surface and extended through the entire cortex abutting against and distorting the right lateral ventricle. The inner surface of the right parietal bone had a slight depression that conformed to the raised aspect of the cyst. The cyst was bordered by an approximately 2 mm thick opaque wall. The cyst contained clear watery fluid with sandy material. The right caudate nucleus was softened and slightly discolored. The brain parenchyma neighboring the cyst was compressed and the midline of the cerebrum was deviated to the left.

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## MICROBIAL ASSOCIATIONS AND SPATIAL PROXIMITY PREDICT NORTH AMERICAN MOOSE (*ALCES ALCES*) GASTROINTESTINAL COMMUNITY COMPOSITION<sup>1</sup>

Nicholas Fountain-Jones<sup>2</sup>, Nicholas Clark<sup>3</sup>, Amy Kinsley<sup>2</sup>, Michelle Carstensen<sup>4</sup>, James Forester<sup>5</sup>, Tim Johnson<sup>6</sup>, Elizabeth Miller<sup>6</sup>, Seth Moore<sup>7</sup>, Tiffany Wolf<sup>2</sup>, and Meggan Craft<sup>2</sup>

### ABSTRACT

Microbial communities are increasingly recognized as crucial for animal health. However, our understanding of how microbial communities are structured across wildlife populations is poor. Mechanisms such as interspecific associations are important in structuring free-living communities, but we still lack an understanding of how important interspecific associations are in structuring gut microbial communities in comparison to other factors such as host characteristics or spatial proximity of hosts. Here we ask how gut microbial communities are structured in a population of North American moose (*Alces alces*). We identify key microbial interspecific associations within the moose gut and quantify how important they are relative to key host characteristics, such as body condition, for predicting microbial community composition. We sampled gut microbial communities from 55 moose in a population experiencing decline due to a myriad of factors, including pathogens and malnutrition. We examined microbial community dynamics in this population utilizing novel graphical network models that can explicitly incorporate spatial information. We found that interspecific associations were the most important mechanism structuring gut microbial communities in moose and detected both positive and negative associations. Models only accounting for associations between microbes had higher predictive value compared to models including moose sex, evidence of previous pathogen exposure, or body condition. Adding spatial information on moose location further strengthened our model and allowed us to predict microbe occurrences with ~90% accuracy. Collectively, our results suggest that microbial interspecific associations coupled with host spatial proximity are vital in shaping gut microbial communities in a large herbivore. In this case, previous pathogen exposure and moose body condition were not as important in predicting gut microbial community composition. The approach applied here can be used to quantify interspecific associations and gain a more nuanced understanding of the spatial and host factors shaping microbial communities in non-model hosts.

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## MYOCARDIAL CYSTICERCOSIS (PRESUMPTIVE *CYSTICERCUS TARANDI*/TAENIA OVIS KRABBEI) IN A MOOSE (*ALCES ALCES*)<sup>1</sup>

Arno Wünschmann<sup>2</sup>, Anibal G. Armien<sup>2</sup>, and Michelle Carstensen<sup>3</sup>

### HISTORY

On January 24, 2013, this free-ranging moose cow was captured, equipped with a collar by field biologists of the Minnesota Department of Natural Resources (MDNR) and released. The moose was part of a MDNR study to examine the sudden decline of Minnesota's moose population. The collar had GPS capabilities and a device that triggered a mortality signal when the animal did not move for 6 hours. The animal was aged at 11 years at capture based on analysis of the annual cementum layer of an extracted incisor tooth. A mortality signal was emitted at 12.25AM on November 25, 2015. The carcass of the moose was extracted from the field and a necropsy was performed at the Minnesota Veterinary Diagnostic Laboratory (MVDL) within 36 hours of the first notification.

### GROSS FINDINGS

The animal weighed 369kg. It only had very scant internal (e.g. perirenal) fat stores and no measurable subcutaneous adipose tissue stores. The abdominal cavity contained 5 liters of clear watery colorless fluid with a small amount of delicate beige stringy elastic material (interpreted as fibrin strands). The heart weighed 2.7kg. The myocardium of the left ventricular free wall, septum and right ventricular free wall had numerous (approximately 50 in total), scattered 1 to 2cm long and approximately 1.5cm in diameter cysts. These cysts contained watery clear fluid and a white, spherical, approximately 0.5cm in diameter structure. In addition, rare collapsed cysts, approximately 1cm in diameter, with greenish pasty material were present within the myocardium. Similar cysts were present in high number within the skeletal muscles particularly in the movers of the head, the masticatory muscles and the esophagus. Approximately 10% of the liver parenchyma were replaced by up to 5cm in diameter cysts containing brownish pasty material that were bordered by fibrous capsule. This lesion is consistent with fluke (*Fascioloides magna*)-induced hepatitis. Few flukes (presumptive *Paramphistomum cervi*) were attached to the ruminal mucosa. The animal was pregnant with an approximately 8cm long (crown to rump length) fetus.

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<sup>1</sup>Journal of the American Veterinary Association 2020: *in press*.

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## RE-EVALUATING THE THRESHOLD DENSITY HYPOTHESIS FOR MOOSE, DEER, AND *PARELAPHOSTRONGYLUS TENUIS*<sup>1</sup>

Amanda M. McGraw<sup>2</sup>, Ron. N. Moen<sup>3</sup>, Louis Cornicelli<sup>4</sup>, Michelle Carstensen<sup>5</sup>, Véronique St-Louis<sup>5</sup>

### ABSTRACT

A parasite, *Parelaphostrongylus tenuis*, whose definitive host is white-tailed deer (hereafter deer; *Odocoileus virginianus*), is often cited as a significant factor contributing to moose (*Alces alces*) population declines. Although deer are asymptomatic, moose express neurologic symptoms and usually die when infected with *P. tenuis*. The life cycle of *P. tenuis* is complex and requires a secondary host (gastropods) for larvae to develop, after which point incidental ingestion by moose and deer while foraging leads to infection. Researchers posit that deer densities influence the strength of the three-way relationship among moose, deer, and *P. tenuis*, and the resulting negative impact on moose health. Despite its importance for moose and deer management, only one peer-reviewed study has evaluated the relationship between deer and moose densities, and the potential for parasite-mediated competition between the species. Using polynomial regression modeling, that study identified a deer density threshold above which moose populations declined; however, the nature of the data and apparent outliers suggest the approach used to develop that threshold may not have been appropriate. We used the data from the original study to test whether alternative models, including linear models and negative binomial models would be less sensitive to outliers and could better explain this relationship. We found no evidence in our analysis that moose density decreases as deer density increases. We conclude that while the proposed moose-deer-*P. tenuis* relationship could be partially density dependent, additional factors such as frequency dependence of disease transmission, gastropod abundance, and shared use of resources by moose and deer should also be considered.

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## USING MOVEMENT ECOLOGY TO INVESTIGATE MENINGEAL WORM RISK IN MOOSE<sup>1</sup>

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### ABSTRACT

Anthropogenic habitat change and moderating climatic conditions have enabled the northward geographic range expansion of white-tailed deer (*Odocoileus virginianus*) and the parasitic nematode (meningeal worm) it carries, *Parelaphostrongylus tenuis*. This expansion can have consequences in dead-end host species for other ungulates because meningeal worm reduces health, causes morbidity or direct mortality, and has been attributed to population declines. In northeastern Minnesota, which marks the southern extent of the bioclimatic range for moose (*Alces alces*), the population has declined more than 50% in the last decade, with studies detecting *P. tenuis* in 25 to 45% of necropsied animals. We took a novel, top-down approach for assessing the factors that are most associated with meningeal worm infection by linking moose movement ecology with known *P. tenuis* infection status from necropsy. Moose were outfitted with GPS-collars to assess their space use and cause-specific mortality. Upon death, a necropsy was performed to determine cause of death and document meningeal worm infection. We then created statistical models to assess the relationship between meningeal worm infection and exposure to hypothesized factors of infection risk based on the space-use of each moose by season. Predictors included landcover types, deer space use and density, environmental conditions, and demographics of individual moose (age and sex). Moose had a greater risk of infection when their home ranges contained higher proportions of wetter environments and their fall home ranges included more upland shrub/conifer. In contrast, the strongest relationships showed that higher proportions of mixed and conifer forest within spring home ranges resulted in lower risk of infection. Relationships between exposure and infection were strongest in the spring models, potentially due to moose foraging on ground vegetation during spring. By incorporating the movement of moose into disease ecology, we were able to test hypothesized components of infection risk with actual spatial and temporal exposure of individual necropsied moose. The probability of infection for moose in northeastern Minnesota was not influenced by deer density, although deer densities did not vary greatly within the study area (2 – 4 deer/km<sup>2</sup>), highlighting the importance of both moose space use and environmental conditions in understanding infection risk. We suggest management strategies that use a combination of deer and land management prescriptions designed to limit contact rates in susceptible populations.

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## 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 are exploring the use of Light Detecting and Ranging (LiDAR) data to identify multiple habitat components and to develop this method as a tool to monitor future changes in these components. We also will examine temporal 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 forest-cover type, deciduous v. coniferous] diameter-at-breast height [DBH], tree canopy density, wetland vegetation 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, and aerial photographs, (2) ascertain the optimal point density of LiDAR needed to accurately measure or classify each habitat component of importance to wood ducks, (3) determine the generalizability of the LiDAR-based models 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 323 forest plots during Fall 2016, Spring 2017, Fall 2017, and Spring 2018. 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 7,869 trees during forest surveys, and classified 223 cavities as suitable and 111 as marginally suitable for nesting wood ducks. Because data were sparse for relatively large DBH trees of multiple species ( $\geq 40$  cm for early and mid-successional species,  $\geq 50$  cm for late successional species), we surveyed additional forest plots to obtain sufficient data on large-DBH trees with suitable cavities.

Flights to collect LiDAR data originally scheduled to occur during Fall 2016 were postponed until Fall 2017. This data became available during Summer 2018, and we began

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associating ground-level aquatic and forest vegetation measurements to LiDAR data during Winter 2019.

We used our probabilities of cavity-occurrence and FIA-based estimates of the populations of stems in corresponding species-DBH-Health Status bins to make an inference about any temporal change in the population of stems with suitable nesting cavities that occurred in Cass County, Minnesota between 1990 and 2014–2018. We initially focused this analysis on 7 tree species that were common in our study area and had some proclivity to produce suitable nesting cavities.

We used and evaluated the accuracy of several approaches to develop models for predicting which forest stands were with or without suitable nesting cavities for wood ducks. We used our empirical forest-plot, LiDAR, and other aerial imagery data in these analyses. The Random-Forest models that best predicted the location of such stands used the presence-absence of cavities as a response variable, and 33 LiDAR-derived metrics, forest cover type, and LiDAR intensity as predictors. The classification accuracy of this model was 74%.

We conducted a preliminary examination of the temporal change in the population of potentially suitable nesting cavities in Cass County between 1990 and 2014–2018 using both FIA and our empirical tree cavity data. Results generally suggest that population decreased during the period of analysis, and that this varied by tree species. In the final analysis, we will attempt to use 1977 FIA data and expand the geographic area of analysis, if we ascertain that these steps will generate reliable results.

## INTRODUCTION

Some terrestrial and aquatic habitats used by wood duck (*Aix sponsa*) 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 have been 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. 2012b), there has been recent concern among Minnesota Department of Natural Resources (MNDNR) managers that harvesting relatively large diameter-at-breast-height (DBH) trees of economically valuable species (e.g., aspen [*Populus* spp.]) in northern Minnesota will reduce the availability of cavity trees (R. A. Norrgard and D. P. Rave, MNDNR, personal communication) frequently used for nesting by some waterfowl (e.g. wood duck, common goldeneye [*Bucephala clangula*], hooded merganser [*Lophodytes cucullatus*]).

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 characterize structural (generally vegetation) attributes over large spatial scales. It also would be beneficial to develop such methodologies so that long-term trends in habitat components can be analyzed in a consistent manner.

It is unlikely that all of these needs can be met with a single methodology or existing dataset. Consequently, we will develop 2 methods for obtaining better knowledge regarding

spatiotemporal changes in wood duck breeding-habitat components. First, we will develop methodology to identify multiple habitat components and to monitor changes in these components from the contemporary period forward. This will entail building and evaluating spatial predictive models of these habitat components based on LiDAR-generated metrics and other spatial datasets (e.g., satellite imagery, radar data, National Wetland Inventory [NWI]). This methodology also could be used to provide habitat trend information that can be used in MNDNR administrative subsection planning and research efforts (e.g., estimating habitat availability in resource selection studies; see Aebischer et al. [1993]).

Second, we will examine historical changes in potential nesting habitat by analyzing Forest Inventory and Analysis (FIA) data with a quantitative method we are developing. Reliable FIA surveys have been conducted in Minnesota since the 1970s. We will 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 (Denton et al. 2012a, b), and over a greater time period (i.e., since the 1970s, if possible). This method 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. 2012b) 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-nesting, nesting, and brood-rearing life cycle phases) and perhaps other species that use similar habitat components. Meeting this goal requires that we (1) identify the location and areal extent of breeding-habitat components in our 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 are using multiple sources of data (e.g., empirical field data, FIA, LiDAR, and associated remote sensing imagery) to meet this goal. 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 or alternatively forest-cover type, DBH, tree canopy density, wetland vegetation type, and water depth based on LiDAR-generated metrics and other sources of spatial data). This evaluation will include determining the accuracy with which each spatial model can predict the locations of habitat components. Some specific questions we will examine include:
  - a) Which specific LiDAR metrics are most important predictors of suitable cavity presence-absence or abundance?
  - b) Does including ancillary remotely-sensed data and derived forest inventory attributes as predictors (e.g., satellite imagery, cover-type, timber volume estimates) improve accuracy?
  - c) Which spatial scale of analysis produces the most accurate predictions of cavity occurrence (e.g., comparing 5, 10, 20, and 40 m pixels)?
2. Ascertain the optimal point density of LiDAR needed to accurately characterize each habitat component of importance to wood ducks.
3. Determine the generalizability of the LiDAR-based models 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, and composes 40.6% of this county (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 Bird Conservation Region 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 9 major wetland types in the study area: 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  $\geq 100$  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 that wetland was 0.81–8.09 ha,  $\leq 402$  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  $\leq 100$  m from both a public road and water feature. From sites that met these criteria, we then randomly selected  $\leq 5$  sampling locations per wetlands that were  $\geq 4.05$  ha, with the- points  $\geq 100$  m apart.

Because potential loafing sites were encountered infrequently at randomly selected plots during 2016, we chose to non-randomly 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  $\leq 1.37$  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  $\geq 1.37$  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 and 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 U.S. 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  $\geq 50$  years, all other stand types  $\geq 80$  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  $\geq 50$  m apart and  $\geq 30$  m from the nearest stand boundary and (2)  $\leq 2$  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 (2014) methodology (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 (both live and dead) 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.,  $\geq 22.0$  cm DBH [Haramis 1975]), and tall enough for the DBH to be measured (i.e.,  $\geq 1.37$  m). Starting at the  $0^\circ$  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; U.S. Department of Agriculture Forest Service 2014).

All field crew members (ranging from 2 to 4 per site visit) then circled each stem  $\geq 22.0$  cm in the plot, and used binoculars to conduct a preliminary visual search of each tree  $\geq 22.0$  cm DBH in the plot to identify cavities that potentially were suitable for nesting by wood ducks. During the preliminary 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.,  $\geq 0.6$  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  $\geq 10.2$  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  $\geq 14$  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.,  $\geq 1$  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 identification 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 ( $\pm 0.1$  m), Leupold RX-800i rangefinder ( $\pm 0.1$  m), or Suunto clinometer ( $\pm 0.5$  m).

### **LiDAR Data Collection and Analysis**

The MNDNR Resource Assessment Program (RAP) originally planned to acquire airborne LiDAR and simultaneous 4-band near-infrared imagery data during Fall 2016, but this did not occur until Fall 2017. Data became available for analyses during late Summer 2018. The high-density LiDAR was acquired by a private vendor (Quantum Spatial Inc.), who used a single-photon LiDAR sensor.

We first clipped the LiDAR point-cloud data corresponding to our forest plots using Program FUSION/LDV version 3.80 software (McGaughey 2018). The clipped data of each plot then were summarized into numerous metrics representing canopy-elevation distributions, percent cover, and densities of vertical strata (i.e., slices). These metrics were associated with forest data in each plot for all stems  $\geq 22.0$  cm DBH. We then developed a Random Forest model (Liaw and Wiener 2002) using these data. We conducted preliminary analyses both to identify important predictors to be used in further analysis and to identify any analytical issues. We used the analytical approach developed by Murphy et al. (2010, p.255–256), in which the number of predictor variables is minimized and the amount of variation explained is maximized via a process of eliminating unimportant variables based on their model improvement ratios. The Random Forest algorithm in the R statistical package (Liaw and Wiener 2002, R Core Team 2020) was used to predict the presence/absence or abundance of a suitable cavity in plots based on the LiDAR metrics and RAP-generated stand-type classifications (i.e., random-forest analyses can be performed in either classification or regression mode [Liaw and Wiener 2002]).

We conducted separate analyses with 3 different response variables derived from our forest and cavity surveys: (1) the presence-absence of suitable cavities, (2) 3 levels of cavity occurrence (none, low [1 suitable cavity per plot], and high [ $>1$  suitable cavity per plot]), and (3) abundance of suitable cavities (0-5 cavities per plot on a continuous scale). We initially included 177 LiDAR-derived metrics that occurred in 40-m grid-cells overlaying the plot locations as potential predictors. The predictors used in the next step of the analysis were 33 metrics related to the intensity of the LiDAR returns (note: these returns were not normalized across the study area) and forest cover-type (classified from Landsat and LiDAR data). After removing collinear variables using QR decomposition (Murphy et al. 2010), 71–78 variables were retained for further analyses.

Random Forest models were run with 10,000 trees 10 separate times, and the accuracy metric was averaged among runs. Murphy et al. (2010) only ran 1 model with 5,000 trees, but we observed that accuracy was variable between model runs without some form of replication.

We then compared the accuracy of Random Forest models for each of the 3 response variables with (1) LiDAR metrics only, (2) LiDAR metrics and the cover type variable, and (3) LiDAR metrics, the cover type variable, and LiDAR intensity metrics. Thus, there were 9 analytical comparisons (i.e., 3 response variables X 3 groups of predictor variables). We also assessed the classification accuracy of response variables (1) and (2) using out-of-bag (OOB) error. OOB is calculated through internal cross-validation in each Random Forest model, with  $1 - \text{OOB}$  = proportion of correct classification (Cutler et al. 2007). We evaluated response (3) using the proportion of variance explained. Last, we used the `rf.modelSel` function in the `rfUtilities` package (Evans and Murphy 2019) in R to rank random-forest models based on performance.

## FIA Analysis

We used results from Zlonis et al. (2020) in conjunction with FIA data to infer the extent to which the density and population of suitable nesting cavities changed in Cass County during 1990–2018. Zlonis et al. (2020) found that tree species, DBH, and health status were important predictors on the occurrence of suitable nesting cavities. More specifically, the probability of cavity occurrence among the 7 species examined was greatest in sugar maple, followed by American basswood, red maple, northern red oak, bigtooth aspen, quaking aspen, and finally, paper birch. The probability of cavity occurrence was positively associated with DBH. Suitable cavities were most prevalent in dead stems, followed closely by live health-impacted stems, and finally live healthy trees.

Next, we queried the FIA database to obtain an estimated population of stems that corresponded to the species-DBH-health status bins used in our analysis of empirical data. We limited our query to plots classified as 'timberland' by the U.S. Department of Agriculture Forest Service, which is defined as "forest land capable of producing in excess of 20 cubic feet per acre per year and not legally withdrawn from timber production, with a minimum area classification of one acre" (U.S. Department of Agriculture Forest Service 2019). We used FIA data only from Cass County, Minnesota because it likely had a forest composition and structure (i.e., species, age classes, health condition) similar to that of our study area in the northern portion of this county.

The approaches for applying damage codes (i.e., a metric of health status) to live stems and tallying dead trees changed in FIA over 6 evaluation cycles (i.e., 1977, 1990, 1999–2003, 2004–2008, 2009–2013, 2014–2018). Thus, it was necessary to construct a health-status crosswalk among these evaluation cycles and our cavity survey. Relevant changes in FIA methodology and the data we used are as follows.

The only standing dead stems tallied during the 1977 FIA evaluation cycle were if the mortality of a tree had occurred within the previous 3 years or if it was deemed salvable; thus dead trees during this cycle did not include all of the stems that would have been measured in our empirical study or in subsequent FIA inventories, which had no requirements regarding years since mortality or salvability. Consequently, we did not use data from the 1977 FIA evaluation cycle in the preliminary analysis. A subsample of undisturbed plots were modeled (i.e., not remeasured) during the 1990 FIA evaluation cycle. We included data from this evaluation cycle in the preliminary analysis so that the plausibility of results can be assessed. Another important change in FIA methodology is that all plots in Minnesota were surveyed within approximately 1 year during 1977 and 1990, but only a subset of 20% of available plots were surveyed during any 1 year under the annual evaluation system (i.e., beginning in 1999, all plots in Minnesota are measured in 5 years). Further, damage codes were recorded during 2000–2003 but not 1999. Thus, we decided to include data from the 1999–2003 cycle in the preliminary analysis because of the need for blocks of 5 consecutive years for coverage of all plots in Minnesota.

For each year-group  $i$ , we summed FIA estimates by species  $j$ , health status  $k$  (live-healthy, live-health impacted, dead), and DBH bin  $l$  to compute estimates of  $\hat{N}_{ijkl}$  (total trees by subgroup) and  $\text{Var}(\hat{N}_{ijkl})$ . We used the cavity model from Zlonis et al. (2020) to estimate the mean probability of a tree in each subgroup having a suitable cavity,  $E(P)$ , given predictors for species, health status, and DBH bin. We used the product of  $\hat{N}$  and  $\hat{P}$  to generate estimates of total potential cavities in each subgroup,  $\hat{t}_{ijkl}$ , and the delta method (Seber 1982, Goodman 1960) to compute  $\text{Var}(\hat{t}_{ijkl})$  based on the assumption that  $\hat{N}$  and  $\hat{P}$  were independent random variables. We then summed  $\hat{t}_{ijkl}$  and  $\text{Var}(\hat{t}_{ijkl})$  by year and species for investigating temporal changes in suitable cavities over time. Summing variances across the subgroups is based on the assumption that the estimates were independent, which is not true. However, an

exploratory Monte Carlo simulation suggested this approach provided a reasonable approximation of the primary sources of uncertainty in the estimation process.

## 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 documented tree presence 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  $\leq 100$  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. We observed 6 beaver lodges, 2 logs or stumps, and 1 muskrat house in the 15 non-randomly selected plots.

### Forest Surveys

We conducted surveys at 323 forest plots during Fall 2016, Spring 2017, Fall 2017, and Spring 2018 (Figure 3). Of these plots, all stems  $\geq 22.0$  cm DBH were examined in 213 and only larger stems were measured in 110.

Most results of the forest surveys were reported in a previous issue of *Summaries of Wildlife Research Findings* and in Zlonis et al. (2020). We reported that the best predictive variables of the presence and absence of suitable nesting cavities were tree species, DBH, and health status. We will use the findings of Zlonis et al. (2020) in follow up analyses of FIA and LiDAR data.

### LiDAR Data Collection and Analysis

Single-photon LiDAR data and simultaneous aerial imagery were collected during peak fall color in 2017, usually at about 30 return pulses / m<sup>2</sup> (minimum of 12, up to 40–50; J. Corcoran, MNDNR, unpublished data). Single photon LiDAR operates with green laser and in principle can penetrate the water surface. However, LiDAR data was not as good as anticipated, and did not have bathymetric capability. Thus, identifying the presence/absence and density of submergent vegetation and depth of water in relatively shallow locations likely will not be possible.

Classification accuracy was highest when using all of the predictor variables and the binary cavity presence-absence response variable (74% plots correctly classified; Table 2). This model was better able to classify plots that did not have suitable cavities (80% correctly classified) versus plots that did have suitable cavities (67% correctly classified). When the intensity metrics and the cover type variable were removed consecutively, there was a drop in overall classification accuracy from 74% to 68% to 66%, respectively (Table 2).

Classification accuracy was also lower when using three levels of cavity occurrence (56–59% correctly classified; Table 2). Notably, these models were relatively poor at classifying plots with low and high numbers of suitable cavities (<30% correctly classified for each category). However, when compared to models with a binary response, plots without suitable cavities were correctly classified at a higher rate (85% correctly classified). Generally, regression models did

not explain a high percentage of variation in the cavity data ( $\leq 21\%$  variance explained; Table 2). Similar to the binary response variable, regression models performed best when using all of the available predictor variables.

### **FIA Analysis**

Preliminary results suggest that there was a substantial decrease in the estimated population of suitable nesting cavities between 1990 and 1999–2003, followed by a rise and approximate stabilization of such stems (Figure 4). However, not all the tree species examined followed this pattern. Further, the estimated proportion of stems with suitable nesting cavities in each health status class appeared to vary among evaluation cycles (Figure 5).

## **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).

### **LiDAR Data Analysis**

We will continue exploring the use of Random-Forest models and LiDAR data to predict the locations of suitable nesting cavities for wood ducks, given the promising preliminary results. Accuracy levels to-date have been acceptable, but could be improved by refining our methods. We will proceed with the binary presence-absence response variable and incorporate a further comparison of accuracy among several pixel sizes of this response (e.g., presence-absence at the scales of 5-, 10-, 20-, and 40-m pixels). Initial forest-inventory modelling using the same LiDAR predictors we used suggests that a pixel size smaller than our plot size (i.e., <40-m pixels) will have greater predictive accuracy (RKD, *unpublished results*). Because of the highly-

detailed and fine-scale nature of the LiDAR data, we expect that spatial information would become more generalized at the broader scales examined and predictability would be reduced. Cavities are inherently linked to individual stems, which likely only affect structural attributes of LiDAR data at scales <20 m in the forests of northcentral Minnesota.

We also will incorporate new predictor variables into random-forest models. MNDNR RAP personnel have modelled and mapped 8 common forest inventory metrics that may be useful for predicting suitable nesting cavity locations (i.e., biomass, basal area weighted height, quadratic mean DBH, basal area, site index, trees per acre, volume, cover type; *unpublished results*). We also will explore modelling tree health-status using variables such as snag density and proportion health-impacted trees. With these additional predictors, we would then have 3 metrics that are analogous to forest inventory attributes that have been used to predict suitable nesting cavities (Zlonis et al. 2020). More specifically, the variables cover type, quadratic mean diameter, and proportion health impacted generated by RAP are comparable to the predictors tree species, DBH, and health status, respectively, used by Zlonis et al. (2020), respectively. Finally, a variety of ancillary remotely-sensed data could be used as predictors (e.g., radar). We will restrict the predictors used to those that are available for the entire study area. We plan to continue using QR decomposition and the random-forest model selection function to reduce this large number of potential predictors to a manageable subset. The objective of ascertaining the pulse density needed to accurately classify forest and aquatic vegetation characteristics likely will not be possible because of the characteristics of single-photon linear LiDAR. Last, we are unlikely to use LiDAR intensity to predict cavity presence or absence in the final analysis because this intensity was so uneven across the study area.

We likely will use a similar approach to analyze wetland plot data during the upcoming year. However, it may be necessary to use remotely sensed data to classify wetland habitat if the scattering of LIDAR pulses off the water surface is problematic.

### **FIA Analysis**

Preliminary results suggest that the population of suitable nesting cavities generally decreased between 1990 and 2014–2018. This purported reduction in the population of stems with suitable nesting cavities may be attributed in-part to forest succession, forest practices, and changes in FIA methodology. The idea of forest succession influencing tree-species composition and ultimately the population of stems with suitable cavities is supported by the increase in the estimated population of stems with such cavities in some mid- or late successional species (i.e., red maple, sugar maple) and a decrease in an early successional species (i.e., quaking aspen). We have not examined species-specific removals in FIA data, but will do so to develop a better understanding of whether forest practices may have had some influence on the population of stems with suitable cavities. The lack of recorded damage codes in FIA during 1999 may partially explain the relatively low population estimate of stems with suitable cavities, especially those that were classified as damaged, during the 1999–2003 evaluation cycle.

During the next year, we will take several steps to complete this phase of the study. Specifically, we will finalize a health-status crosswalk and identify data that warrants inclusion in the final analysis (e.g., additional tree species beyond the 7 examined by Zlonis et al. [2020], evaluation cycles, spatial scales). For example, we will include data from the 1977 and 1990 evaluation cycles if the issues of undercounting dead stems and use of modeled data, respectively, can be resolved. We will use the 2000–2004, 2005–2009, 2010–2014, 2015–2019 time blocks for the final analysis now that 2019 data has become available (i.e., 1999 data will not be used). We also will ascertain whether our findings can be extended reliably into nearby

ecological strata (e.g., subsections), based on similarity of forest characteristics at the spatial scales of our study area and those strata.

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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 <sup>a, b</sup>	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

National Wetland Inventory system, subsystem, class and subclass of sampled plots <sup>a, b</sup>	Number of plots surveyed
Riverine upper perennial emergent nonpersistent	4

<sup>a</sup> Wetlands in the palustrine system are not assigned a subsystem classification in the National Wetland Inventory classification scheme.

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

Table 2 The percent (range) of plots correctly classified for the presence/absence or abundance of suitable nesting cavities for wood ducks in 9 different analyses. In these analyses, we used 3 response variables (presence/absence, classes of cavity occurrence (none, low [1 suitable cavity per plot], high [ $>1$  suitable cavity per plot], abundance [0-5 cavities per plot on ]) and 3 predictors (LiDAR metrics, LiDAR metrics + forest cover type data, LiDAR metrics + forest cover type data + LiDAR intensity). Plots were 40-m grid cells.

Response variable	% Correct classification (range) of plots for 3 groups of predictors		
	LiDAR metrics	LiDAR metrics + cover type	LiDAR metrics + cover type + intensity
Presence-absence	66% (63–68)	68% (66–69)	74% (69–75)
Three classes – none-low-high	59% (58–62)	57% (56–60)	56% (53–60)
Regression – abundance	9% (7–11)	14% (13–15)	21% (20–21)

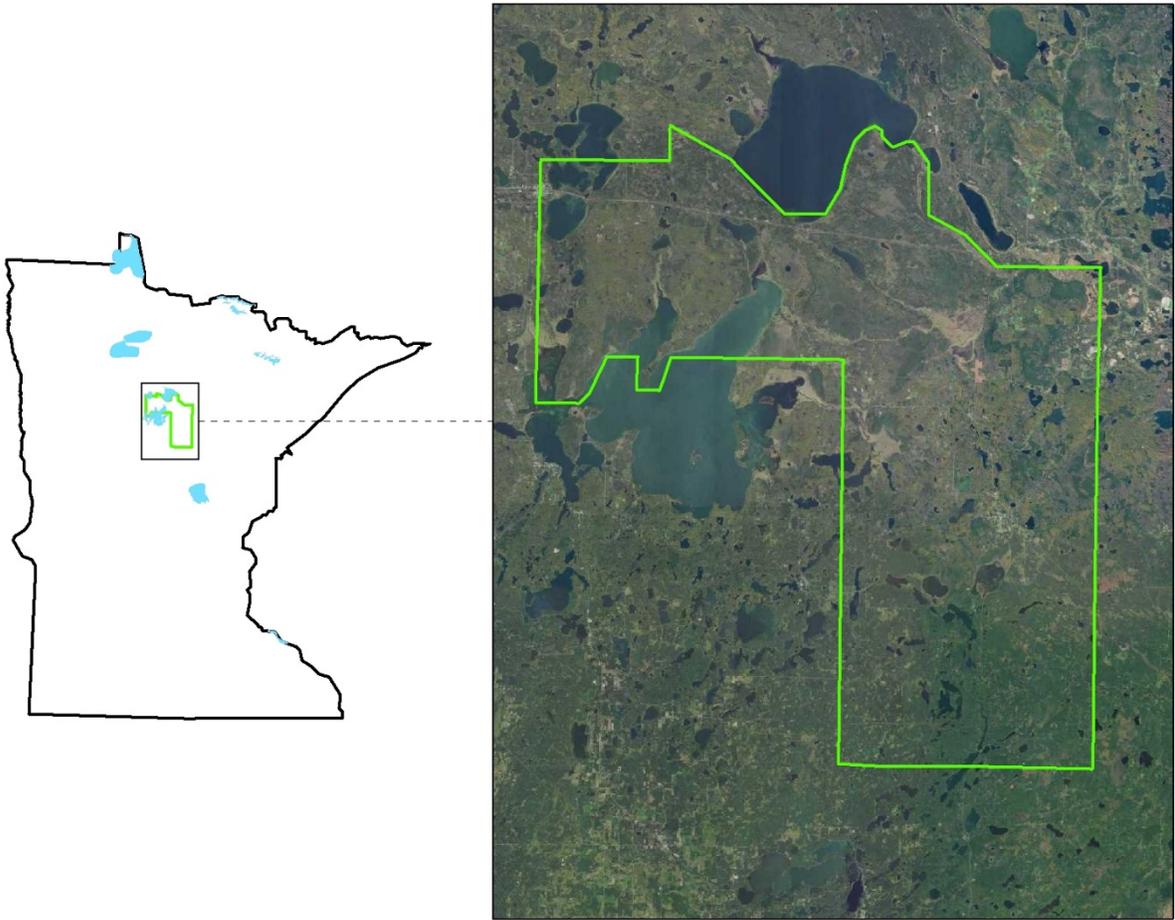


Figure 1. Location of the wood duck-LiDAR project in Cass County, Minnesota, USA.

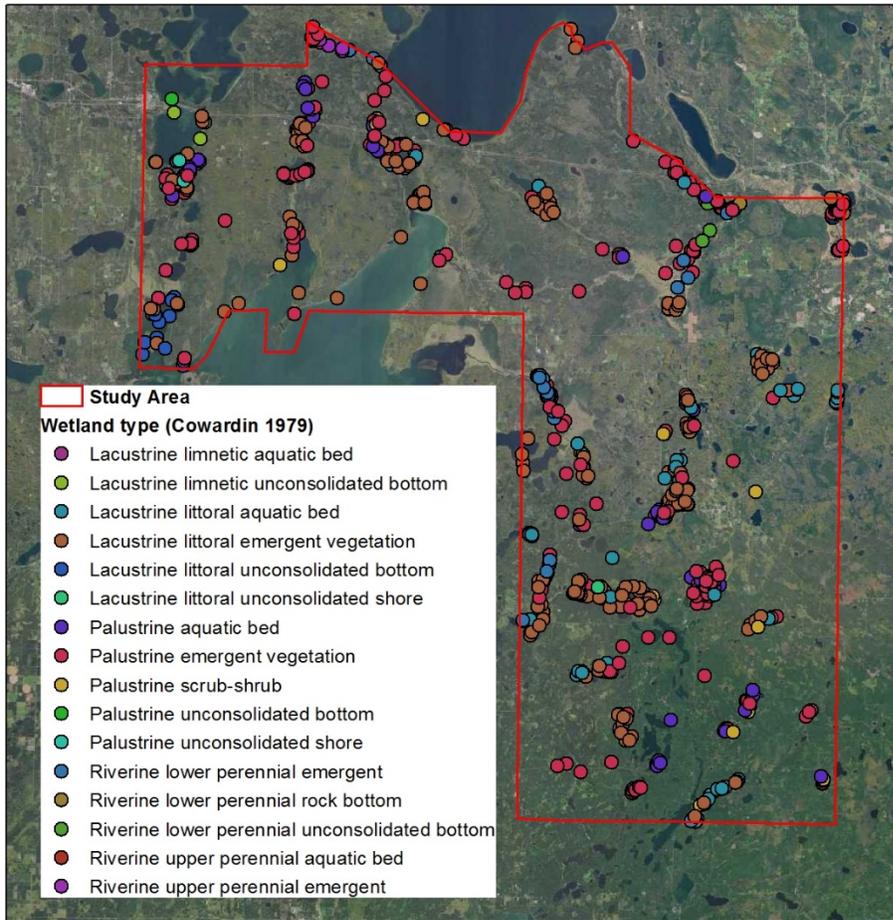


Figure 2. Location of wetland plots of different National Wetland Inventory types (Cowardin et al. 1979) surveyed in in Cass County, Minnesota, USA during Summer and Fall 2016 and 2017.

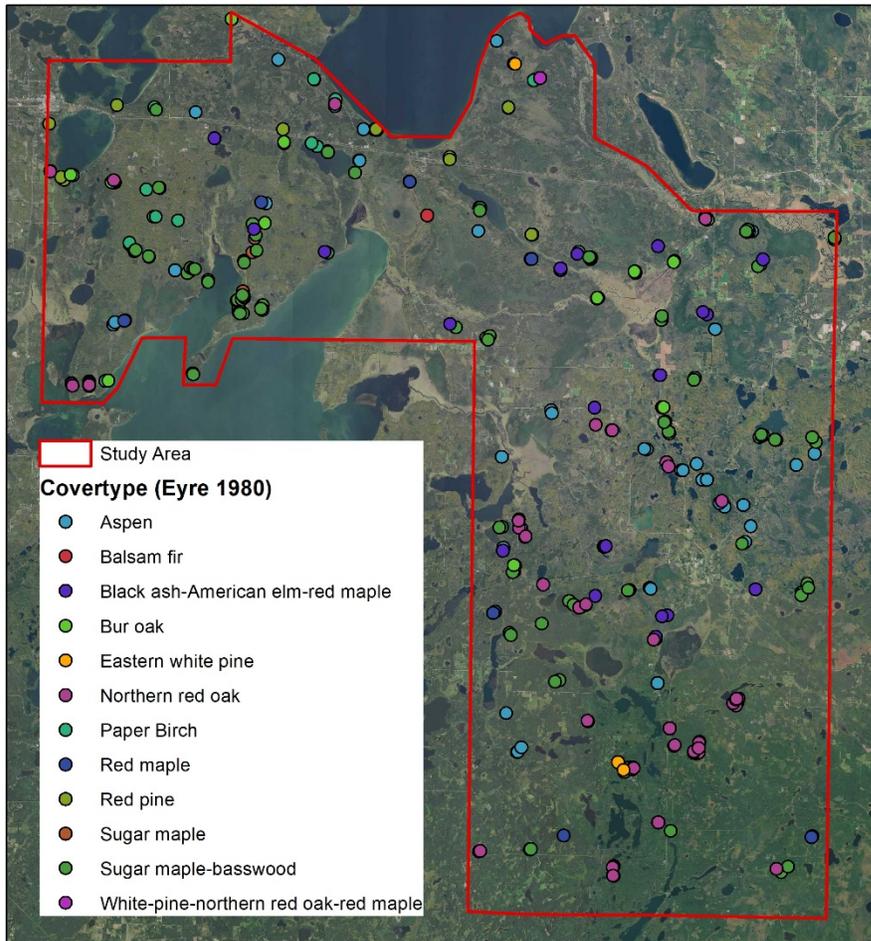


Figure 3. Location of forest plots of different cover types (Eyre 1980) that were surveyed in Cass County, Minnesota, USA during Fall 2016, Spring 2017, Fall 2017, and Spring 2018.

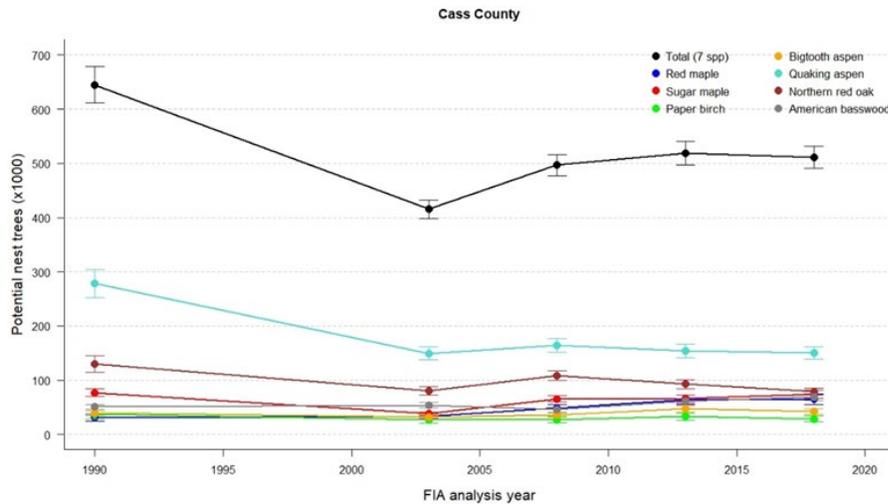


Figure 4. The number of potential nest cavity trees  $\geq 22$  cm (8.7") DBH of 7 species individually and in aggregate in Cass County, Minnesota during 5 FIA survey periods. It is important to note that FIA changed methodology over time. For example, some plots in Minnesota were measured during 1990, except for a subset of plots that had been undisturbed since 1977. Estimates from this subset of undisturbed plots were generated via modeling. Another important methodological change beginning in 1999 was that only about 20% of plots were measured each year, resulting in 5-year evaluation cycles for generating estimates (e.g., 1999–2003, 2004–2008, 2009–2013, 2014–2018). These cycles are labelled as the last year in the 5-year cycle (e.g, 2003 represents the 1999–2003 cycle).

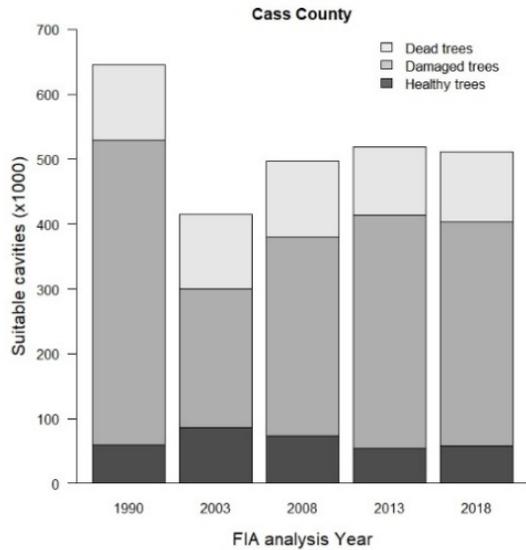


Figure 5. The number of potential nest cavity trees  $\geq 22$  cm (8.7") DBH of 7 species with suitable cavities in Cass County, Minnesota during 5 FIA evaluation cycles. Estimates during each survey period are aggregated into 3 health-status classes: Live-healthy, Live-damaged, and Dead. Some plots were measured during 1990, except for a subset of plots that had been undisturbed since 1977. Estimates from this subset of undisturbed plots were generated via modeling. Beginning in 1999, only about 20% were measured each year, resulting in 5-year evaluation cycles for generating estimates (e.g., 1999–2003, 2004 –2008, 2009–2013, 2014–2018). These cycles are labelled as the last year in the 5-year cycle (e.g, 2003 represents the 1999–2003 cycle). Further, damage codes were not recorded during 1999, which may explain why the number of damaged stems with suitable cavities was relatively low during 1999 –2003.

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

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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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## FALL MOVEMENTS OF MALLARDS MARKED IN MINNESOTA

Bruce E. Davis

### SUMMARY OF FINDINGS

During August through 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 3,506 tracking locations. Locations within the state of Minnesota ( $n=2,848$ ) were used to examine habitat use. Marked birds stayed in Minnesota longer than expected and 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 of 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 combined for 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 southern hunting zone than for birds marked in the northern 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 through September of 2017, I marked 90 mallards with GPS tracking units; 79 of these units used the Argos system and 11 units were GPS dataloggers. During August through September of 2018, I marked 45 mallards with GPS tracking units that transmitted through the Argos system. We were planning to end fieldwork for the project after the 2018 field season, but after the season, we had 20 tracking units on hand that had not yet been deployed. During August through September of 2019, I marked the final 18 mallards with GPS tracking units that transmitted through the Argos system; 2 tracking units failed to start and could not be deployed.

Herein, I present summaries of preliminary analyses for birds marked in 2016, but I have not yet completed analyses for birds marked in 2017, 2018 or 2019. Beyond summary statistics, I provide here some example data as well as some comparisons with band recovery data for mallards marked in Minnesota. I expect to complete analyses by late 2020 to early 2021.

### 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 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 conducted to understand the ecology 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 (*Anas platyrhynchos*) 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 2<sup>nd</sup> 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 and 2018. 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 and 2018.

### **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 estimate product-limit emigration rate (Kaplan and Meier 1958) for the marked sample and examine directionality of emigration trajectories.

### **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 into 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 2,848 location estimates from 44 birds using compositional analyses (Aebischer et al. 1993). I determined diurnal and nocturnal proportional bird use of habitat during each time period and 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 ( $P > 0.018$ ). Retention rates of marked birds in Minnesota remained  $> 80\%$  until early November then declined to about 45% by mid-November, with remaining birds leaving 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). One noteworthy exception to the southeasterly emigration pattern was that in 2016, 6 birds marked in northwestern Minnesota moved north into southern Manitoba (Figure 5). This movement pattern took place in only a small percentage of birds, but represents movement that is not usually seen when examining band recovery data due to low hunting pressure and dilution of the banded sample by large numbers of other mallards.

### **Use of Habitats**

We attained location data for birds marked with GPS-Argos units and assigned location data habitat attributes (Figure 6). For birds marked in 2016, I did not detect differences among

proportional use of habitats by seasons or cohorts ( $P > 0.090$ ), 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 7). Use of open water habitats were highest during the day; use of crop habitats were highest during the night (Figure 8).

Use of areas closed to hunting varied by zone of marking and season ( $P < 0.0029$ ). 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 and 2018 will be conducted in 2019. An additional sample of 25 Mallards are scheduled to be marked in August-September 2019 using GPS-Argos type tracking units.

## ACKNOWLEDGMENTS

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REGULAR SEASON DUCK AND GOOSE ZONES

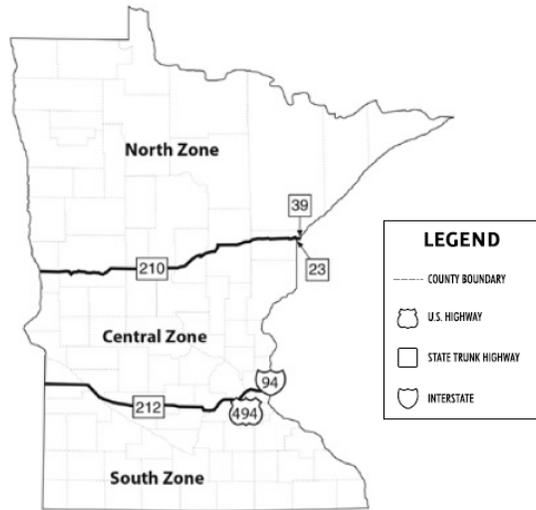


Figure 1. Minnesota waterfowl hunt zones boundaries, 2016.

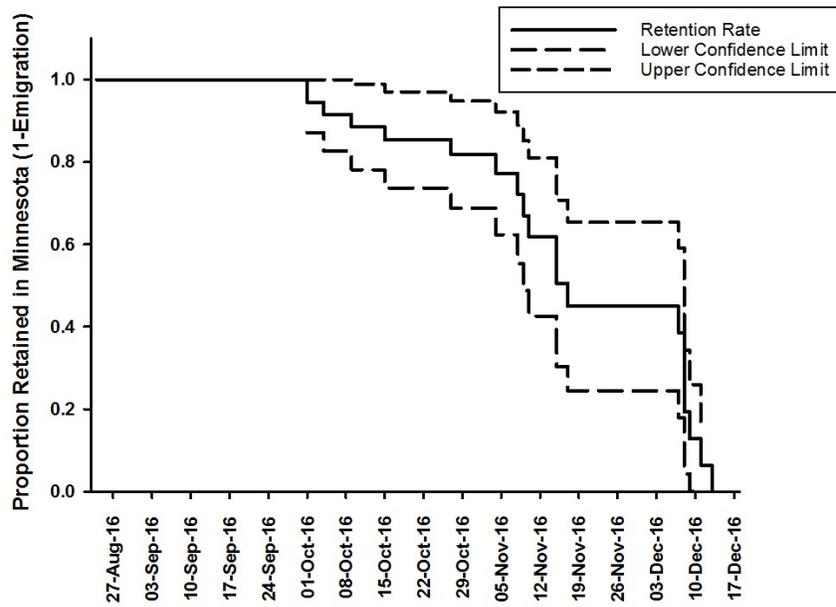


Figure 2. Retention curve for mallards marked with tracking units in Minnesota, 2016.

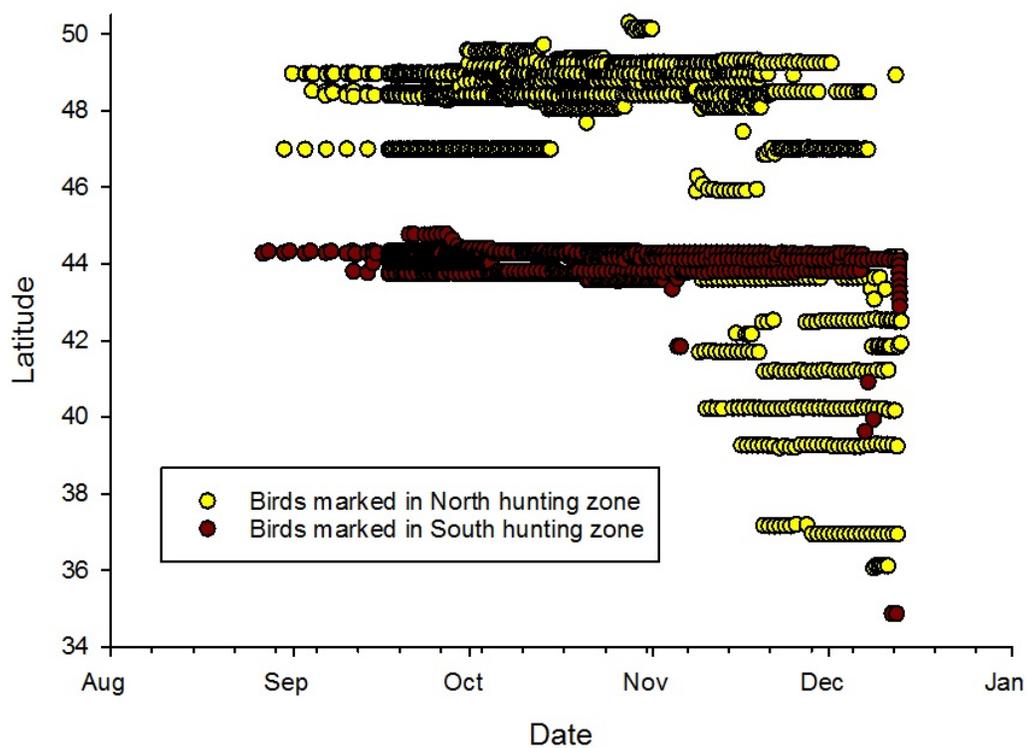


Figure 3. Scatter plot of location latitudes by date for mallards marked with tracking units in Minnesota, 2016.

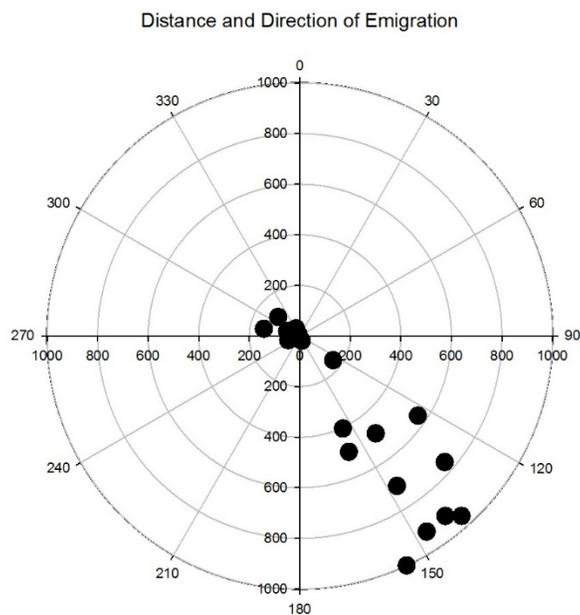


Figure 4. Polar plot of distance and direction of movement on permanent emigration (black dots) for mallards marked with tracking units in Minnesota, 2016. Concentric rings represent distances (km); azimuth (degrees) of movements are indicated on the outermost ring of the plot.

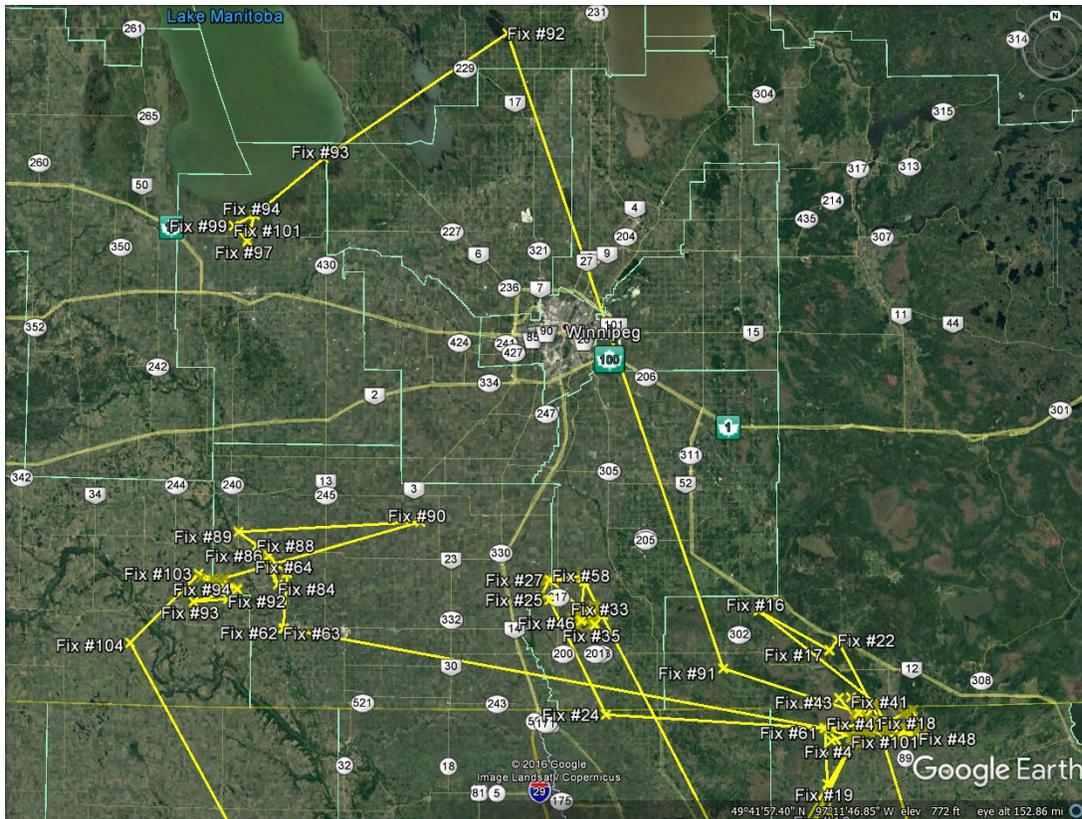


Figure 5. Movements of marked individuals into southern Manitoba from northwest Minnesota in 2016. Yellow Xs on the map represent telemetry fixes and consecutive fixes are connected by yellow lines.

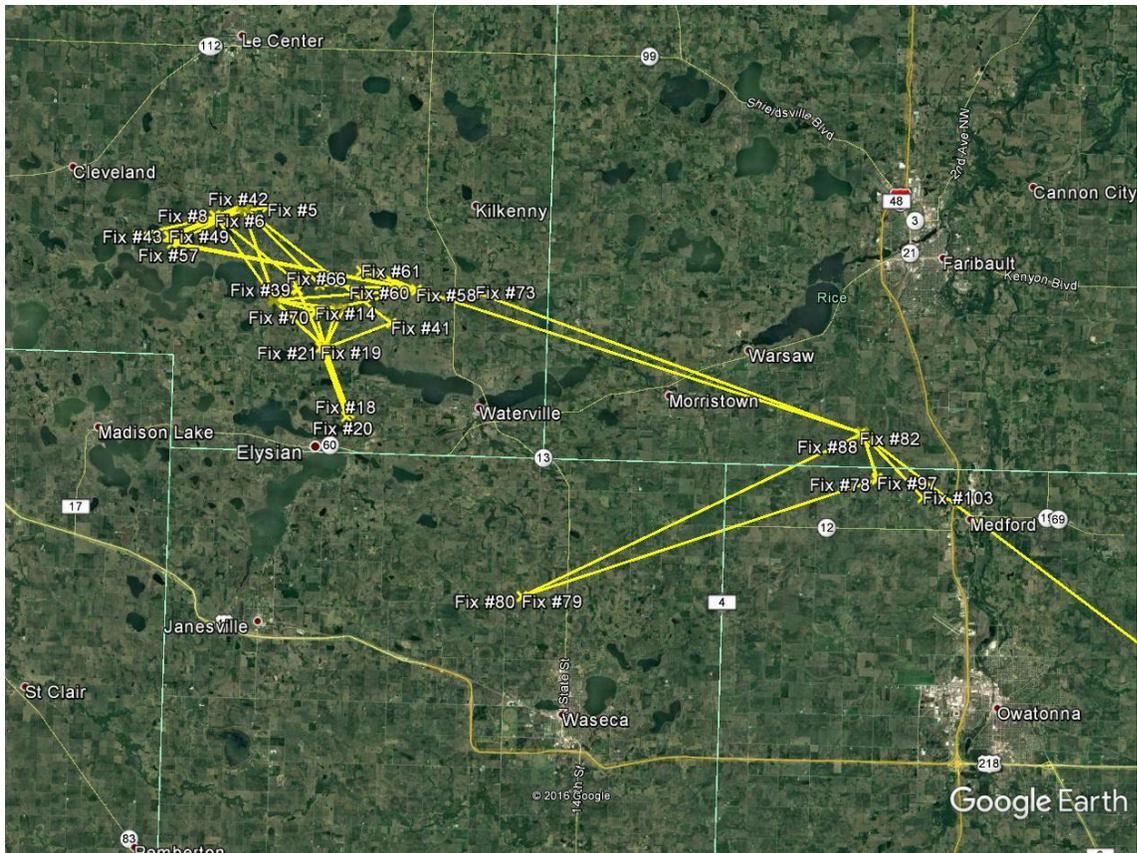


Figure 6. An example of tracking data for a single marked female used for habitat use analyses from Minnesota in 2016-2018. Yellow Xs on the map represent telemetry fixes and consecutive fixes are connected by yellow lines.

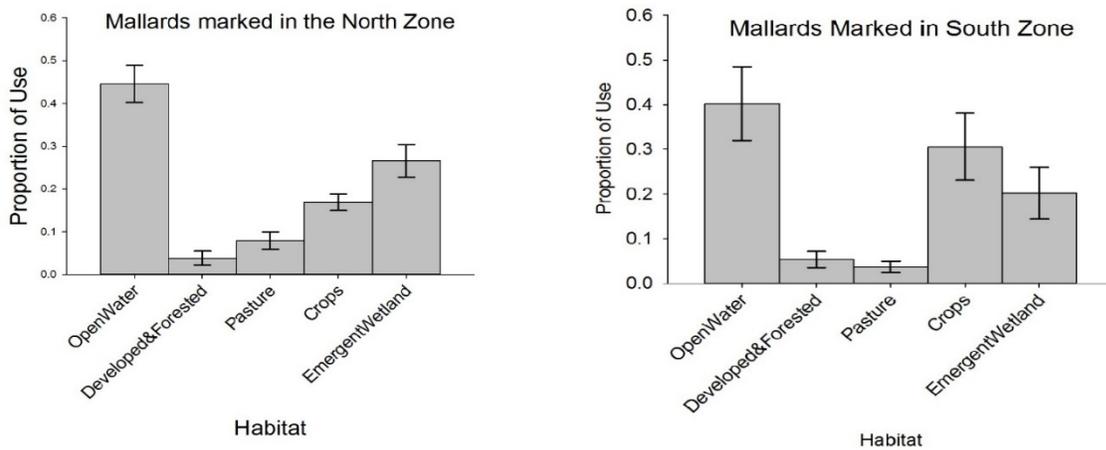


Figure 7. Proportional use of habitats by mallards marked in the Minnesota's north and south hunting zones, 2016. Proportions are expressed as an average across birds.

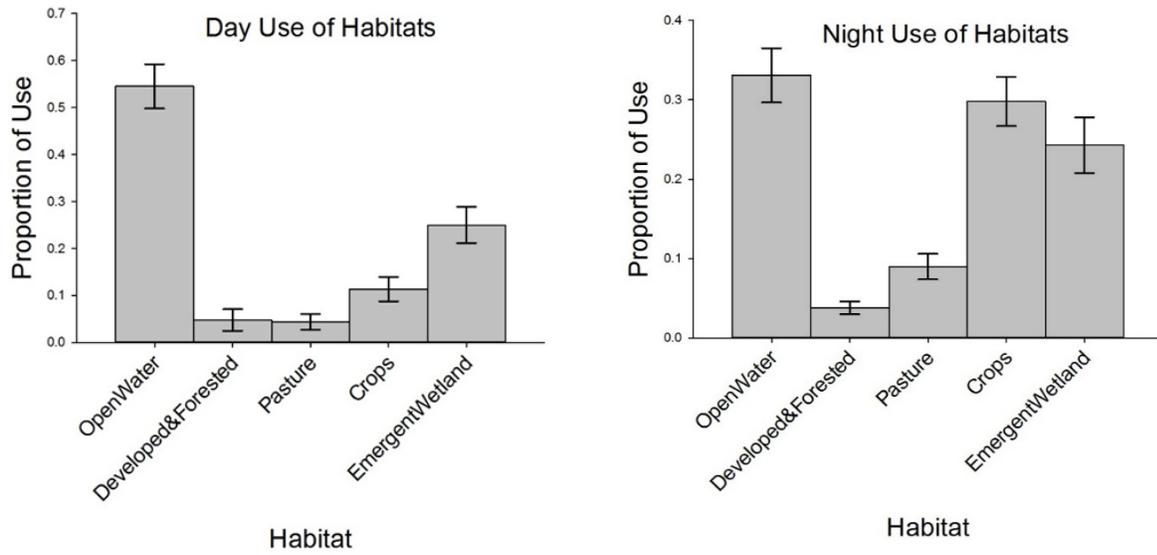


Figure 8. Proportional day or night use of habitats by mallards marked in the Minnesota's north and south hunting zones, 2016. Proportions are expressed as an average across birds.



## Restoring Wetland Invertebrates to Revive Wildlife Habitat: 2019 Research Summary

Megan J. Fitzpatrick<sup>1</sup>, Breanna Keith<sup>2</sup>, Jake Carleen<sup>2</sup>, and Carl Isaacson<sup>2</sup>

### SUMMARY OF FINDINGS

Freshwater amphipods are an important component of prairie pothole wetland food webs. In some wetlands, they reach densities of thousands of individuals per square meter. However, amphipod populations have declined in recent decades, and this trend has been implicated as a cause of decline of the continental lesser scaup (*Aythya affinis*) population. Despite the importance of amphipods to wildlife, their habitat requirements are understudied, particularly in wetlands with very high amphipod densities (hundreds to thousands per cubic meter of water). In this study, we are surveying amphipod densities and wetland characteristics (fish communities, plant communities, water chemistry, sediment characteristics, sediment pyrethroid levels, buffer strip extent, and watershed-level agricultural intensity) throughout the Minnesota prairie-pothole and forest-transition zone to improve understanding of habitat characteristics that support amphipod occurrence and abundance. We are also surveying waterfowl during spring migration and the summer brood period to better understand the ties between habitat characteristics, amphipods, and waterfowl use of wetlands. As a second component of the project, we are assessing the efficacy of amphipod stocking as a management technique to establish self-sustaining populations of *Gammarus lacustris* amphipods, using a Before-After/Control-Impact study design. We are simultaneously surveying characteristics of stocked wetlands to assess factors that support successful stocking, and carrying out waterfowl surveys to detect impacts of stocking on waterfowl use of wetlands. This research summary describes project progress through December 2019. We have completed one of two field seasons of assessing wetlands with naturally-occurring amphipod populations. Preliminary findings suggest that amphipod densities may be negatively associated with numbers of benthivorous fish and relative densities of some plant species (carnivorous *Utricularia macrorhiza*), and positively associated with other plant species (some narrow-leaved *Potamogeton* spp, *Myriophyllum sibiricum*, and *Lemna trisulca*) and buffer strip extent. In waterfowl surveys, migratory scaup (*A. affinis* and *A. marila*) occurred primarily in basins with high amphipod densities, but mallard (*Anas platyrhynchos*) breeding pairs and waterfowl broods did not show obvious patterns associated with amphipods. We caution that all results are preliminary, and will be reassessed with formal analysis with a larger sample size following our second field season. In our stocking assessment, we have completed two of three field seasons, stocking 10 wetlands in winter 2017-18 and five wetlands in winter 2018-19. We have not found stocked *G. lacustris* in any wetlands in the fall following stocking, when we assess for persisting population size. However, winter monitoring following stocking in January 2020 indicated that stocked amphipods survived for at least one week post-stocking. *G. lacustris* may be persisting in undetectable numbers and appear in future surveys. After compiling results of a final season of stocking (seven new wetlands), the need for on-going surveys will be assessed after our final field season.

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## INTRODUCTION

Freshwater amphipods, an important component of food webs in prairie pothole wetlands, have declined in recent decades (Anteau and Afton 2006, 2008a, b, Huener 2011). Prairie pothole amphipods (hereafter “amphipods”; primarily *Gammarus lacustris* and *Hyaella azteca*) consume coarse particulate organic matter, algae, and bacteria from the benthos and aquatic macrophytes, and are consumed by amphibians, fish, waterfowl, and other invertebrates (Mathias and Papst 1981, Olenick and Gee 1981, Brown and Fredrickson 1986, Benoy et al. 2002, Strand et al. 2008). Amphipods reach densities of thousands of individuals per square meter in some wetlands (Mathias and Papst 1981, Wen 1992, Pickard and Benke 1996), where they may be a particularly important wildlife food resource. Declines in amphipods have been linked in particular to declines in the continental population of lesser scaup (*Aythya affinis*), which specialize on amphipods as a food resource during migration and breeding (Lindeman and Clark 1999, Anteau and Afton 2004, 2006, 2008a, c, 2009a, 2011, Huener 2011).

Numerous wetland and community characteristics may influence amphipod occurrence and abundance in the Prairie Pothole Region (PPR). Amphipods occur primarily in semi-permanent and permanent wetlands, as they lack a desiccation-resistant winter stage (Murkin and Ross 2000) and have limited dispersal ability. Water chemistry (e.g. temperature, pH) and toxin levels (e.g. pesticides from agricultural run-off) must remain within physiological tolerance limits. Vertebrate and invertebrate predators may impact amphipod occurrence and abundance. Fish in particular may factor in amphipod declines, as fish occurrence in prairie pothole wetlands has increased in recent decades due to a combination of increased wetland connectivity, changing precipitation patterns, and human introduction (Hanson et al. 2005, Anteau and Afton 2008b, McLean et al. 2016). Aquatic macrophytes, on the other hand, may provide cover to escape or hide from predators. Increased turbidity due to presence of bottom-feeding fish or agricultural run-off, along with algae growth promoted by nutrient run-off, may impact amphipods directly via changing water temperature and oxygen content, or indirectly via impacting aquatic vegetation. Landscape level factors, such as the presence of buffer strips - the strip of upland vegetation between the wetland edge and adjacent cultivated crop - may mitigate effects of agriculture on amphipods.

Despite the decline in amphipod populations, their habitat requirements are understudied. To our knowledge, Anteau et al. (2011) is the only prior study to examine the relationship between amphipods and community/habitat characteristics in a large number of prairie pothole wetlands. In a study of 283 PPR wetlands in North Dakota, Minnesota, and Iowa, Anteau et al. (2011) found amphipod density to be related to relative fish density, relative submerged aquatic vegetation density, levels of organic mineral suspended solids (*G. lacustris* only), and width of upland vegetation buffer (*H. azteca* only). Relevant fish groups included fathead minnows, other small fish (*H. azteca* only), large Cypriniformes (*H. azteca* only), and other large fish.

In our study, we aim to further explore habitat characteristics associated with amphipod occurrence and density, including fish and aquatic plant communities, water quality and clarity, and landscape-scale characteristics (buffer width extent, percentage of watershed composed of cropped land cover). Previous studies of similar factors using random-site selection techniques (Anteau and Afton 2008b, Anteau et al. 2011) were limited by the relatively small proportion of basins containing high amphipod densities, with *G. lacustris* and *H. azteca* densities of  $\leq 5 \text{ m}^{-3}$  in 92% and 61% of wetlands surveyed, respectively. To explore habitat characteristics associated with especially high-density basins (which may provide exceptional habitat for migrating waterfowl), we scouted for study sites containing high amphipod densities in the year prior to our study, and surveyed them in addition to randomly selected wetlands. Additionally, we will examine amphipod abundance/occurrence in relation to relative density of submerged aquatic vegetation at the species- to genus-level. Star duckweed (*Lemna trisulca*) and large-leafed

pondweed (*Potamogeton amplifolius*) may particularly support high amphipod population densities (B. Thoele, Lincoln Bait LLC, personal communication). Finally, we will examine influences of pyrethroid pesticide levels on amphipod population densities, along with relationships to agricultural intensity. To further understand the relationship between waterfowl, amphipods, and underlying habitat characteristics, we will also carry out spring migratory waterfowl surveys and summer waterfowl brood surveys at our study wetlands.

A second aim in our study is to assess the efficacy of amphipod stocking as a wildlife management technique for establishing persistent amphipod populations. Conservations groups and private landowners have been stocking *G. lacustris* for over 20 years as a method of providing forage for fish and waterfowl (Mathias and Papst 1981; B. Thoele, personal communication). However, the persistence of amphipods post-stocking has not been evaluated. Some aspects of amphipod biology may support amphipod persistence (e.g. maternal egg/brood care), whereas other aspects (e.g. tendency toward local adaptation) may present challenges (Jourdan et al. 2019). Consequently, we are measuring habitat characteristics (fish and plant communities, water chemistry) in stocked wetlands to understand what factors support stocking success. To document potential influences on waterfowl, we will carry out spring and summer waterfowl surveys at stocked basins in comparison to randomly-selected reference basins.

## OBJECTIVES

1. Identify habitat characteristics associated with amphipod occurrence and abundance in Minnesota prairie-pothole and forest-transition zone wetlands, including fish and plant communities, water quality and clarity, sediment characteristics, sediment pyrethroid levels, and landscape-scale characteristics (buffer width extent, percentage of watershed composed of cropped land cover).
2. Identify associations between waterfowl occurrence/abundance and amphipod occurrence/abundance in spring and summer, to better understand the importance of amphipods to waterfowl.
3. Evaluate the effectiveness of amphipod stocking as a management technique to establish self-sustaining amphipod populations and improve waterfowl habitat.

## METHODS

### **Objectives 1 and 2: Identify habitat characteristics and waterfowl occurrence/abundance associated with amphipod occurrence and abundance**

#### *Study design, study area and site selection*

Our study focuses on wetlands in the prairie-pothole and forest-transition regions of Minnesota, which encompasses approximately the western half of the Minnesota (Figure 1). Study sites were located primarily in four clusters to reduce travel time while incorporating a range of latitudes: near Windom, St. Cloud, Fergus Falls, and Detroit Lakes, MN. All study wetlands were permanent or semi-permanent. We set a 200-acre limit on wetland size for logistical reasons (limiting time spent per wetland to maximize sample size of wetlands).

We surveyed 49 wetlands for amphipod population densities, waterfowl, and wetland characteristics in spring-summer 2019. In spring-summer 2020, we will survey another sample of wetlands. We planned to incorporate another 50 wetlands in the same geographic regions, but are currently making adjustments to our study design to maintain crew safety during the COVID-19 pandemic.

Half of our 2019 sample consisted of wetlands known to have relatively high amphipod densities (hereafter “high density wetland”) and half were randomly selected. We included known high

density wetlands because conclusions in previous studies in the Prairie Pothole region (Anteau and Afton 2008b, Anteau et al. 2011) were limited by the limited number of high-density basins found via random sampling techniques (M. Anteau, personal communication). High density wetlands were identified via preliminary scouting of 105 wetlands in western Minnesota in 2018, guided by knowledge of local land managers, landowners, and aerial imagery. The 2019 field season prioritized sampling of wetlands with the highest amphipod density in each geographic region. For each high density wetland, we randomly selected a Type 4 or 5 wetland (“inland deep freshwater marsh or inland open water”) of similar size ( $\pm$  25% area), within the high density basin’s geographic region, from the National Wetlands Inventory Circular 39 wetlands classification layer (Minnesota Department of Natural Resources, Shaw and Fredine 1956) Geographic regions were defined by creating 15-mile circular buffers around each basin’s centroid, and merging overlapping buffers (Figure 1). Reference wetlands were included to reduce potential influence of subconscious bias in our choice of wetlands and to ensure a range of amphipod densities (including basins without amphipods) for assessment.

### *Surveying amphipods, waterfowl, and wetland characteristics*

We visited basins, working from south to north as much as possible, during each of three sampling periods.

First, as soon as possible after ice-out (April-May), we surveyed for amphipods and waterfowl. Our survey timing was aimed to capture amphipods prior to or shortly after the appearance of visible juveniles (which is followed by mortality of adults) and to survey waterfowl during migration. For waterfowl, our goal was to focus on migrating scaup (*Aythya affinis* and *A. marila*) and mallard (*Anas platyrhynchos*) breeding pairs in early spring because scaup are known to rely on amphipods as a food source, mallards are abundant in Minnesota, and both species are easy to detect. However, because it added minimal extra time in the field, we collected data on all waterfowl. We will analyze their abundance or presence/absence of additional species in relation to amphipod abundance if sample sizes permit.

We surveyed waterfowl immediately upon arrival to the site. When possible, we conducted surveys from the roadside. When necessary, observers walked in towards the wetland (preferably) or surveyed from canoes positioned just inside the ring of emergent vegetation surrounding the wetland. We used the independent double-observer method to collect data (Pagano and Arnold 2009). Two observers simultaneously scanned the wetland with binoculars and a spotting scope from one best vantage point and documented any birds observed for a fixed 10 minutes. Bird species and social group type (Table 1) were recorded in a data table and on a map of the wetland. Observers did not communicate and used various methods to ensure their actions did not provide useful information to the other observer, such as mock data recording, delayed recording, and mock or prolonged use of binoculars. After 10 minutes, observers communicated and recorded which waterfowl groups they both observed and missed, making use of maps, without changing the original data in their data tables. (Preservation of original data will allow for detectability modeling.) Observers also recorded estimated percentage of the wetland visible from their position, precipitation (light or none), estimated cloud cover (<50% or >50%), and air temperature and wind speed measured with a hand-held weather meter (Kestrel 3000).

We sampled amphipods at 8 sample points per wetland (Figure 2). Sample points were placed on 8 evenly spaced transects across the wetland. Transects were oriented north-south or east-west, whichever direction was closest to perpendicular to the longest axis of the basin. On each transect, a sample point was placed either 5 m or 50 m from shore. The position of the first point (northern/eastern-most or western/southern-most transect, 5 m or 50 m from shore, measured from the west/south shore or the north/east shore) was randomly selected. Points were

distributed from there by altering 5 m and 50 m from shore on adjacent transects, and by switching shores every 2 transects. Amphipods were sampled using dip nets (1200 micron mesh) from canoes or sport boats (hereafter 'boats'). At each point, water depth was measured to the nearest 5 cm using depth markings on a dip net handle lowered vertically into the water. If water was too deep for sampling (>120 cm), we moved along the transect gradually toward shore until water was shallow enough for sampling. A benthic sample was taken by lowering a D-frame dip net vertically into the water, sweeping it along the top 1-2 cm of sediment for 0.5 m, and raising it vertically. Water column samples were taken in an undisturbed area (e.g. other side of the boat) with a modified D-frame or round-frame dip net, with the net frame bent perpendicular to the net handle. The net was lowered vertically to a depth 30 cm above the sediment (corresponding to the top of the area sampled by the benthic dip net), shifted horizontally to avoid disturbed water, and raised vertically to collect a sample. Samples were stored in 1-L plastic bottles or whirl-paks with 99% ethanol at a ratio of 30% sample volume to 70% ethanol volume. Differences in net frame area between D-frame and round nets were measured and incorporated into amphipod density calculations.

We sampled wetlands for fish in June-July. Fish abundance and presence was indexed via catch in one gill net (70' length, comprised of seven, 10' panels of different mesh sizes: 0.75", 1", 1.25", 1.5", 1.75", 2", 3") and 3 mini-fyke nets (6.5mm bar mesh with 4 hoops, 1 throat, 7.62 m lead, and a 0.69 m x 0.99 m rectangular frame opening into the trap) set overnight (12-24 hours) The gill net was set along one of the transects used for amphipod surveys (randomly selected), with the smaller mesh end anchored 10 m from shore or the innermost ring of emergent vegetation when relevant. Mini-fyke nets were also set along randomly-selected transects, with the lead secured to shore or the innermost ring of emergent vegetation. Nets were set perpendicular to shore with the bag end anchored off-shore. Following net retrieval, fish were sorted by species, weighed (total mass of each species), and counted. Field crews subsampled nets if the time required to process the fish in the net was estimated to be greater than 30 minutes. In these cases, fish >30 cm were sorted to species, counted, and weighed. Smaller fish (majority of the catch) were grouped together and weighed. A subsample equivalent to  $\geq 20\%$  of the weight was retained and processed (total weight of each species and species-specific counts).

We visited wetlands a third time (July-early August) for aquatic vegetation surveys, waterfowl surveys aimed to incorporate waterfowl broods, and collection of water chemistry data. Timing of this visit was aimed to capture mature vegetation that could be identified to species, in addition to waterfowl broods. For summer waterfowl surveys, we followed the same methods used to survey waterfowl in spring.

We modeled vegetation survey methods after Minnesota DNR Shallow Lakes survey protocols (Perleberg et al. 2019). In brief, we surveyed sample points distributed across each basin according to the point-intercept method (grid of sampling points; Madsen 1999) from boats. We surveyed a minimum of 10 points per basin, with an additional point for every 1-acre increase in size above 10 acres ( $\pm 10\%$ ) up to a maximum of 50 points. At each point, we collected the following five measurements: water depth, relative biomass of floating vegetation, relative biomass of submerged vegetation, and presence/absence of floating and submerged plant taxa. Plants were identified to species-level except in the case of macroalgae (*Chara* spp.), naiads (*Najas* spp.) and some narrow-leaved pondweeds (*Potamogeton* spp.). Water depth was measured using a weighted rope with depth markings. Floating vegetation biomass was indexed visually in a 1-m<sup>2</sup> sample area next to the boat on a 0-to-4 scale (0 = no floating vegetation, 1 = 1-25% area covered with floating vegetation, 2 = 26-50% covered, 3 = 51-75% area covered, 4 = 76-100% area covered), and presence of all floating plant taxa in the sampling area was recorded. Submerged vegetation was sampled by dragging a plant rake 1.5 m along the

wetland bottom alongside the boat. The rake was raised and biomass indexed on a 0-4 scale (0 = no vegetation on rake, 1 = 1- 25% of prong area filled with vegetation, 2 = 26%-50% filled, 3 = 51%-75% filled, 4 = 75-100% filled). All living plant taxa on the rake were recorded. Type of emergent vegetation was recorded for each of the shore-adjacent points in the grid. Field crews recorded the dominant emergent vegetation taxon (covering >50% of the area filled with emergent vegetation) in a 1-m wide swathe between the boat and shore, along the transect created by the gridded sampling points. The boat was moved closer to shore as needed. Emergent vegetation was classified to broad group: cattail, sedge, or rush/bulrush.

We collected sediment samples for analysis of pyrethroid concentrations, total organic carbon, and grain size. We collected wetland sediments following procedures described in Shelton and Capel (1995). We collected sediment samples from a minimum of 5 randomly-generated points within 3 meters from shore (McMurry et al. 2016), adding an additional point for each 20-acre increase in wetland size for basins >50 acres. We collected approximately 192 mLs from the top 5 cm of the bottom substrate at each sampling location using a stainless steel sediment corer, and transported samples to a field processing station in a chilled stainless steel bowl. The samples were processed following the procedures of Radtke (2005). In brief, we homogenized each sample with a clean stainless steel spatula, subsampled it (if necessary), and separated it into two 500-mL amber glass jars: one for pyrethroid analysis, and one for total organic carbon and grain size analysis.

We measured near-surface water temperature and pH (Hach HQ40d multimeter) and water turbidity (Hach 2100P turbidimeter). For chlorophyll-a measurements, we used a 60 mL syringe and Swinnex-sylte filter holder to filter a measured volume of water (60-240 mL) through a glass microfiber filter (Whatman 1822-025 GF/C 25-mm circular filter with 1.2  $\mu\text{m}$  pore size). The filter was shaded from light and wrapped in stored in aluminum foil. We collected approximately 25mL of water into a plastic centrifuge tube for total phosphorous testing. Water samples and chlorophyll filters were transferred back to the lab on ice and frozen until analysis.

#### *Amphipod processing and identification*

For each wetland, we combined the contents of all benthic sample bottles before picking out invertebrates. We picked invertebrates from 25% (volumetric) of the sample at a time until at least 400 invertebrates were obtained, or the entire sample had been picked. We followed the same procedure with water column samples, but water column samples never included >400 invertebrates, and so were always picked entirely.

To process the samples, we filtered ethanol from sample bottles over a 500 $\mu\text{m}$  sieve. We poured bottle contents into a plastic tub, added water until the sample could be thoroughly stirred, poured the homogenized sample onto a subsampling apparatus (rectangular wooden frame containing 500- $\mu\text{m}$  metal mesh), and rinsed it further with water. We floated the sample/mesh in shallow water (below the level of the wooden frame) to aid in dispersing the sample evenly across the mesh with a plastic spoon. To split the sample into 25% portions, we fitted a frame containing outlines of four grid squares to the subsampling apparatus and randomly selected a grid square for processing. Contents of the grid square were transferred to a tray using a plastic spoon and floated in water. The subsample was inspected with a 3x lighted magnifier and all invertebrates removed. Amphipods were stored separately from other invertebrates, and all invertebrates were stored in 70% ethanol.

Amphipods were identified (*Hyalella azteca*, *Gammarus lacustris*, or *Crangonyx* spp.) using a stereomicroscope (Laxco, 6.5-53x magnification) with a digital monitor attachment. We measured amphipod length along a curved line following the dorsal edge of the amphipod, from the base of the first antennae to the base of the telson, using Tcapture software (Tucsen Phototonics Co.) Other invertebrates are being stored for future identification.

### *Wetland buffers and watershed land use*

Wetland buffer strips (also known as filter strips) are defined as the strip of upland vegetation between the wetland edge and adjacent cultivated crops. We assessed buffer strip coverage using remote sensing techniques. Briefly, we used ArcMap tools to construct a buffer zone (30 m width; Sweeney and Newbold 2014) around each wetland edge. Using unsupervised cluster analysis and 2019 NAIP imagery (infrared, 1-m resolution), we then quantified land cover (cropped vs non-cropped) within the 30-m buffer zone. This allowed for the quantification of 1) the proportion of shoreline with a buffer strip  $\geq 30$  m wide, and 2) cultivation disturbance in the immediate upland area.

Land use/land cover characteristics in the upland area surrounding each wetland were evaluated at a watershed scale. We used 1-m resolution digital elevation models and ArcHydro tools to delineate the immediate upland catchment associated with each study wetland; these catchment perimeters were very similar to those corresponding with DNR level 08 automated catchments. We then used the 2016 national land cover dataset, 2017 MDA cropland data layer, and 2019 NAIP imagery to quantify dominant land use/land cover types within the wetland catchment area.

### **Objective 3: Identify habitat characteristics and waterfowl occurrence/abundances associated with amphipod occurrence and abundance**

#### *Experimental design, study area, and site selection*

Objective 3 of our study focuses on evaluating amphipod stocking efficacy in the prairie-pothole and forest-transition regions of Minnesota. We used a before-after/control-impact study design, such that each stocked wetland was paired with a nearby control wetland of similar size and the same management type (MNDNR Wildlife Management Area, US Fish and Wildlife Service Waterfowl Production Area, or privately owned), and we sampled amphipod density in wetlands both before and after stocking. Wetlands were incorporated into the study in pairs, and the choice of which wetland to stock was randomized whenever possible. The decision was made non-randomly when accessibility for stocking (which required a snowmobile) was only possible on one wetland. All wetlands had semi-permanent or permanent hydrology and at least 1 m maximum depth (to avoid amphipod winterkill). Wetland pairs were chosen based on recommendations from managers, private landowner volunteers, and preliminary scouting.

Objective 3 is a three-year study (fall 2017 through fall 2020). We stocked wetlands in each winter of the study: 10 wetlands in winter 2017-18, 5 wetlands in 2018-19, and 7 wetlands in winter 2019-20. Excepting the first year of the study (see below), for each wetland and its associated control wetland, we sampled amphipod density in the fall (August-October) prior to stocking ("before" sample). We stocked wetlands the following winter (see below), and sampled amphipod population density in each fall following stocking through fall 2020 ("after" samples). Thus winter 2017-18 basins will have 3 after-stocking samples, winter 2018-19 basins will have 2 after-stocking samples, and winter 2019-20 basins will have 1 after-stocking sample, by the end of the study. Habitat characteristics (fish communities, water chemistry, and aquatic vegetation) are assumed to be stable throughout the three years, and are only sampled once per wetland in a year after stocking. Spring and summer waterfowl surveys are also only conducted in one year following stocking. In this research summary, we describe results through December 31, 2019.

#### *Amphipod stocking*

We hired a private contractor (Lincoln Bait, LLC) to stock locally collected *G. lacustris*. Amphipods were stocked in winter at the contractor's recommendation for maximum amphipod survival and ease of collection. In winter, *G. lacustris* cluster on the underside of the wetland's

surface ice and begin mate-guarding (precopulatory amplexus). Amphipods were collected using a remote operated, underwater vehicle towing a net beneath the ice surface. Amphipods were held in tanks (<1 week) prior to stocking. On the day of stocking, amphipods were transported to the wetland in water-filled coolers. We stocked amphipods at a rate of 2 gallons/acre of wetland area, based on the contractor's recommendation. Coolers contained 10,871 +/- 4,441 (standard deviation) amphipods per gallon, based on four subsamples. Thus, we stocked at a rate of 6.4 +/- 5.4 amphipods per m<sup>2</sup>.

Coolers were dragged onto the ice on a sled via snowmobile. We stocked amphipods by collecting them in a pitcher and pouring them into holes drilled through the ice. A pump connected to a hose, drawing from a second hole, was used to gently flush amphipods away from the hole after pouring. We divided amphipods among multiple holes: two holes for wetlands <10 acres, with an additional stocking hole added for every 10-acre increase in wetland size. The contractor spread stocking sites across the wetland, but all sites were away from shore (deep enough that water had not frozen to the substrate), and where ice was safe for snowmobile access.

#### *Amphipod sampling*

We sampled amphipods in each stocked wetland, and its associated control, in the fall (August-October) of each year, beginning the year before the wetland was stocked. We sampled amphipods along transects that radiated out from the center of the wetland (Figure 2). Wetlands <10 acres had two transects, and a transect was added for each 10-acre increase in wetland size. The first transect was placed a randomly selected bearing (0-359), and the remaining transects were distributed evenly around the wetland (e.g. two transects 180° apart, three transects 120° apart, etc.) On each transect, amphipods were sampled at a site 5 m from shore and a site 50 m from shore.

Amphipods were sampled using D-frame dip nets (1200 micron mesh) from boats. At each point, water depth was measured to the nearest 5 cm using depth markings on the dip net handle, which was lowered vertically into the water. If water was too deep for sampling (>120cm), we moved along the transect gradually toward shore until water was shallow enough for sampling. We then used the net to sweep horizontally along the top 1-2 cm of sediment for a distance equal to the water depth, and then brought the net to the surface at a 45 degree angle. (That is, we sampled the benthos and water column together.) Samples were stored in 1L plastic bottles or whirl-paks with 99% ethanol at a ratio of 30% sample volume to 70% ethanol volume.

One exception to the sampling procedure was that we did not conduct fall before-stocking sampling in the first year of the study. Amphipods informally sampled in winter, prior to stocking, via drilling an ice augur through the ice and into the water, such that water with amphipods washed onto the ice surface.

#### *Amphipod processing and identification*

Amphipods were processed and identified as in Objectives 1 and 2, with the following exceptions. Rather than combining sample matter from all sampling points in a wetland, each sampling point was processed separately. We picked invertebrates from 25% (volumetric) of the sample at a time until at least 100 invertebrates were obtained, or the entire sample had been picked.

#### *Waterfowl surveys, fish surveys, water chemistry, and aquatic vegetation*

For Objective 3, we will survey waterfowl, fish, water chemistry, and aquatic vegetation at all wetlands following the methods described for Objectives 1 and 2.

## PRELIMINARY RESULTS

### **Objectives 1 and 2: Identify habitat characteristics and waterfowl occurrence/abundances associated with amphipod occurrence and abundance**

#### *Amphipod occurrence/abundance*

We found amphipods in 42 of 49 wetlands surveyed in 2019. As expected based on previous studies in the prairie pothole region, the most common species were *H. azteca* ( $n = 39$  wetlands) and *G. lacustris* ( $n = 25$  wetlands). In all but 3 wetlands where *G. lacustris* were present, *H. azteca* were also present. We also identified a small number of *Crangonyx* amphipods (identified to genus only) in 9 wetlands, where they co-occurred with *G. lacustris*, *H. azteca*, or both. A small number of amphipods (<1%) could not be identified to genus due to damaged or missing body parts.

In basins with amphipods, total densities (summed across species, averaged across depths and sample points) ranged from 1.4 to 2,061.32 amphipods per cubic meter of water, or 1.5 to 2,203.0 amphipods per m<sup>2</sup> of wetland area (Figure 3). For all taxa, most amphipods were found in benthic sweeps (bottom 0.3 m of water), with densities ranging up to 7,140.6 amphipods per cubic meter (all taxa combined). Water column sweep density ranged up to 60% of benthic density (maximum 785.4 amphipods per cubic meter; all taxa combined).

#### *Amphipod relationships to fish*

We found fish in 41 of 49 wetlands surveyed in 2019. A total of 23 species were sampled, with the most common species being fathead minnows (*Pimephales promelas*;  $n = 33$  wetlands), brook stickleback (*Culaea inconstans*;  $n = 25$  wetlands), and black bullheads (*Ameiurus melas*;  $n = 16$  wetlands). In basins with fish, counts ranged from 6 to 2,586 fish sampled and biomass ranged from 14 g to 35,550 g. Probability of *G. lacustris* occurrence decreased significantly with increasing fish biomass (Figure 4;  $\chi^2 = 4.70$ ,  $df = 48$ ,  $p = 0.03$ ). However, preliminary linear regression models assessing fish abundance or biomass as an explanatory variable for *G. lacustris* or *H. azteca* abundance were not significant ( $p > 0.05$ ). Analyzing fish by primary feeding characteristic with redundancy analysis revealed significant, negative associations of amphipod community to benthivores, but not with planktivores or piscivores ( $F=6.46$ ,  $df = (1,45)$ ,  $p < 0.01$ ). Results will be reassessed with a larger sample size of wetlands following the 2020 field season.

#### *Amphipod relationships to aquatic vegetation*

Emergent vegetation data have not yet been analyzed. Preliminary analysis of submerged and floating aquatic vegetation indicated that *H. azteca* densities were positively correlated to submerged plant prevalence. However, *G. lacustris* were not associated with submerged vegetation prevalence, relative biomass, or species richness. The amphipod community was significantly predicted by the vegetation community according to redundancy analysis. Benthic counts of amphipods (all species together) were positively associated with relative density of narrow-leaved pondweeds (*Potamogeton* spp.) and northern watermilfoil (*Myriophyllum sibiricum*). *G. lacustris* counts were positively associated with star duckweed (*Lemna trisulca*). Amphipoda were negatively associated with common bladderwort (*Utricularia macrorhiza*), a carnivorous plant.

#### *Amphipod relationships to water chemistry*

Preliminary analyses evaluating relationships between water chemistry and amphipod abundance did not reveal distinct trends; overall, both species of amphipods appeared to exist at a wide range of densities across a wide gradient of turbidities, nutrient concentrations, and

pH levels. Data will be reanalyzed with a larger sample size of basins following the 2020 field season.

#### *Amphipod relationships to sediment characteristics and pyrethroids*

Analysis of sediment characteristics and pyrethroid levels are on-going in BSU labs, following delays due to COVID-19.

#### *Amphipod relationships to buffer widths and watershed scale land use*

Preliminary landscape-level results showed that *H. azteca* were positively associated with upland buffer strip coverage (Figure 5), whereas neither *H. azteca* nor *G. lacustris* exhibited a strong relationship with watershed-scale land use/land cover (Figure 6). We will continue examining these trends with a larger sample size of basins following the 2020 field season.

#### *Waterfowl relationships to amphipods*

In April-May 2019, we carried out spring waterfowl surveys at 48 of the 49 basins sampled for amphipods. Waterfowl were not detected at 5 basins. We counted 250 waterfowl (duck, goose, or swan) social groups (e.g. pairs, lone individuals, flocked males, groups), including 1830 adults and 191 indicated breeding pairs in the other 43 basins. Canada geese and mallards were the most common species (occurred on the most basins). Scaup occurred in 10 basins, primarily with high amphipod densities (Figure 7). Mallards occurred on 26 basins, but did not show an obvious trend in occurrence or abundance related to amphipods (Figure 7).

In July-August, we carried out surveys at all 49 basins. We observed waterfowl in 36 basins, including 23 basins with waterfowl broods. Mallards and “unknowns” (observers could not identify to species) were the most common species of adults and broods in summer. Number of broods did not show an obvious trend in occurrence or abundance related to amphipods (Figure 7).

Spring and summer survey outcomes will be reassessed with larger sample size following the 2020 field season.

### **Objectives 3: Evaluate the effectiveness of amphipod stocking as a management technique to establish self-sustaining amphipod populations and improve waterfowl habitat**

As of December 2019, we had stocked 15 wetlands with *G. lacustris*. At two sites, 2-3 stocked basins were associated with the same control basin, such that we had 12 control basins. Objective 3 amphipod counts are currently being incorporated into our project database for analysis. *G. lacustris* were not found in any basins prior to stocking, with the exception of one site (Old Red Lake Trail Wildlife Management Area) stocked in the first year of the study. *H. azteca* were found in some basins, and their densities will be analyzed in the future. However, *G. lacustris* have not been found in any basins post-stocking, with the exception of Old Red Lake Trail WMA.

Spring waterfowl surveys were conducted at all 27 basins in spring 2019, and fish surveys were conducted at 12 basins. These data remain to be analyzed.

An additional seven wetlands were stocked with amphipods in winter 2019-20. We will conduct spring waterfowl surveys at these basins, aquatic vegetation and summer waterfowl surveys at all basins, and fish surveys at all remaining basins, in spring-summer 2019-20.

## DISCUSSION

### **Objectives 1 and 2: Identify habitat characteristics and waterfowl occurrence/abundances associated with amphipod occurrence and abundance**

Our preliminary data suggest that our method of including of preliminarily-scouted basins will allow us to assess relationships between habitat characteristics and amphipod density in high-density basins. 53% of our 2019 study wetlands contained amphipods at densities greater than 100 individuals per cubic meter.

Preliminary results suggest that fish generally have a negative relationship with amphipod density, whereas plants have a positive relationship. Our findings that *G. lacustris* are less likely to occur in wetlands with higher fish biomass, and that benthivorous fish counts are negatively associated with amphipod counts, align with Anteau et al.'s (2011) findings that fish at high densities are negatively correlated with amphipod densities. Benthivores may consume amphipods directly, or affect amphipods indirectly by stirring up wetland turbidity (Anteau et al. 2011). While previous studies have documented a positive relationship between relative density of submerged aquatic vegetation and amphipods, our preliminary data suggest that particular species, including northern watermilfoil, narrow-leaved pondweeds, and star duckweed may play a role in supporting high amphipod populations. On the other hand, negative correlations with common bladderwort may indicate that this carnivorous plant depresses amphipod populations via predation. Interestingly, our preliminary finding that *H. azteca*, but not *G. lacustris*, densities are related to buffer strip coverage matches Anteau et al.'s (2011) results. This may indicate differing tolerances for pesticide or sediment run-off between the two species, or that *G. lacustris* simply don't occur in landscapes where agriculture is intense enough that upland vegetation has much impact on water quality. However, we caution that all these data are highly preliminary, and will be reassessed with an increased sample size after a second field season.

In waterfowl surveys, scaup appeared to occur primarily in basins with high amphipod densities (>400 amphipods per cubic meter in benthic samples). These preliminary results concur with earlier studies finding a positive relationship between scaup occurrence and amphipod density during migration and breeding (Lindeman and Clark 1999, Anteau and Afton 2009b) However, with a such a small sample size of basins with scaup ( $n = 10$ ), at least one additional field season is needed to establish whether this pattern is consistent. Water column amphipod densities will be incorporated into density values for comparison to Anteau and Afton's (2009b) finding that probability of scaup foraging peaks at 26 amphipods per cubic meter.

### **Objectives 3: Evaluate the effectiveness of amphipod stocking as a management technique to establish self-sustaining amphipod populations and improve waterfowl habitat**

Stocked *G. lacustris* have not been detected post-stocking in any of our wetlands. It is possible that stocked amphipods have 100% mortality within the year due to predation, other aspects of habitat quality, or stress associated with translocation. However, monitoring of newly stocked wetlands via underwater camera in January 2020 indicates that stocked amphipods remain alive under the ice in high numbers for at least 7 days following stocking. Amphipods may be persisting in basins at low, undetectable levels and appear in future years. Monitoring of stocked wetlands will continue for at least another year. Additionally, habitat characteristics in stocked wetlands will be compared to wetlands where *G. lacustris* naturally occur (Objective 1) to assess potential reasons for stocking challenges.

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Table 1. Social groups counted by observers during spring and summer waterfowl surveys in Minnesota in 2019.

Social group	Description
Lone male	Single isolated male without visible associated female
Lone female	Single isolated female without visible associated male
Lone unknown	Single isolated bird of unknown sex. For sexually monomorphic species and lone birds where species is unknown.
Pair	One male and one female of the same species in close association
Flocked males	2-4 male individuals of same species in close association
Group	Any other group of adults <sup>1</sup>
Brood hen with brood	Brood associated with an adult female
Brood	Brood without associated adult
Pair with brood	Two adults closely associated with brood. Only for geese and swans.
Lone Unknown with Brood	One adult with a brood. Only for geese and swans (sexually monomorphic).
Unknown	Group cannot be classified because species or sex cannot be identified

<sup>1</sup>One female associated with two males is considered a pair and a lone male.

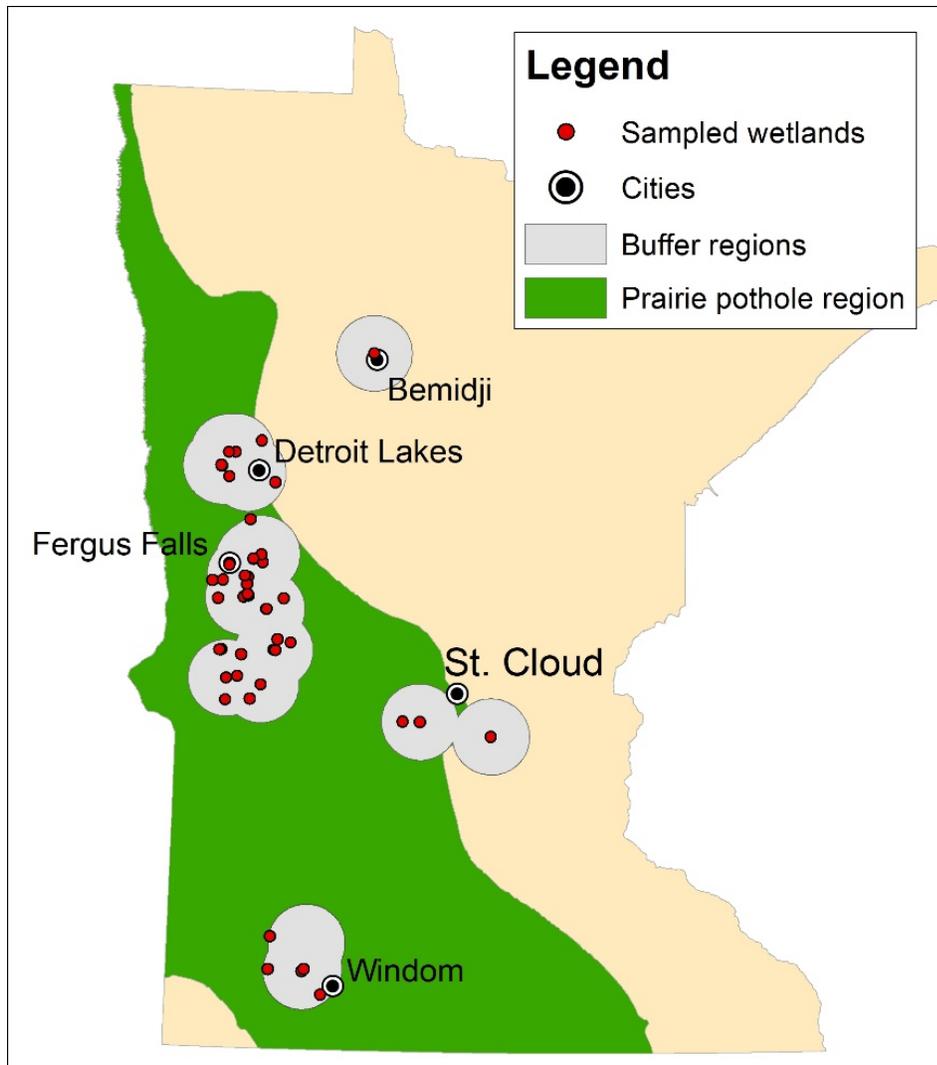


Figure 1. Prairie pothole and forest-transition zone wetlands sampled for amphipods, waterfowl, and habitat characteristics (fish communities, aquatic vegetation, soil and water properties) in 2019. Wetlands were primarily distributed in four sampling clusters near Windom, St. Cloud, Fergus Falls, and Detroit Lakes, MN. Study sites included wetlands of known high amphipod density and randomly selected wetlands. Randomly selected wetlands were chosen from merged 15-mile circular buffer zones around high density basins in each sampling cluster.

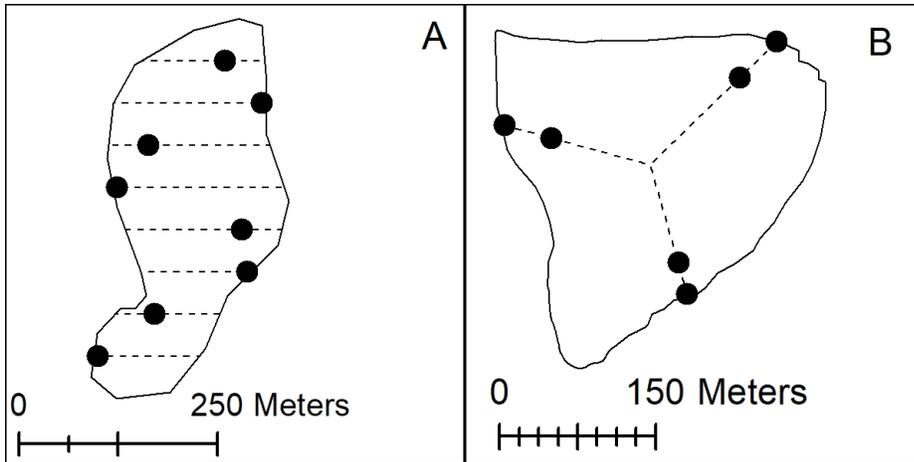


Figure 2. Amphipod dip-net sampling point arrangement for (A) Objectives 1 and 2, (B) Objective 3. Solid lines represent sample wetland outlines, dashed lines represent transects, and dots represent survey points.

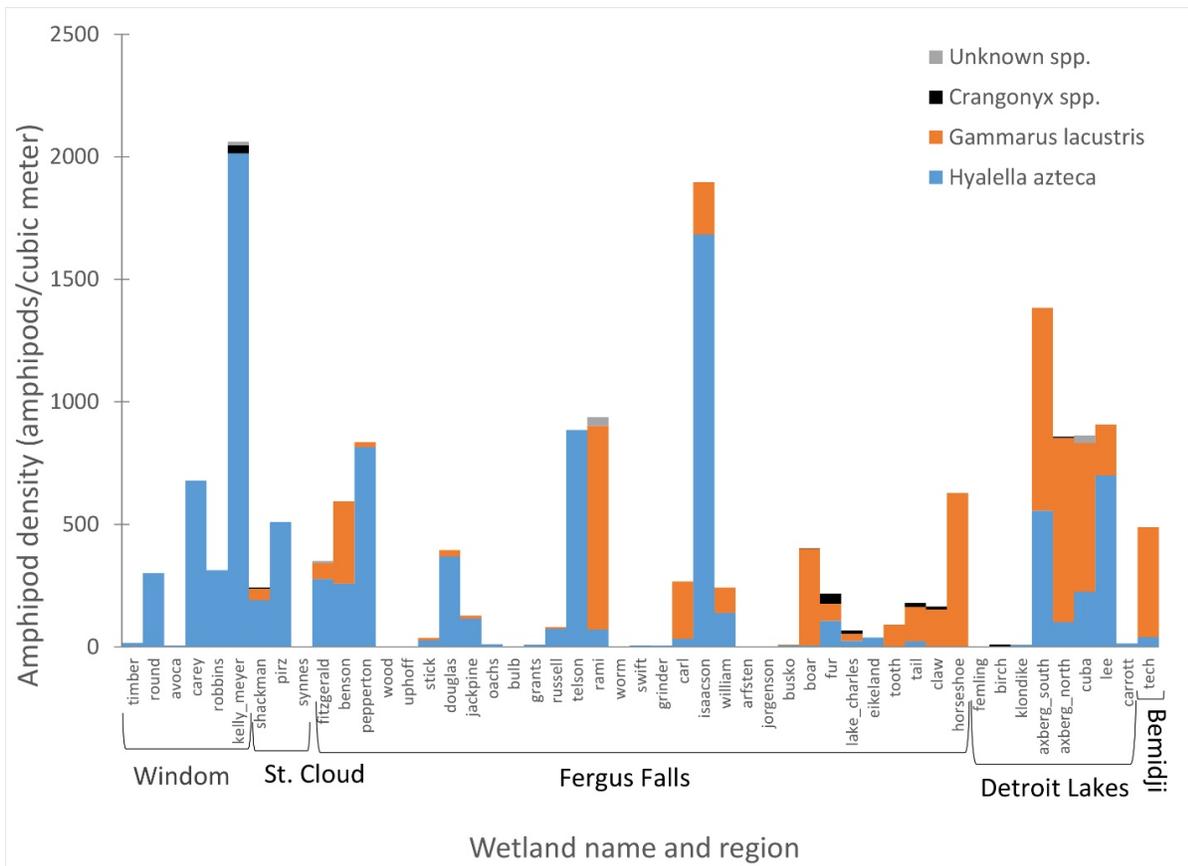


Figure 3. Amphipod densities by species (spp.) in prairie pothole and forest-transition zone wetlands in western Minnesota in spring 2019. Densities are calculated from eight benthic and eight water column dip net sweeps per wetland, accounting for water column depth. Wetlands were in geographic clusters named for nearby cities (Windom, St. Cloud, Fergus Falls, Detroit Lakes, Bemidji) and are ordered from lowest to highest latitude (left to right) on the x-axis.

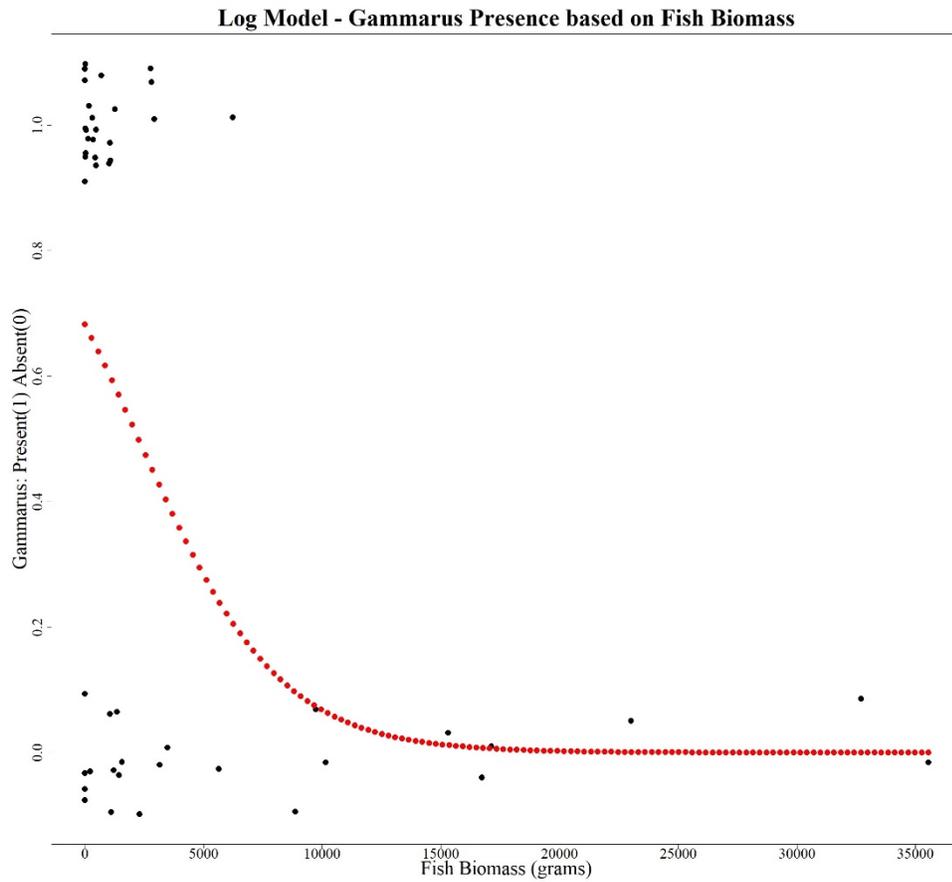


Figure 4. Relationship between probability of *G. lacustris* occurrence and total fish biomass in prairie pothole and forest transition-zone wetlands sampled for fish and amphipods in Minnesota in 2019. Red dots represent the best-fit logistic regression line. Probability of *G. lacustris* occurrence decreased significantly with increasing fish biomass ( $\chi^2(48) = 4.70, p = 0.03$ ).

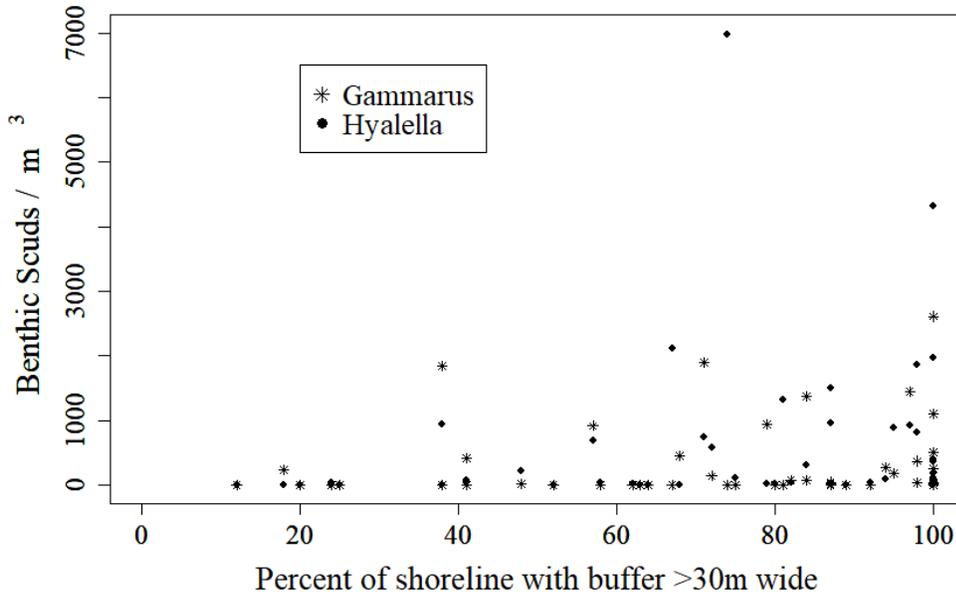


Figure 5. Relationship between benthic-zone amphipod density and percent of shoreline length with a buffer strip at least 30 m wide in 49 prairie pothole and forest-transition zone wetlands sampled in Minnesota in 2019.

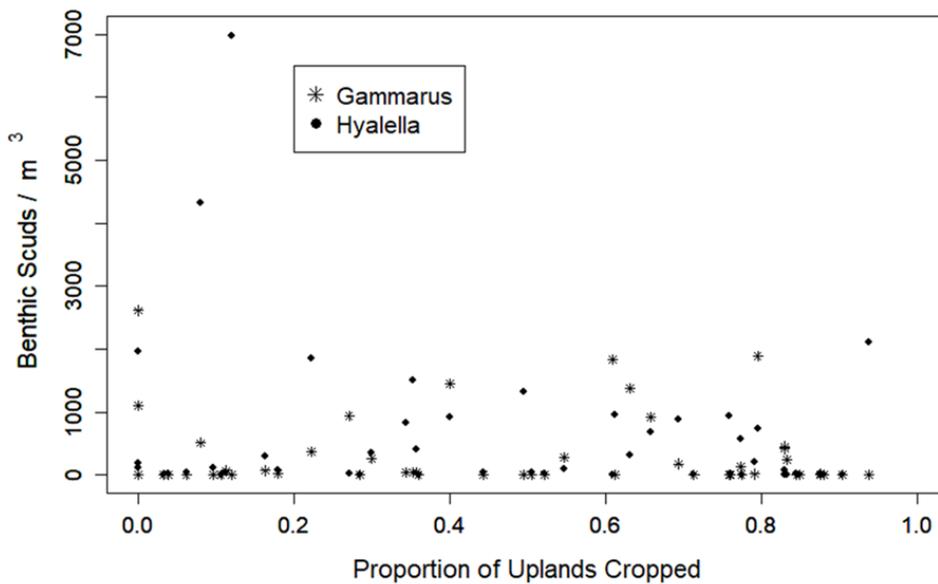


Figure 6. Relationship between benthic-zone amphipod density and proportion of immediate upland catchment with cropped landcover in 49 Minnesota prairie pothole and forest-transition zone wetlands sampled in 2019.

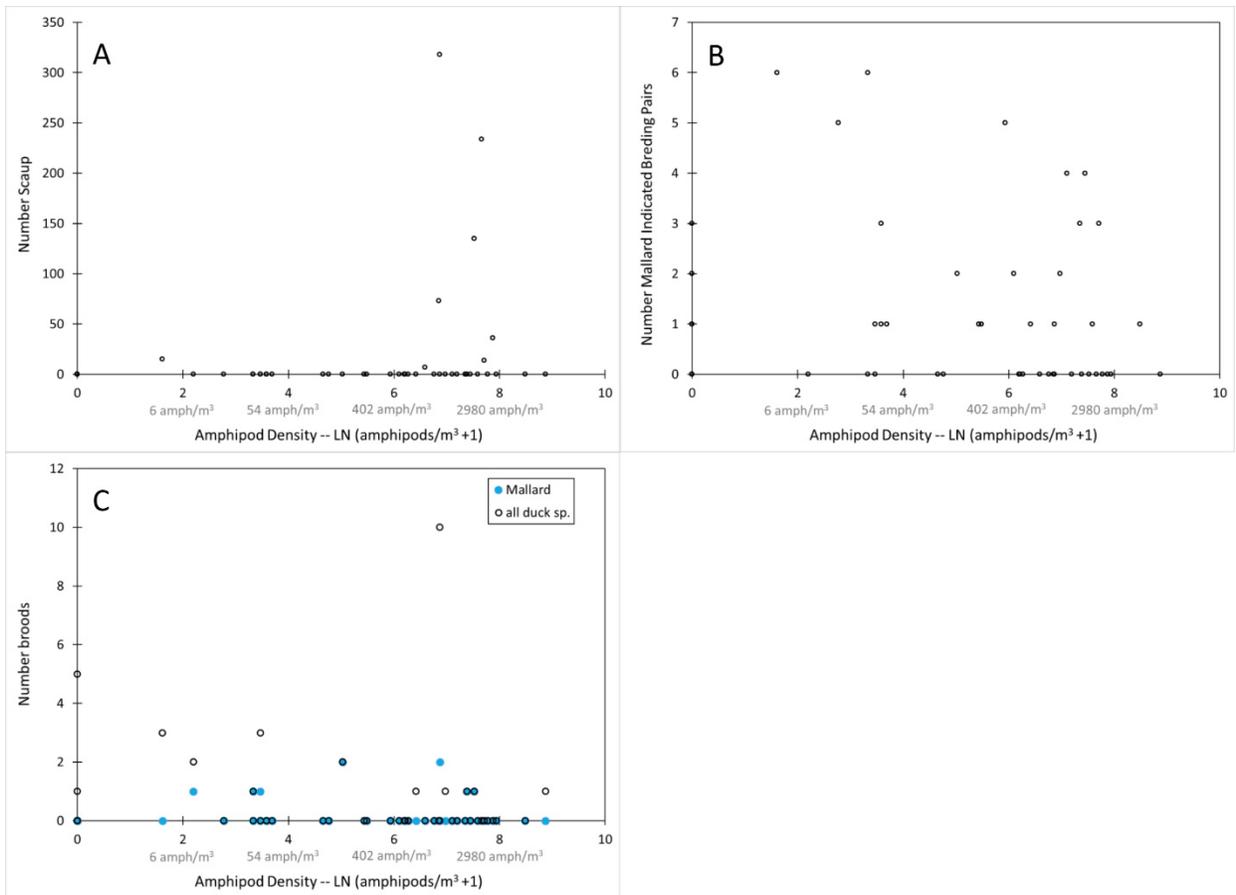


Figure 7. Number of (A) adult scaup (*Aythya affinis* and *A. marila*) in spring, (B) mallard (*Anas platyrhynchos*) indicated breeding pairs in spring, and (C) number of summer waterfowl broods observed on prairie pothole and forest-transition zone wetlands of varying benthic-zone amphipod density in Minnesota in spring 2019. Amphipod densities are transformed to a log scale for visual clarity.



## WATERFOWL BANDING PROJECTS SUMMARY

Ciara R. McCarty, Bruce E. Davis, and Brian J. Hiller

### SUMMARY OF FINDINGS

During July-September 2019 we completed successful field seasons for 3 ongoing waterfowl banding projects. During these projects, we marked birds with aluminum metal leg bands provided by the United States Geological Survey. These marked birds will contribute to the long-term data set used to estimate waterfowl survival, harvest rates, and demographic distribution of harvest in Minnesota and at a continental scale. During the traditional summer banding project (Federal Aid Project 2W475), we were able to mark 1,189 ducks of 14 species with aluminum leg bands during this effort. During our preseason banding project (Federal Aid Project 2W472), we were able to mark an additional 467 ducks of 4 species. Additionally, during our south and central Minnesota expanded banding project (Federal Aid Project 2W466), we were able to mark 2,063 ducks of 6 species.

During 2019, in our summer banding project, 37% of the birds we banded were mallards (*Anas platyrhynchos*) and 23% of birds marked were ring-necked ducks (*Aythya collaris*). For the pre-season banding project, 83% of banded birds were mallards and 15% were wood ducks (*Aix sponsa*). For our south and central Minnesota expanded banding project, 76% of banded birds were wood ducks and 16% were blue-winged teal (*Spatula discors*).

The data produced via Minnesota Department of Natural Resources (MNDNR) banding efforts and subsequent hunter reports of harvested bands contribute to a long-term data set that allows for calculation of demographic parameters important to wildlife managers. Due to reliance on reported harvest of banded birds and structure of survival models, harvest information is available the summer after birds are marked when harvest is complete and records have been entered; survival estimates require 2 or more years of reporting post-banding. Band returns are also used to estimate several other important parameters for ducks, including but not limited to estimates of harvest distribution and identification of patterns in movements.

Currently, analyses are being conducted using band and recovery data for wood ducks marked in Minnesota from 1997-2017 through these ongoing banding project efforts. Information from two chapters of an in-progress Master's Thesis are included in this report; this thesis is unfinished, but substantial progress in analyses have been made and are shared here. These analyses should be regarded as preliminary until the thesis is finalized. This research is being conducted through the Wetland Wildlife Research Populations Group of MNDNR and Bemidji State University. Band and recovery data are being used to understand factors affecting harvest distribution for wood ducks banded in Minnesota and to estimate population size and understand trends over time for wood duck populations in the Mississippi Flyway.

Using linear mixed effects models, we analyzed the effects of age, sex, banding zone, and time to explain changes in distance moved from banding location to recovery location in order to better understand harvest distribution of wood ducks banded in Minnesota. We found that age of bird, time, and the zone of marking were all factors affecting the harvest distribution of wood ducks marked in Minnesota. Preliminary Lincoln estimates for the Mississippi Flyway ranged

from 4,796,061 to 7,581,376; further analyses will be needed to better understand potential trends in this data.

## INTRODUCTION

Wood ducks are a cavity nesting waterfowl species indigenous to North America with an estimated breeding population of three million birds within the Atlantic, Mississippi, and Pacific flyways (Baldassarre 2014). Following a recovery from a large scale population decline in the early 1900s wood ducks are now the second most harvested duck in Minnesota and most of the Mississippi and Atlantic flyways (Bellrose and Holm 1994; Baldassarre 2014; Fronczak 2017). Wood duck breeding distribution differs from, most other waterfowl species because wood ducks breed throughout most of their habitat range (Baldassarre 2014). Wood ducks provide recreational hunting opportunities and waterfowl hunters provide funding for conservation through the purchase of hunting licenses, state and federal habitat stamps, and taxes on hunting related items. Little is known about movement dynamics of wood ducks in Minnesota which motivates this study.

Harvest distribution describes where birds from a specific production area are harvested (De Sobrino et al. 2017). Harvest distribution has helped define boundaries for populations used in the development of adaptive harvest management (AHM) protocols (U. S. Fish and Wildlife Service 1999) (Munro and Kimball 1982). Szymanski and Dubovsky (2013) described harvest distribution patterns for blue-winged teal that helped identify effectiveness of the Conservation Reserve Program and subsequent improvements to production throughout North Dakota and South Dakota (De Sobrino et al. 2017). Harvest distribution analyses have been used in the Pacific Flyway to describe production areas necessary for maintenance of sustainable waterfowl harvest (De Sobrino et al. 2017).

Dependable knowledge of patterns between production and harvest areas is beneficial for management of migratory waterfowl (Osnas et al. 2014). Descriptions of harvest distribution illuminate where birds move, and the factors affecting those movements. Better understanding areas utilized by wood ducks from a defined population enhances the efficiency of habitat conservation efforts and hunting regulations, by providing insight to where funding may best benefit conservation efforts. Some wood ducks inhabit the majority of the species range year round (Baldassarre 2014); this makes distinguishing harvest distributions difficult for local populations.

Habitat and hunting regulations are typically managed on a state scale (within a federal framework) (De Sobrino et al. 2017), but wood ducks have potential to move up and down the flyway throughout the year. Effective management regulations require that managers understand how harvest outside of the state might affect local populations and how local populations are affected by other state's harvest and regulations.

There is limited population data for wood ducks (Shirkey and Gates 2020). Quantitative estimates of population sizes are important for managing wood ducks effectively. Knowledge and understanding of trajectories in population trends will help us better understand wood duck populations. Starting in 1955, annual abundance of North American ducks have been estimated using the Waterfowl Breeding Population and Habitat Survey (Alisauskas et al. 2014). These surveys are conducted using airplanes, helicopters, and ground crews. Traditional population estimates for waterfowl rely on detection from observers, typically from an airplane, but this method is ineffective for wood ducks due to lack of observability in their preferred habitat (Alisauskas et al. 2014). Within the area sampled, birds can be undetected due to observer fatigue or experience, weather conditions, or use of habitats that obstruct view from the air, generally referred to as "visibility bias" (Pollock and Kendall 1987). A common problem resulting from visibility bias is an underestimation of population size (Pollock and Kendall 1987).

Minnesota lies near the northern edge of the habitat range for wood ducks, within the North American Mississippi flyway (Baldassarre 2014). The Mississippi flyway includes Minnesota, Wisconsin, Michigan, Iowa, Illinois, Indiana, Ohio, Missouri, Kentucky, Tennessee, Arkansas, Alabama, Mississippi, and Louisiana. The provinces of Manitoba, Ontario, and Nunavut are also included in the flyway. Wood ducks typical breeding range within the Mississippi flyway is from central Manitoba and southern Ontario all the way down to the Gulf of Mexico (Baldassarre 2014). In fall and winter, at middle and southern latitudes, substantial mixture of breeding populations and populations of more northerly breeding populations that must migrate south to escape harsh winter conditions occurs; Wood ducks are present year round from southern Minnesota along the Mississippi River down to the Gulf of Mexico (Baldassarre 2014).

## **OBJECTIVES**

1. Identify factors affecting harvest distribution for wood ducks banded in Minnesota using waterfowl band and recovery data.
2. Estimate population sizes for wood ducks within the Mississippi Flyway and describe any trends in population estimates for the Mississippi Flyway wood duck populations from 1999-2017.

## **METHODS**

### **Band and Recovery Data Query – Distribution of Harvest**

We obtained wood duck banding and recovery records from the United States Geological Survey (USGS) Bird Banding Laboratory, in Laurel, Maryland (USGS, Gamebirds data set). Recovery records for bands placed on normal wild-caught birds; including birds caught by night lighting or control band birds in a reward study were included. We excluded birds marked with auxiliary markers (i.e., nasal tags, patagial tags) because auxiliary-marked birds often have higher reporting rates (Arnold et al. 2016). Birds marked in Minnesota during the preseason banding period (1 July- 31 September) (Anderson and Henny 1972; Green and Kremetz 2008) were selected to target birds being produced or producing offspring in the state. We selected direct band recoveries, (birds that were harvested in the hunting season immediately following their banding). Ducks banded as locals (i.e., flightless young of the year) and hatch year (flight-capable young of the year) were combined into a juvenile category (De Sobrino et al. 2017). We used only records with all known variables including age, sex, and recovery date. Single federal bands with a toll free or web address band were the only bands used to keep reporting methods and rates consistent.

Minnesota started marking wood ducks with toll free bands in 1997, and data were available through the 2017 hunting season when these analyses were initiated, so we restricted analysis to these years. Banding sites are distributed across the state (Figure 1). Our query produced a total of 2,411 harvest records (Figure 2). A large number of birds harvested at or near their banding location caused a bimodal zero rich data set (Figure 3). This distribution presents challenges for examining harvest distribution data.

### **Band and Recovery Data Query – Population Estimation**

We obtained wood duck banding and recovery records from the United States Geological Survey (USGS) Bird Banding Laboratory (BBL), in Laurel, Maryland (USGS, Gamebirds data set) as outlined above and additionally included banding and recovery records for birds marked and/or harvested in the Mississippi Flyway. Birds marked anywhere during the preseason banding period (1 July - 31 September) (Anderson and Henny 1972; Green and Kremetz 2008) and harvested within the Mississippi Flyway were included. We included only direct band recoveries (birds that were harvested in the hunting season immediately following their banding) in this data set.

## **Harvest data - Population Estimation**

Starting in 1999 with a new survey, the United States Fish and Wildlife Service reports number of waterfowl harvested (Fronzak 2019). Data collected from hunter surveys (Harvest Information Program) and parts collection surveys are used to estimate the number of birds harvested. Based on the data structure the harvest estimated can be split into geographic areas and species each year. These are the estimates we used for our Lincoln estimator calculations for the Mississippi Flyway.

## **Preliminary Examination of Data – Distribution of Harvest**

Examination of the data set reveals a few strong patterns that may help us understand the distribution of data. There is an apparent positive relationship between number of days from the start of the hunting season and distance traveled from banding location (Figure 4). There is a break in the data around 1000 km, where there are relatively few data points; middle latitude states, such as Iowa, Missouri, Illinois, Indiana, and Kentucky, have very few recovery points. Early season movements appear to be in random directions, but movements later in the season appear to be approximately straight south (Figure 5). We classified movements from banding sites to these early recovery points “local movements” because they are short distances from banding location to recovery location and in random directions. We classified movements from banding sites to the more distant recovery points “major movements” because they represent greater distances of recovery and appear to be movements towards wintering grounds. Nearly 41% of reported birds were harvested within 50 km from banding location. To better understand potential broad scale movements past mid-latitude, we present a strip chart (Figure 6). For this chart, recoveries were divided into three zones based on the recovery point latitude. Northern recoveries occurred early, and south zone recoveries occurred late; recoveries at the middle latitudes occurred throughout the season. For subsequent analyses, data were divided into harvest locations within the state of Minnesota and harvest locations that are outside of Minnesota (Figures 7 and 8). This produced an out of state data set with 1,312 recovery points. ArcGIS (10.6.1) and Program R (64 3.6.0) were used to calculate values for analyses and examine models.

## **Modeling- Distribution of Harvest**

Distance traveled from banding location to recovery location (in kilometers), the change in latitude, and the change in longitude from banding location to recovery location were used as dependent variables in our analyses of harvest distribution.

For our independent variables, we classified banding zones within Minnesota based on the state’s current hunting zones (North, Central, or South; Figure 9), we calculated the number of days from hunting season start (hereafter, time), we used age determined at time of banding (adult or juvenile), and sex determined at banding (male or female) as fixed effect variables in the models (Bates et al. 2015). We also included year as a random effect variable in our models to further explain variation in the data (Bates et al. 2015).

## **Analyses – Distribution of Harvest**

Three separate analyses using mixed effects regression models were constructed using distance, change in latitude from banding location to recovery location, and change in longitude from banding location to recovery location as a dependent variables. Together these three metrics of spatial movement will help describe what factors are the most important to harvest distribution. By using the same independent variables in each analysis, we tested what factors affect harvest distribution. As discussed above, wood ducks seemingly travel south when making major movements.

The global models contain an age and sex interaction term and time and zone interaction term. We tested for the interaction of age and sex because adult males molt earlier and have the ability to migrate earliest, juveniles have to fully develop in order to make large scale movements, and females wait to molt until their young fledge (Bellrose and Holm 1994). Figure 4 shows a strong relationship between time and distance traveled. Wood ducks are early migrants (Bellrose and Holm 1994) so we hypothesized an interaction between time and distance. We added banding zones into the model to test for differences in migration for birds marked at different latitudes.

### **Tested models – Distribution of Harvest**

We constructed a global model based on what we know about wood duck biology and movements, and we suspect that age and sex will be important when comparing movements as well as the zone of marking and time. A year effect was included to account for annual variability, but it does not work towards answering our question of what affects harvest distribution, so it was included as a random effect variable.

Global models were tested against null models, models containing only wood duck based factors, and models without interaction terms as well. Model selection was based on Akaike's Information Criterion (AIC) scores. Models within 2 AIC units of the top model were considered to be competitive (Arnold 2010). Models that were more parsimonious (containing the fewest parameters) were considered more competitive if AIC scores were within 2 AIC units (Burnham and Anderson 2002). For the 3 analyses of movements we tested several models (Tables 2, 3, and 4).

### **Analysis – Population Estimation**

Band reporting probability (reporting rate) is the probability that someone who comes in contact with a banded bird will report that bird to the BBL (Garrettson et al. 2014). If all recovered bands were reported, the reporting rate would be one. Garrettson et al. (2014) found that birds banded with a toll free number on their band had a 0.73 probability of being reported to the BBL, conditional on being found. We treated this reporting rate (0.73) as a constant throughout our data set.

We calculated fall flight estimates for the Mississippi Flyway using Lincoln (1930) estimator equations. Fall flight estimates (N) were calculated by taking the reported harvest estimate (E) for the flyway for a given year (Fronzak 2019) and multiplying it by the reporting rate of 0.73 (R) (Garrettson et al. 2014) and by the number of wood ducks marked and released (M) in the Flyway or that state for the given year. That term is divided by the number of bands harvested and reported (H) to the Bird Banding Laboratory. The estimator equation is:

$$N = \frac{E * R * M}{H}$$

### **Modeling – Population Estimation**

Once we calculated fall flight estimates, we graphed them and added a linear trend line through them. The linear trend line is a model of population against year. This model will show if there is a trend over time, but because year is the only variable in the model it does not account for a high amount of variation in the data (low R squared values).

## **RESULTS**

### **Distribution of Harvest - In State Recoveries**

Of the 2,411 records the recoveries were in 25 different states spread over 3 different flyways (Table 6). About 46% of birds marked and recovered were harvested in Minnesota and 90% of

recoveries occurred in the Mississippi flyway (Table 6). The high proportion of recoveries within state lines is important to note because it gives an idea of the effect Minnesota hunters have on the local population of wood ducks in the state due to harvest mortality.

#### **Distribution of Harvest - Distance for out of State Recoveries**

The best fit model describing factors that affect distance from banding location to recovery location was the global model (Table 2). On average, adult wood ducks travel 151 km further than juvenile birds. Male wood ducks travel 143 km more than female wood ducks, on average. Birds marked further north travel further than birds marked south of them but end up in the same wintering locations (Figure 10 and Figure 11). As the season progresses, birds are harvested at greater distances from their banding location.

#### **Distribution of Harvest - Change in Latitude for out of State Recoveries**

The best fit model describing factors that affect the change in latitude from banding location to recovery location was the model containing an additive relationship of zone and days (Table 3). No models were within 2 AIC values of the top model so no other factors are considered for describing factors affecting change in latitude. There is a direct relationship amongst days and change in latitude (Figure 12). As the season progresses the recoveries are reported further from the banding location. Birds marked further north have a larger change in latitude compared to birds marked further south (Figure 13).

#### **Distribution of Harvest - Change in Longitude for out of State Recoveries**

The best fit model describing factors that affect the change in longitude from banding location to recovery location was the model containing an additive relationship of age and zone (Table 4). No other models were within 2 AIC values of the top model. Adult wood ducks on average are being harvested 1.8972 degrees further east than juvenile birds (Figure 14). On average, birds marked in the southern zone (-2.6321 degrees) are harvested at two degrees further west than birds marked in the central (-4.475 degrees) or northern (-4.954 degrees) banding zones (Figure 15).

#### **Population Estimation**

Lincoln estimates for the Mississippi Flyway ranged from 4,796,061 in 2017 to 7,581,376 in 2011 (Figure 16). We failed to detect a significant trend through the data ( $P= 0.462$ ) using a basic linear model testing population size over time in Program R (64 3.6.0) base stats package (R core team, 2017). Since the R-squared value for the population versus time trend was 0.03217, we conclude that there is no linear trend present in this data for the effect of year.

## **DISCUSSION**

#### **Distribution of Harvest**

Nearly half of the harvest distribution for Minnesota marked wood ducks occurs in Minnesota (Table 6). Wood ducks harvested out of the state are reported at long distances almost straight south at their wintering grounds centered on Louisiana. This is interesting because it is known that there are overwintering wood ducks in the mid latitude states that they are “jumping” (Baldassarre 2014).

Age, sex, zone, and time are all indicated as important factors that affect the distance at which wood ducks are harvested from banding location. Adult birds are being harvested at greater distances from banding location to recovery location than juvenile birds, potentially due to a lower susceptibility to harvest (Bellrose and Holm 1994). Males molt earlier in the season than females, due to the incubating and brooding activities of females (Bellrose and Holm 1994). This may be why sex is indicated as an important factor in the model for distance. Zone of

marking can be explained (Figure 11) because the birds from each of the zones all seem to be ending up in or near Louisiana (presumably their wintering grounds). There is no apparent separation of harvest among different marking zones, the primary difference among marking zones is that the birds marked further north are traveling further to get to the wintering grounds.

Zone and days are indicated as the two primary factors affecting change in latitude between marking and recovery locations for wood ducks marked in Minnesota. Similar to the reasons explaining distance can explain these trends for the change in latitude, because as discussed about when the birds initiate major movements they are doing so at a bearing of nearly 180 degrees (straight south). Age and zone are the two indicated factors of importance for change in longitude. The explanation for age and zone affecting the change in longitude may be effects of sampling distribution. The difference in longitude of the north and central banding locations compared to the southernmost banding zone locations is roughly two degrees, the average difference that the model indicates (Figure 14 and Table 5).

### **Population Estimation**

Wood ducks breed, and spend considerable time in forested wetlands, making them difficult to detect during traditional survey techniques. There are not reported population estimates for each state, because of this. Population estimates for fall flight were calculated using Lincoln's (1930) method for estimation of population size. These estimates tend to be higher than more traditional survey estimates (Alisauskas et al. 2014) but depict population sizes at the beginning of the hunting season in a way that eliminates the visibility bias problem for wood ducks. Factors other than time, i.e. weather trends (i.e. wet/dry cycles), harvest regulations, etc. may explain the variation in population better than the model including only time and should be considered in future analyses.

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Table 1. Total ducks banded under Summer Waterfowl Banding project (2W475), Pre-Season Waterfowl Banding Project (2W472), and the Central/Southern Minnesota Duck Banding Project in Minnesota by the Division of Wildlife, 2019.

Species	Summer banding project	Preseason banding project	S/C MN banding project	Total
American Green-winged Teal	18		1	19
American Wigeon	14			14
American Black Duck		1		1
Blue-winged Teal	106		326	432
Canvasback	11			11
Common Goldeneye	161			161
Common Merganser	53			53
Gadwall	4			4
Hooded Merganser	23		1	24
Mallard	438	389	164	991
Northern Pintail	1	7		8
Northern Shoveler	6			6
Redhead	4		7	11
Ring-necked Duck	279			279
Wood Duck	71	70	1564	1705
Total	1189	467	2063	3791

Table 2. – AICc scores, covariates, model weight, change in AICc score, Degrees of Freedom, and model name for each tested model used to examine factors affecting distance from banding location to recovery location for wood ducks banded in Minnesota and harvested out of state from 1997 - 2017. Factors present in each model are indicated with an “x”.

AICc	Age	Sex	Age*Sex	Zone	Days	Zone*Days	Weight	ΔAICc	Degrees freedom	Model name
18702.8	x	x	x	x	x	x	0.992	0.00	11	Mod7
18712.6	x	x		x	x	x	0.007	9.88	10	Mod9
18717.0	x	x	x	x	x		0.001	14.23	9	Mod113
18721.8	x			x	x	x	0.00	19.07	9	Mod11
18728.8		x		x	x	x	0.00	26.06	9	Mod10
18742.8				x	x	x	0.00	40.02	8	Mod8
18995.4	x	x	x		x		0.00	292.67	7	Mod12
19537.2	x	x	x	x			0.00	834.42	8	Mod112
19744.1							0.00	1041.33	3	ModNull

Table 3. AICc scores, covariates, model weight, change in AICc score, Degrees of Freedom, and model name for each tested model used to examine factors affecting change in latitude from banding location to recovery location for wood ducks banded in Minnesota and harvested out of state from 1997 - 2017. Factors present in each model are indicated with an "x".

AICc	Age	Sex	Age*Sex	Zone	Days	Zone*Days	Weight	$\Delta$ AICc	Degrees freedom	Model name
6583.3				x	x		0.807	0.00	6	Mod133
6586.3	x	x	x	x	x		0.182	2.97	9	Mod199
6594.0				x	x	x	0.004	10.72	8	Mod14
6594.8	x			x	x	x	0.003	11.49	9	Mod17
6595.1		x		x	x	x	0.002	11.78	9	Mod16
6596.9	x	x		x	x	x	0.001	13.61	10	Mod15
6597.2	x	x	x	x	x	x	0.001	13.94	11	Mod13
6806.0	x	x	x		x		0.00	222.71	7	Mod18
7404.1	x	x	x	x			0.00	820.84	8	Mod188
7522.4							0.00	939.08	3	ModNull

Table 4. AICc scores, covariates, model weight, change in AICc score, Degrees of Freedom, and model name for each tested model used to examine factors affecting the change in longitude from banding location to recovery location for wood ducks banded in Minnesota and harvested out of state from 1997 - 2017. Factors present in each model are indicated with an "x".

AICc	Age	Sex	Age*Sex	Zone	Days	Zone*Days	Weight	$\Delta$ AICc	Degrees freedom	Model name
6699.4	x			x			0.987	0.00	6	Mod26
6708.1	x			x	x		0.013	8.71	7	Mod25
6721.3	x	x		x	x	x	0.00	21.94	10	Mod21
6723.0	x	x	x	x	x	x	0.00	23.60	11	Mod19
6725.2	x			x	x	x	0.00	25.77	9	Mod23
6739.1		x		x	x		0.00	39.68	7	Mod27
6756.3		x		x	x	x	0.00	56.94	9	Mod22
6774.1				x	x	x	0.00	74.67	8	Mod20
6806.0	x	x	x		x		0.00	106.59	7	Mod24
6843.7							0.00	144.33	3	ModNull

Table 5. Number of recoveries per age classification from each banding zone for wood ducks marked in Minnesota from 1997 - 2017.

Zone	Adults	Juveniles
North	348	124
Central	280	148
South	179	197

Table 6. Number and percentage of reported direct recoveries for wood ducks banded in Minnesota 1997-2017 by state.

State of recovery	Harvest records	Percent of recoveries
Minnesota	1099	45.58%
Louisiana	400	16.59%
Arkansas	206	8.54%
Texas	146	6.06%
Mississippi	103	4.27%
Wisconsin	74	3.07%
Illinois	59	2.45%
Iowa	58	2.41%
Alabama	48	1.99%
Missouri	46	1.91%
Tennessee	42	1.74%
Georgia	26	1.08%
Florida	23	0.95%
Oklahoma	20	0.83%
South Carolina	15	0.62%
Indiana	11	0.46%
Michigan	9	0.37%
Kansas	6	0.25%
North Carolina	6	0.25%
Kentucky	6	0.25%
Ohio	3	0.12%
Virginia	2	0.08%
Nebraska	1	0.04%
North Dakota	1	0.04%
Delaware	1	0.04%
Grand Total	2,411	

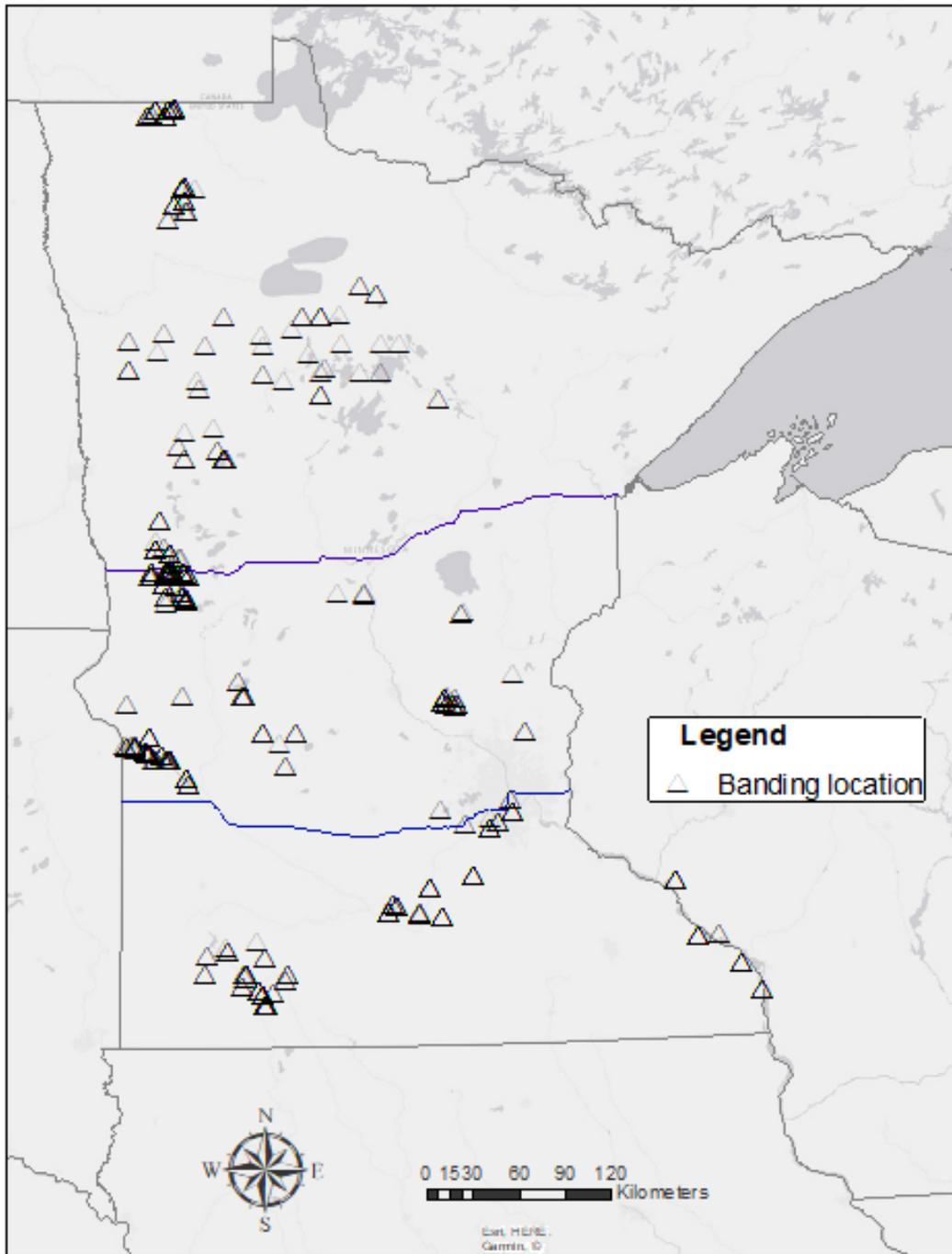


Figure 1. Map showing banding locations (open triangles) for wood ducks in Minnesota from 1997-2017.

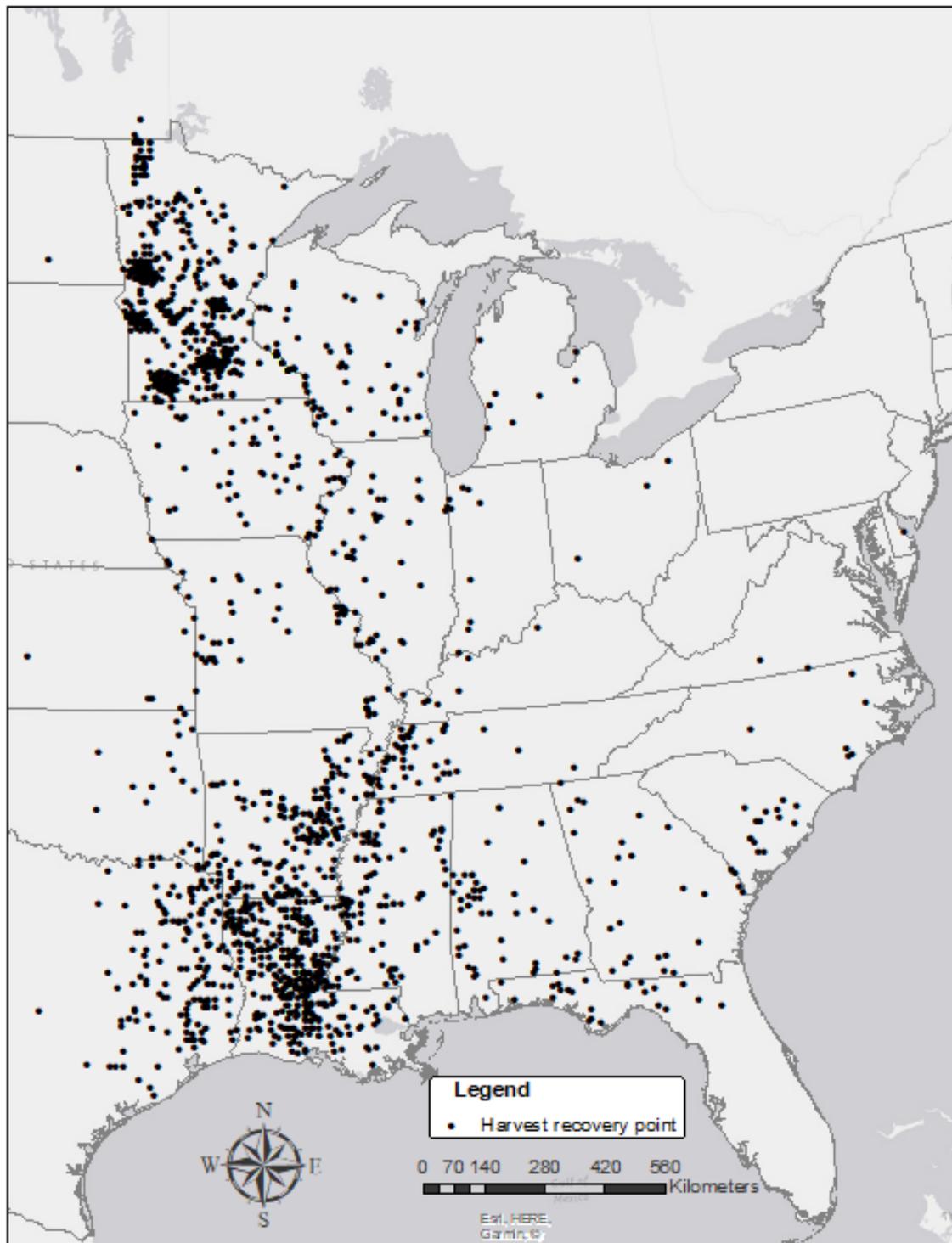


Figure 2. Recovery locations for 2,411 wood ducks banded in Minnesota 1997- 2017.

### Histogram of distance traveled

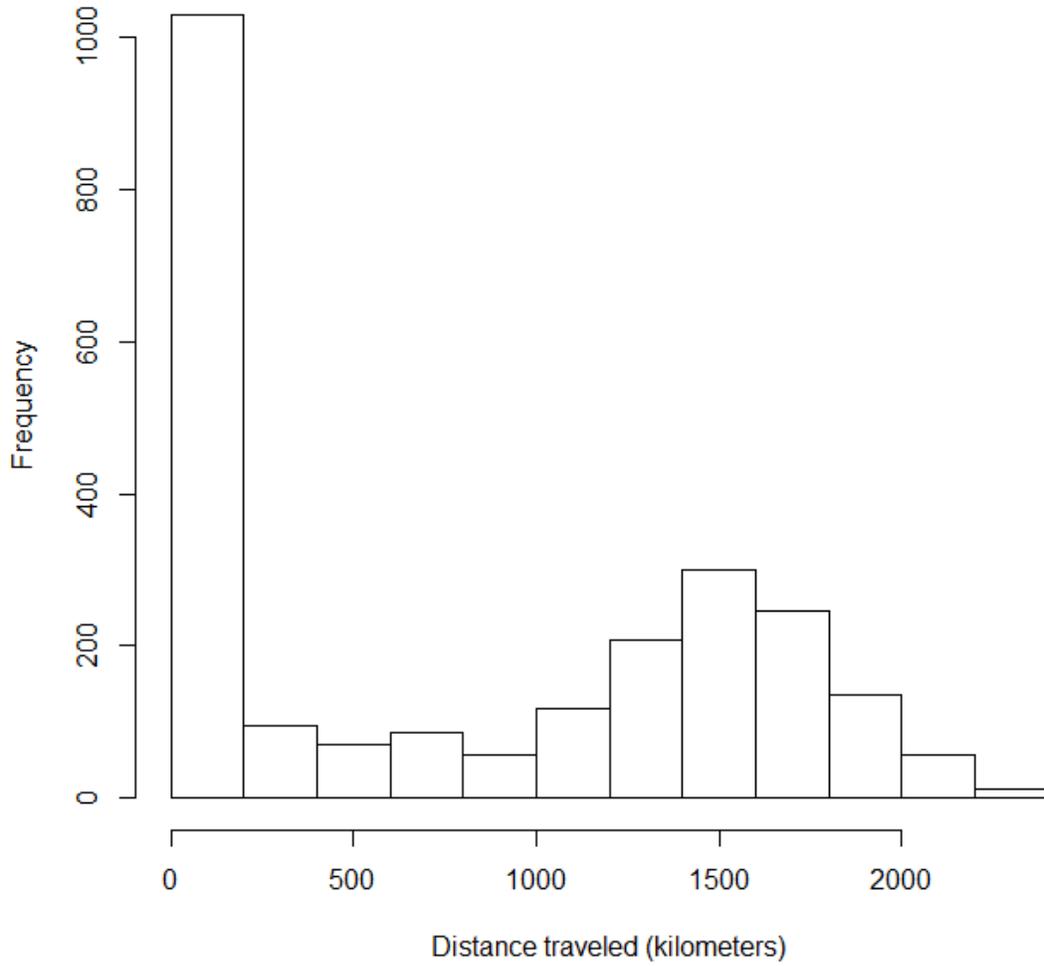


Figure 3. Histogram of distance from banding location to recovery location for wood ducks banded in Minnesota, 1997-2017 and recovered in the immediate hunting season after banding.

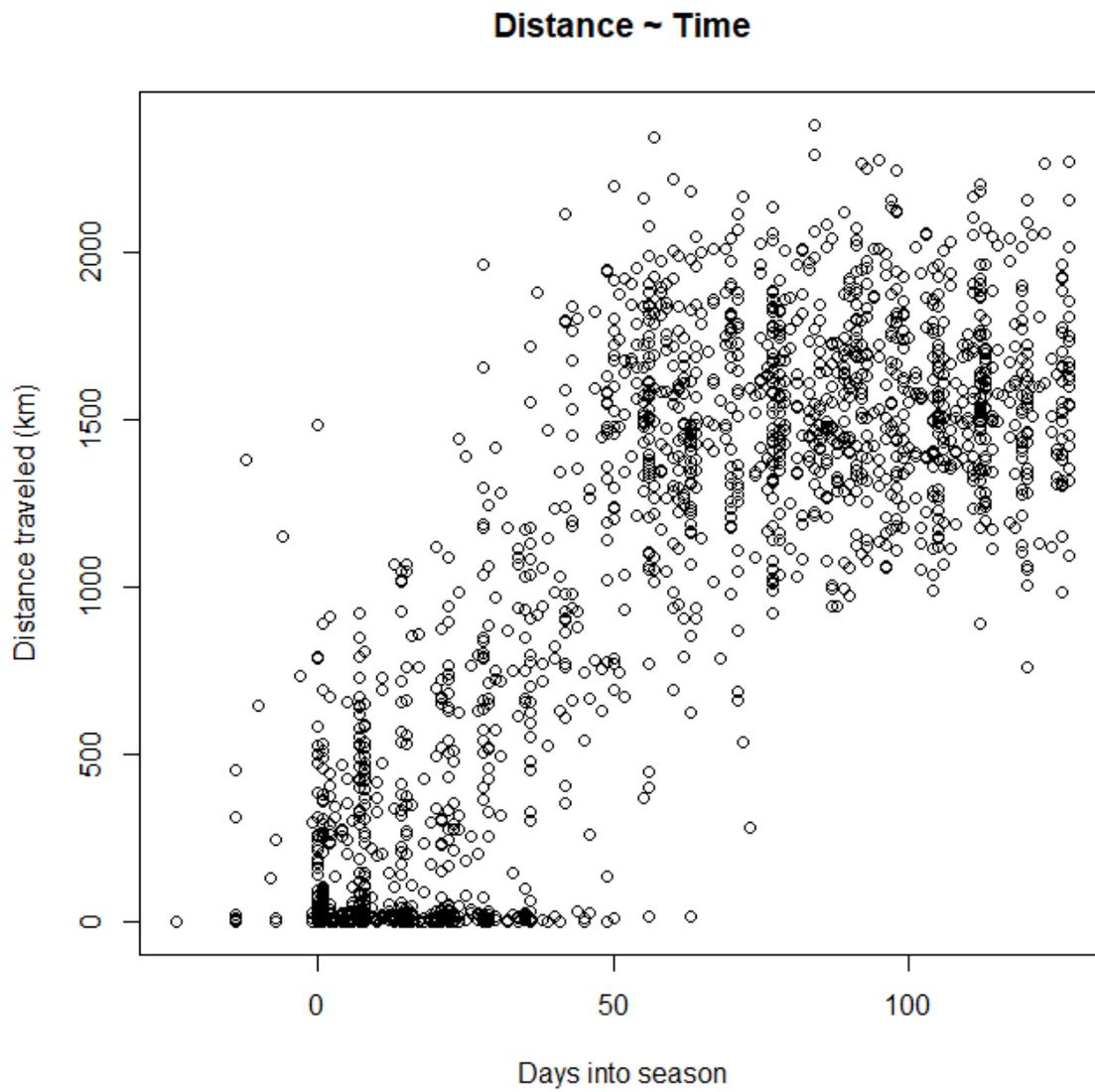


Figure 4. Relationship between time and distance traveled from banding location to recovery location for wood ducks banded in Minnesota, 1997 - 2017.

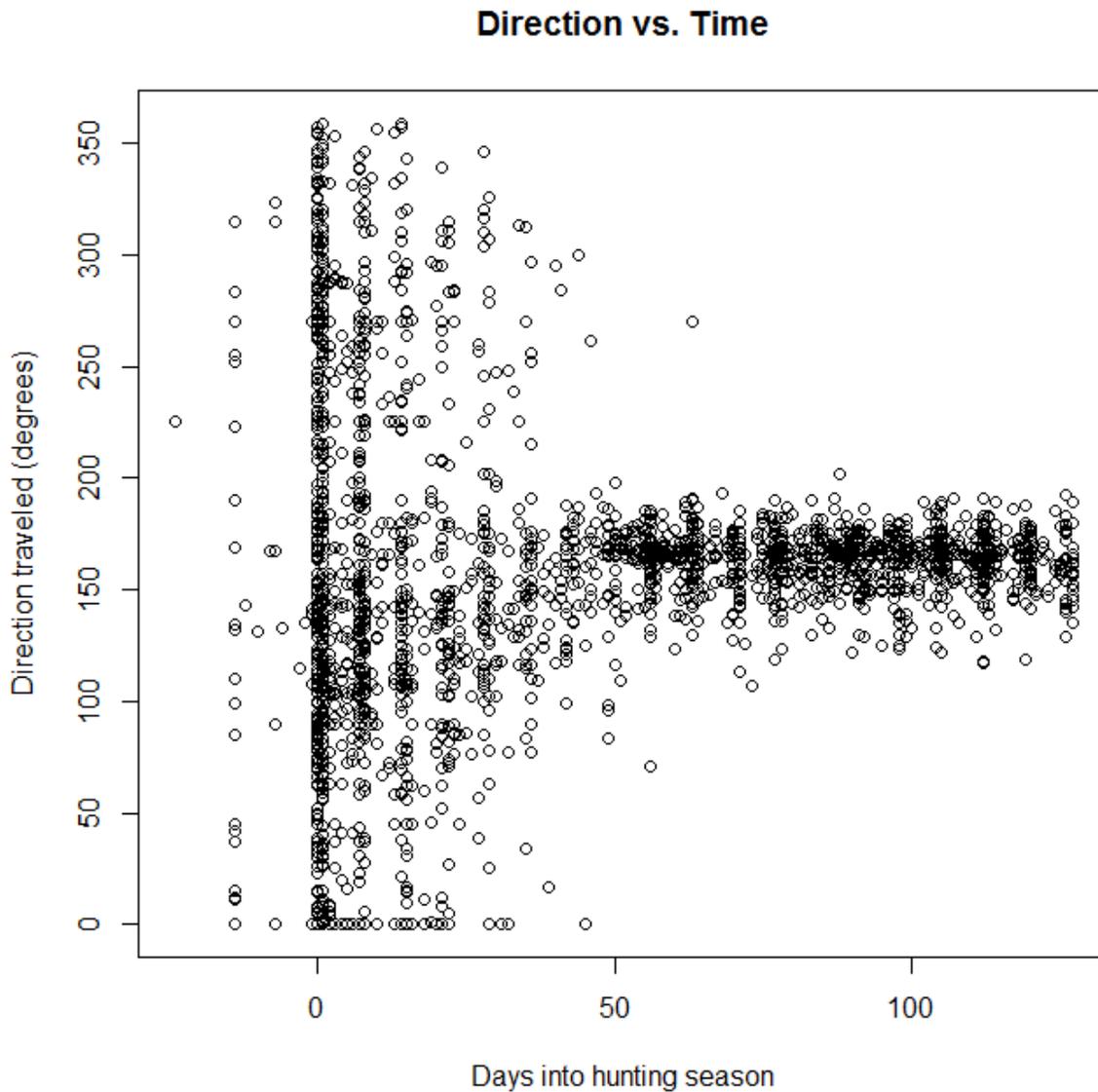


Figure 5. Relationship between time and direction of travel from banding location to recovery location for wood ducks banded in Minnesota, 1997 - 2017.

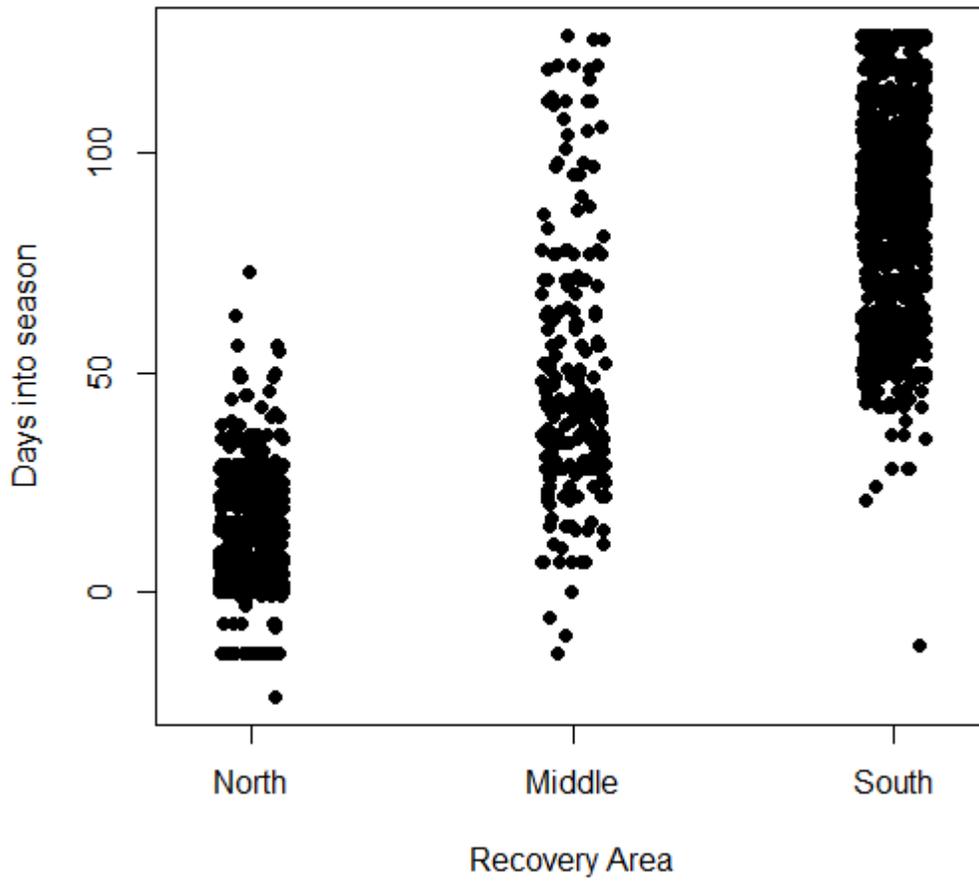


Figure 6. Strip chart of the relationship between harvest in each region and time for wood ducks banded in Minnesota, 1997 - 2017.

### Histogram of distance traveled for in state recoveries

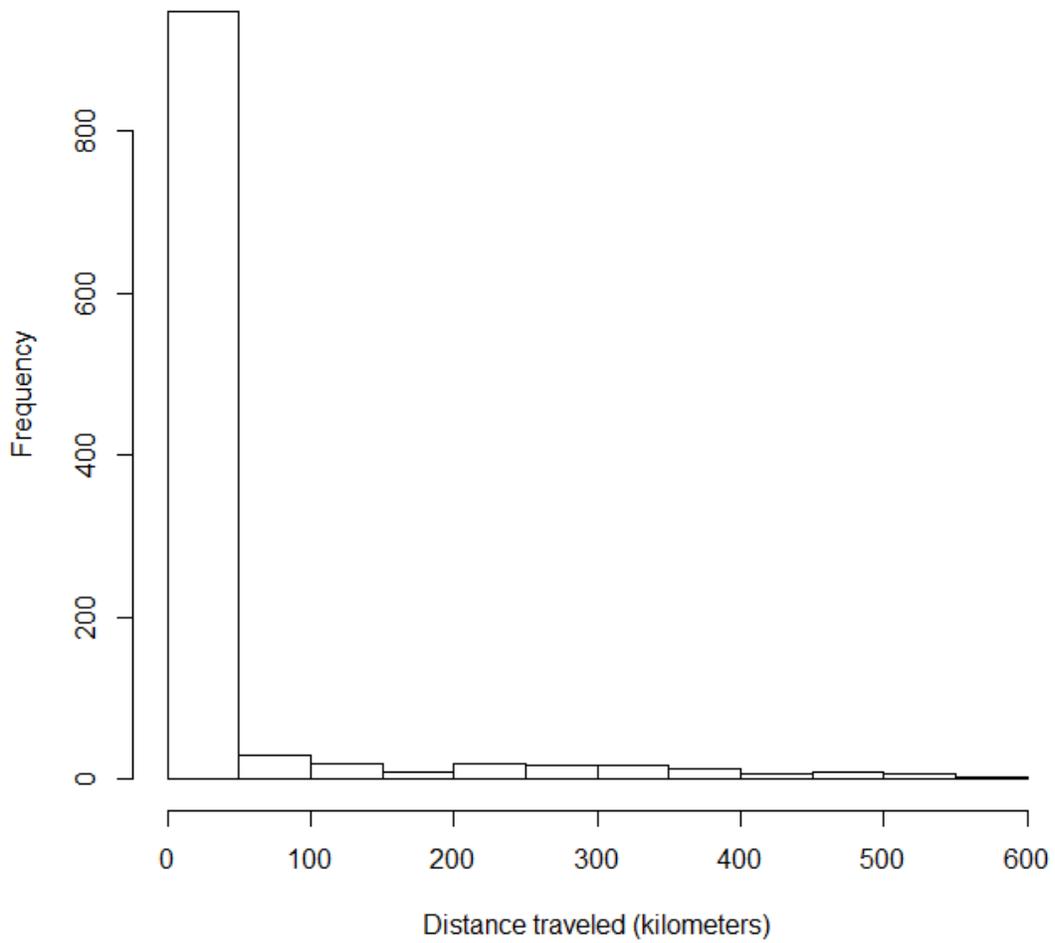


Figure 7. Histogram of distances from banding locations to recovery locations for wood ducks both banded and recovered in Minnesota from 1997 - 2017.

**Histogram of distance traveled for out of state recoveries**

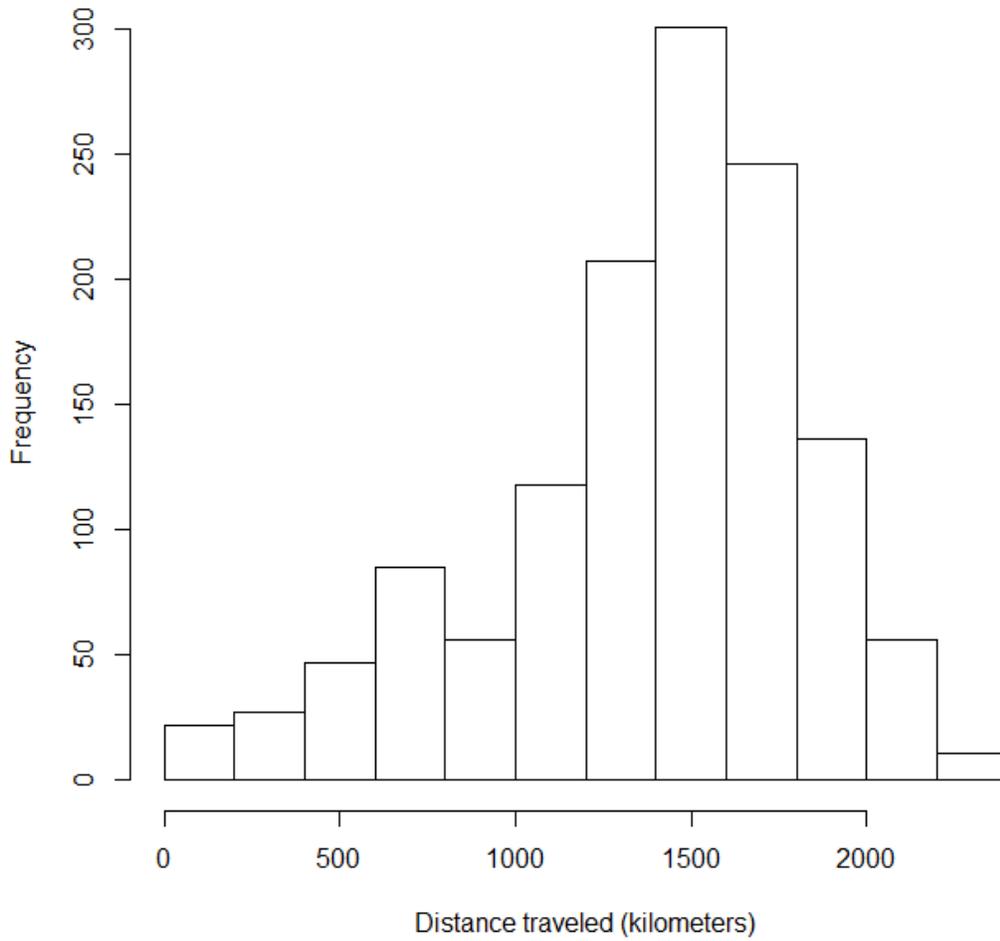


Figure 8. Histogram of distance between banding and recovery locations for wood ducks banded in Minnesota, but recovered out of state from 1997-2017.

# Banding Zones for Minnesota

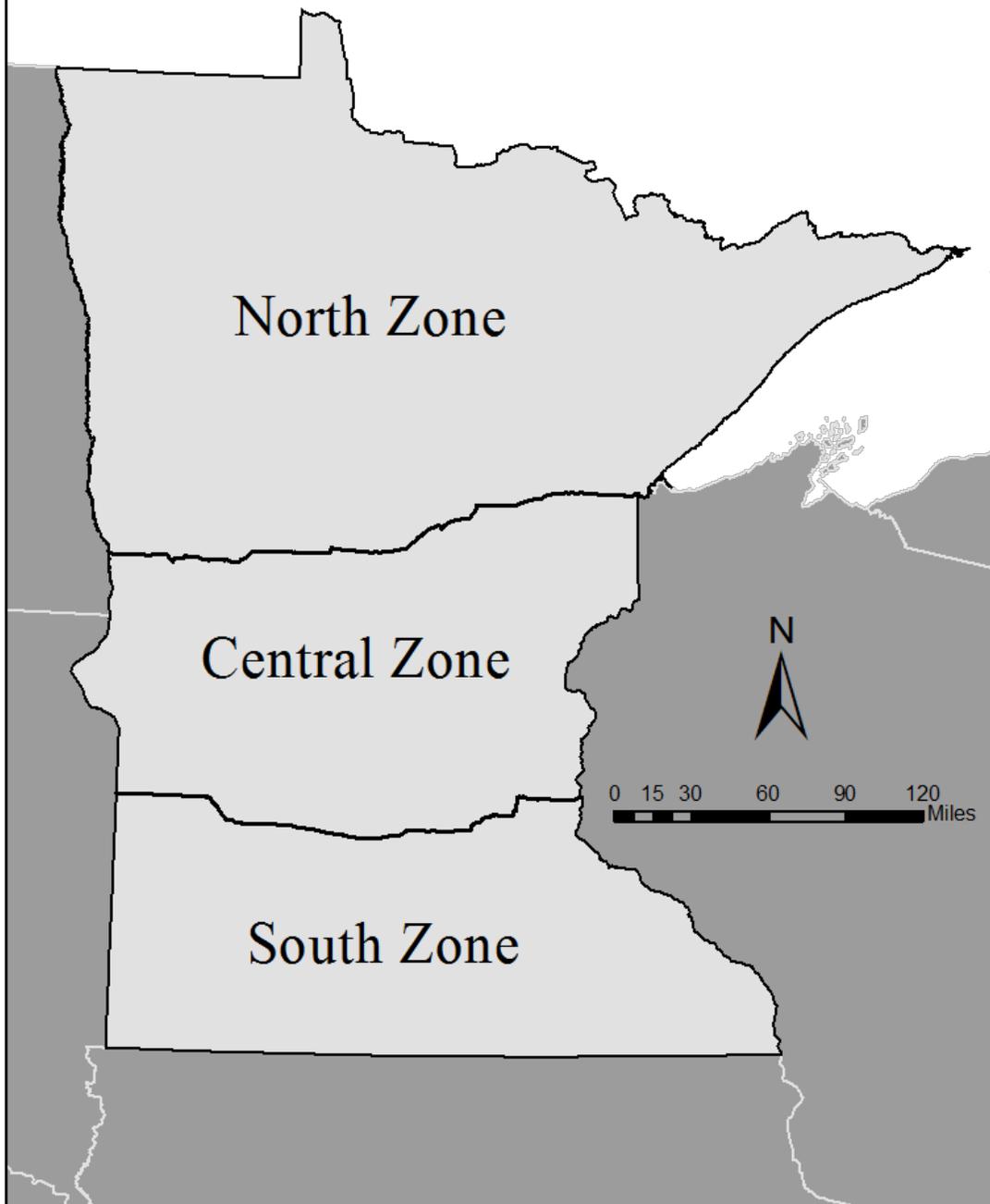


Figure 9. Map indicating the three hunting zones for Minnesota waterfowl; these were used as banding zones for analyses.

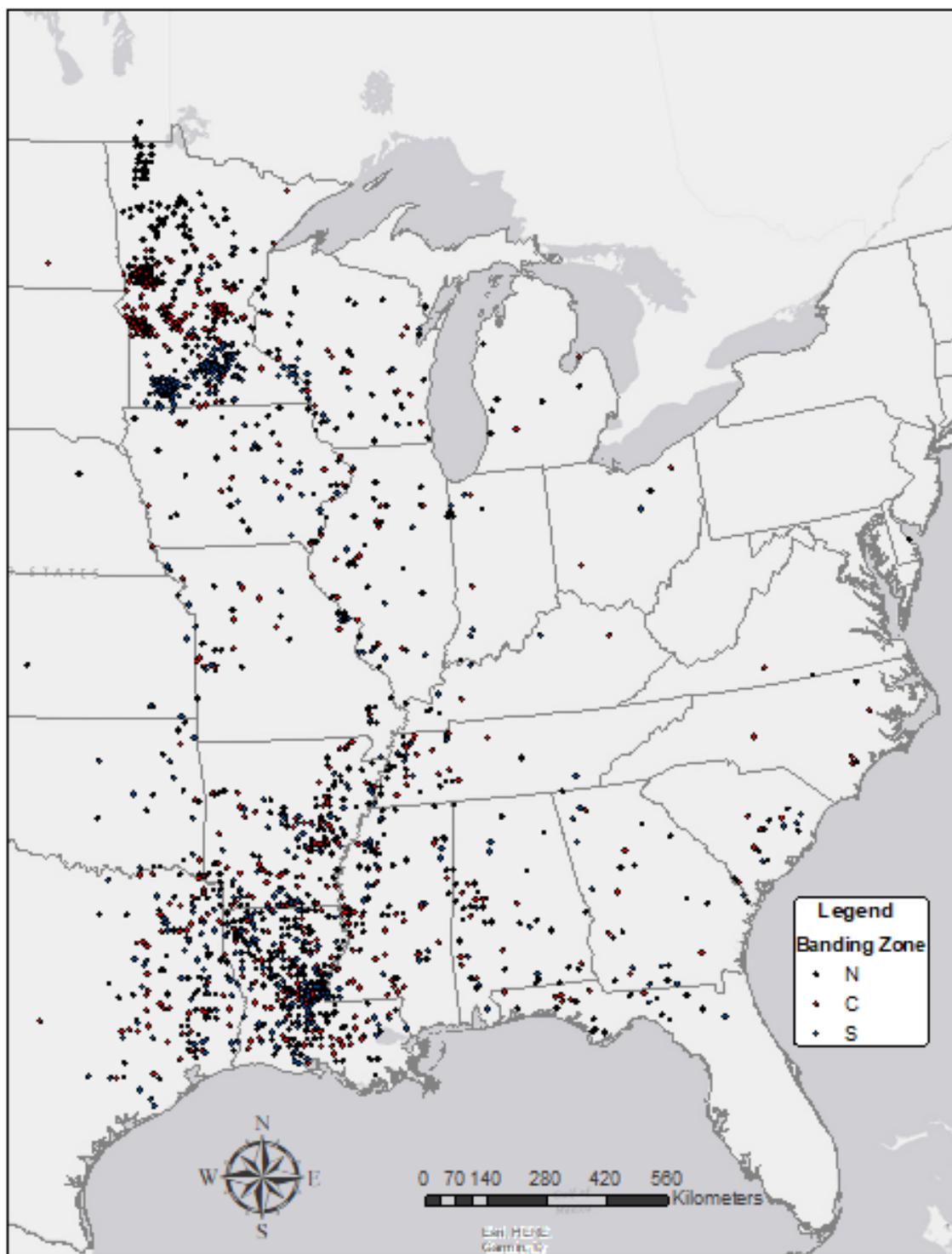


Figure 10. Map showing the recovery locations of banded wood ducks marked in each of the three banding zones in Minnesota from 1997-2017, North (N), Central (C), and South (S).

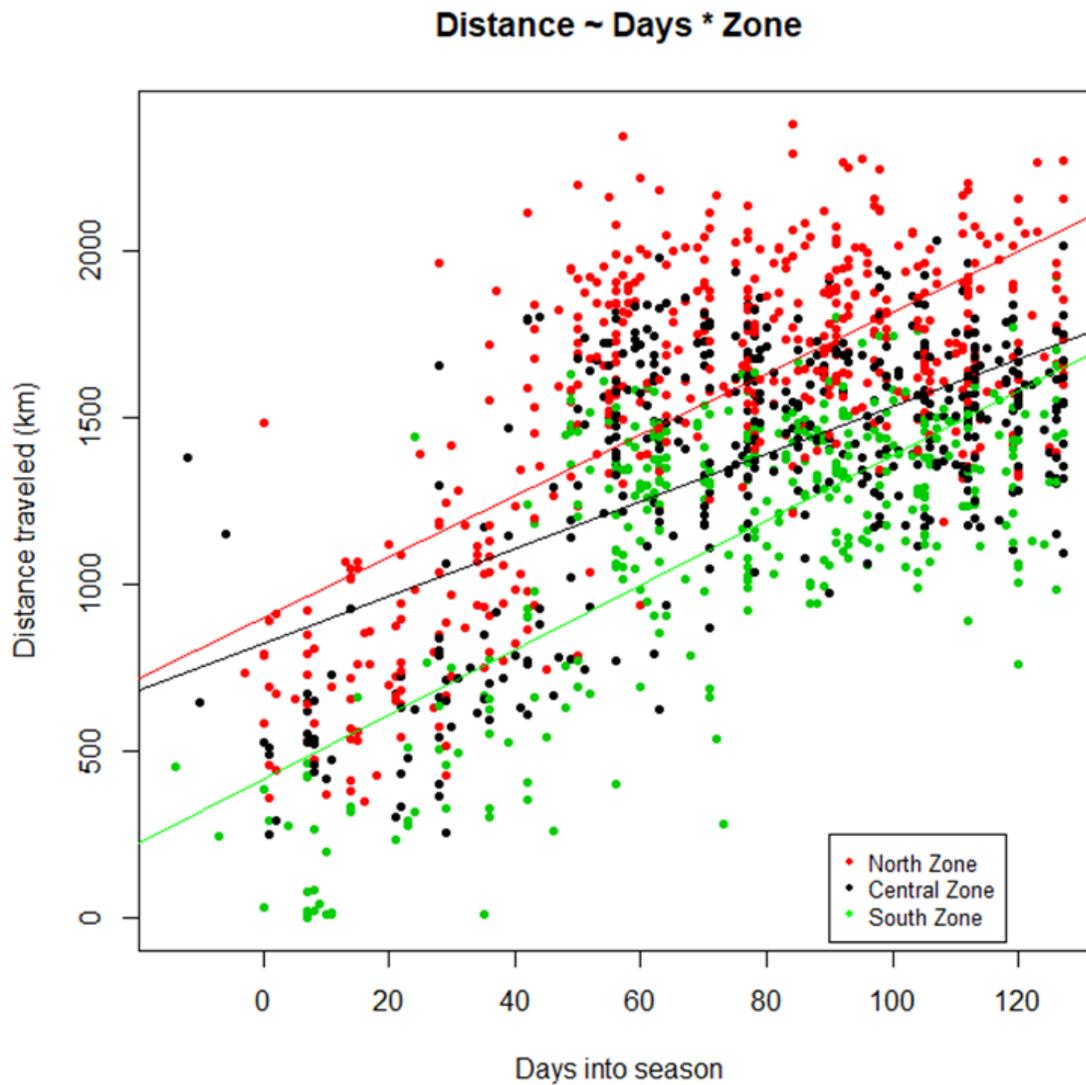


Figure 11. Distance traveled between marking and recovery locations by days into season for wood ducks banded in Minnesota, 1997 - 2017. Birds marked in the North, Central, and South Zones are indicated in red, black, and green respectively. Regression lines are fit for each zone, separately.

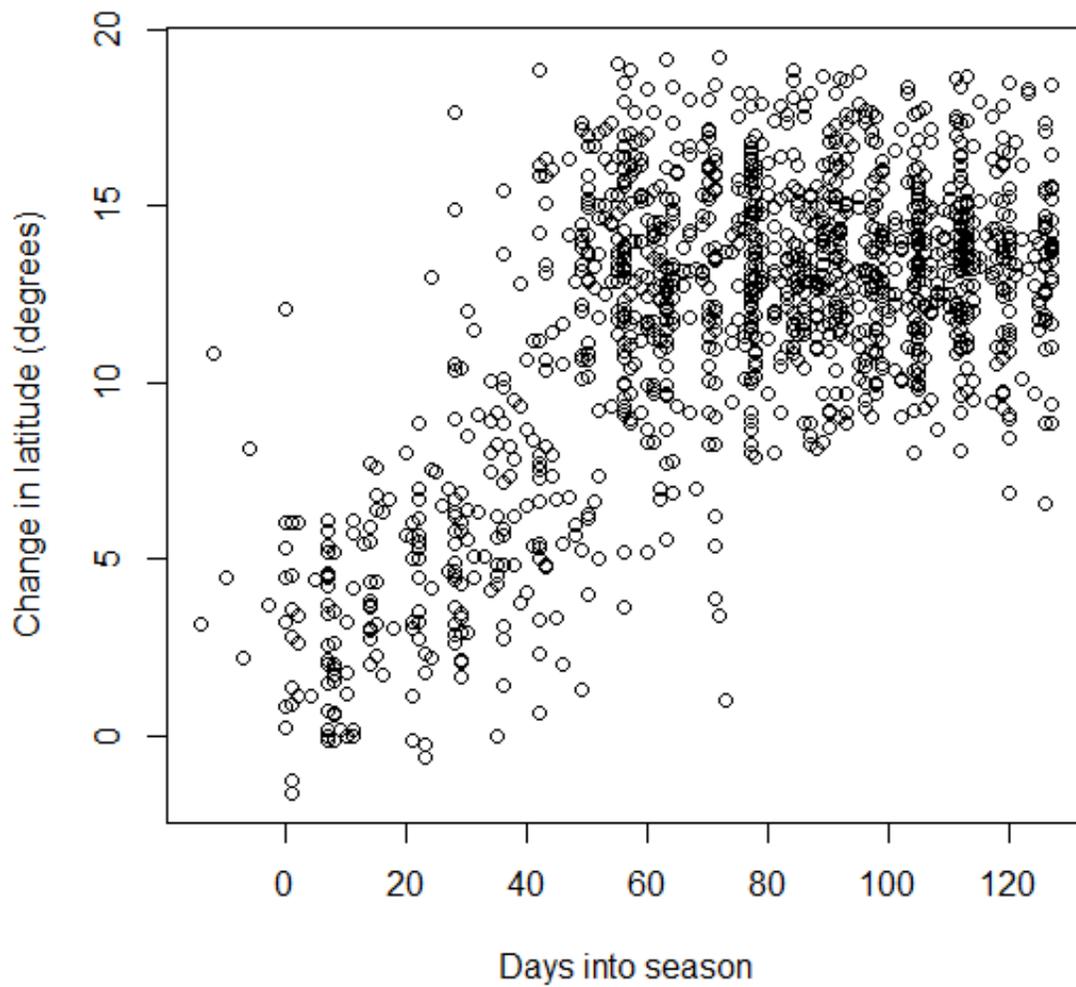


Figure 12. Days into the season (time) and the change in latitude from banding location to recovery location for wood ducks banded in Minnesota, 1997 - 2017.

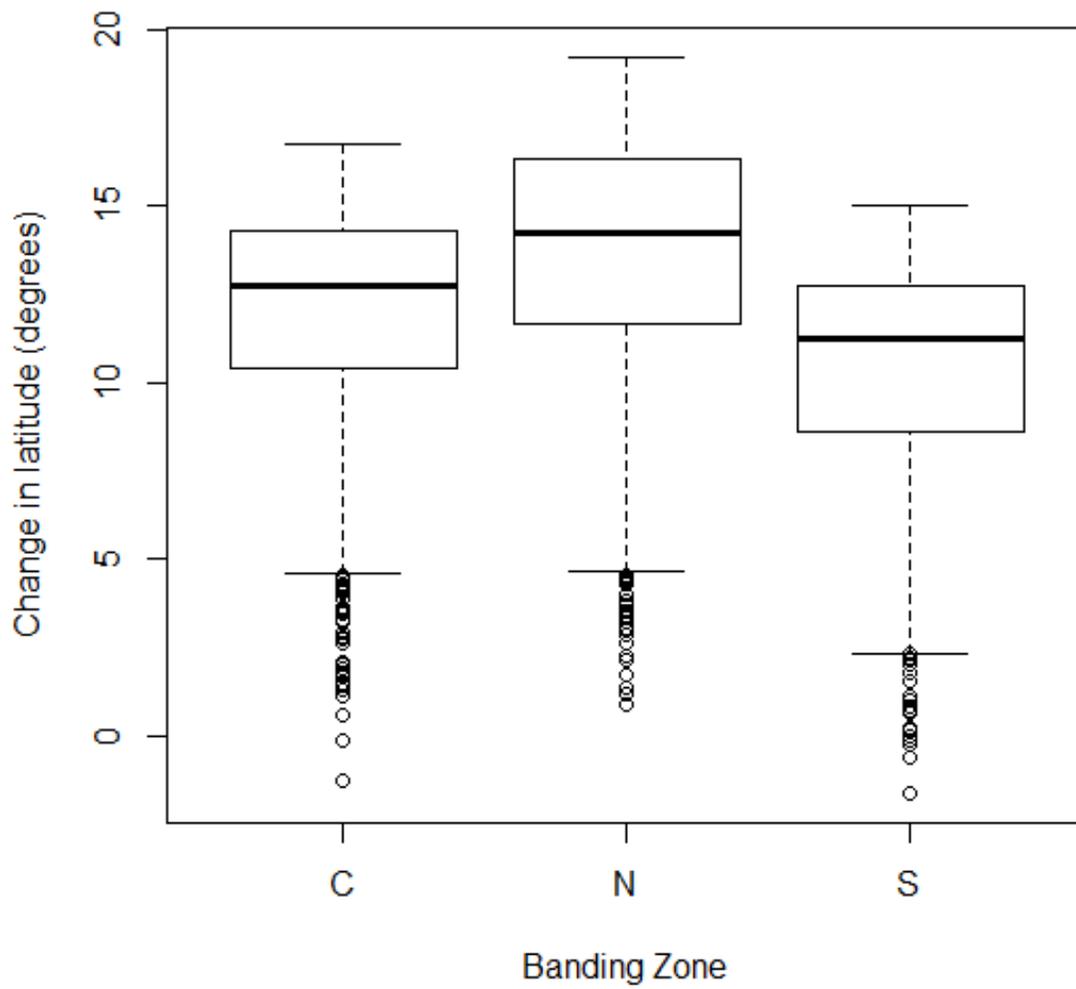


Figure 2. Change in latitude by banding zone for wood ducks marked in Minnesota from 1997 - 2017.

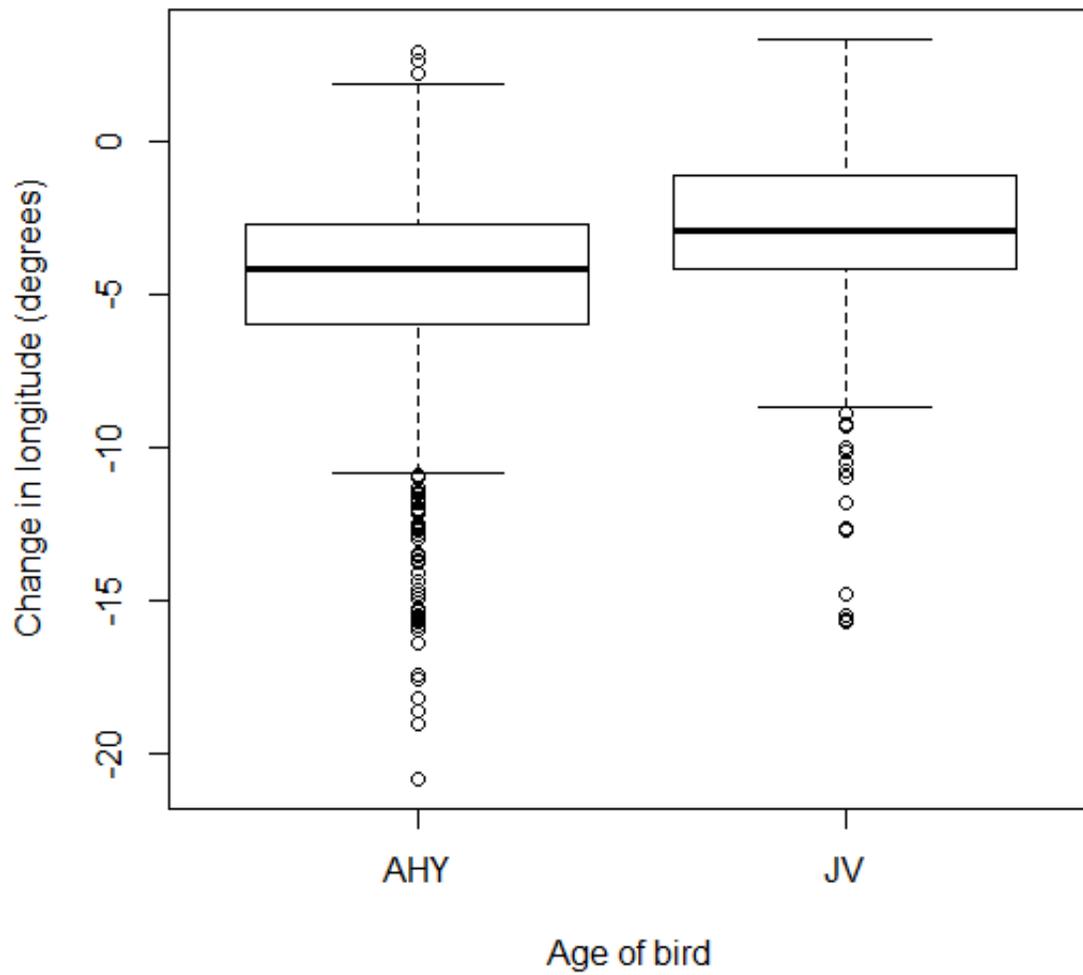


Figure 14. Change in longitude by age for wood ducks marked in Minnesota from 1997- 2017.

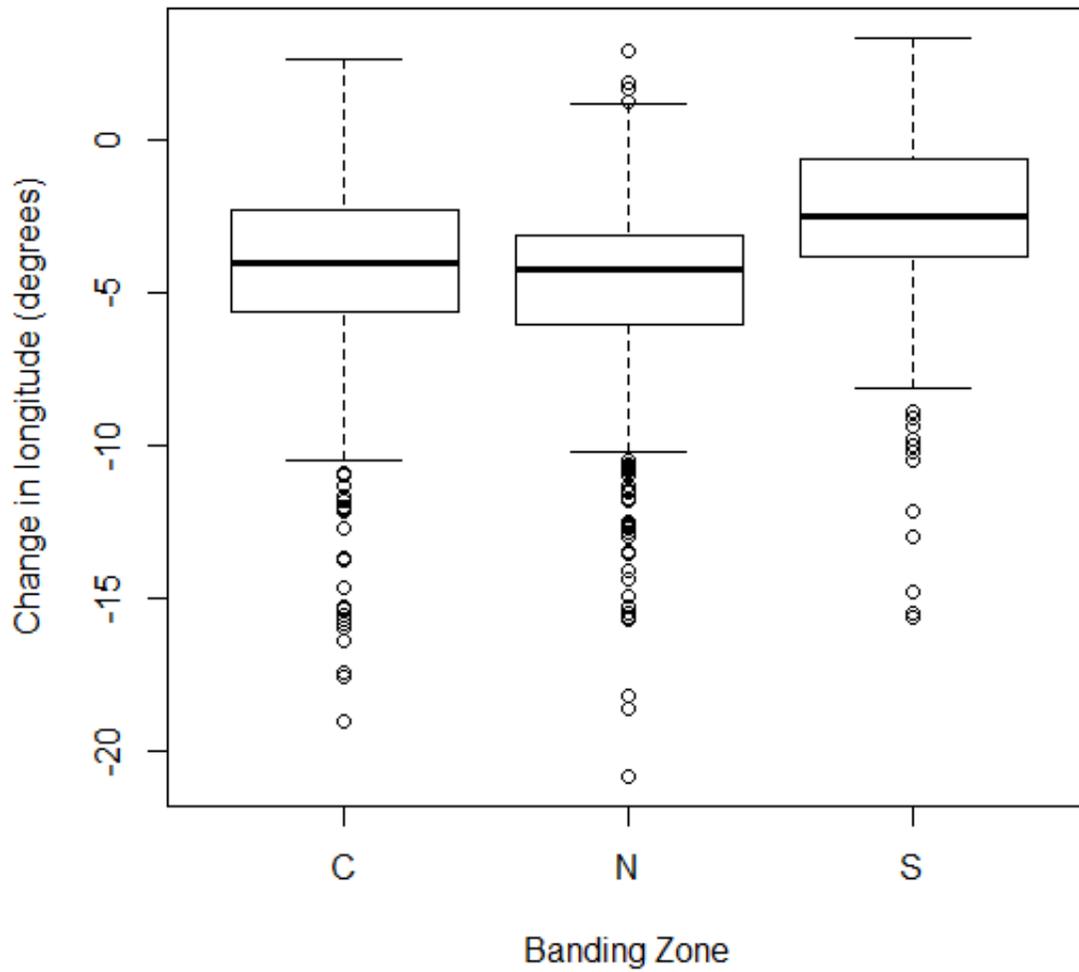


Figure 15. Change in longitude by banding zones for wood ducks marked in Minnesota from 1997 - 2017.

### Fall flight estimates for Mississippi Flyway

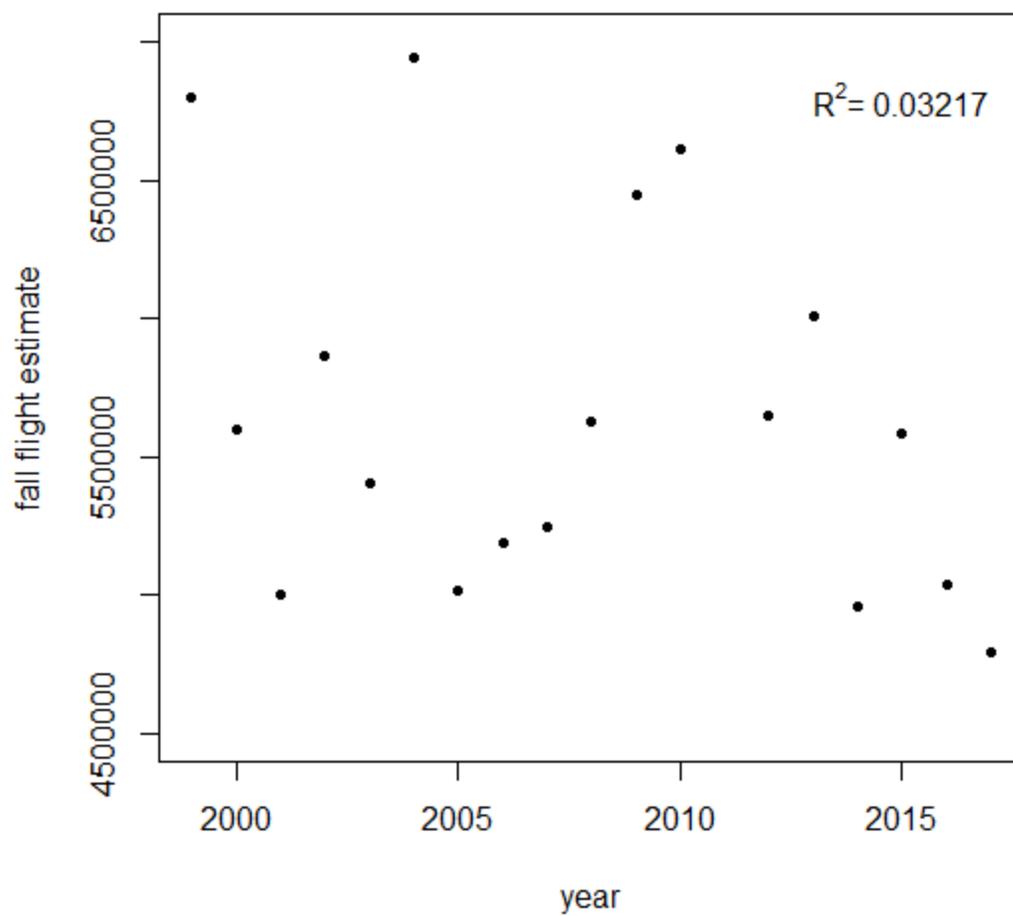


Figure 16. Fall flight estimates of wood ducks for the Mississippi Flyway 1999 - 2017.



## WATERFOWL PRODUCTION IN THE CHIPPEWA NATIONAL FOREST: A HISTORICAL PERSPECTIVE

Edmund Zlonis<sup>1</sup>, Tanya Roerick<sup>2</sup>, Steve Mortensen<sup>2</sup>, Dave Rave<sup>1</sup>, Todd Tisler<sup>3</sup>

### SUMMARY OF FINDINGS

We repeated a historical waterfowl production survey on the Chippewa National Forest in 2019. The survey had previously been conducted from 1937-1972. The methodology was straightforward and repeatable; we plan to conduct the survey for 4 additional years to compare waterfowl production between historical and modern time-periods. In 2019, total waterfowl abundance was similar to the historical average. However, overall waterfowl productivity and production per brood appeared to have decreased. In addition, results from 2019 indicate a long-term shift in species composition of waterfowl breeding in this region. Findings from this study will be used to identify research needs and management solutions for sustaining or increasing forest-breeding waterfowl populations in Minnesota.

### INTRODUCTION

The principle goal of Minnesota's Long Range Duck Recovery Plan is to restore breeding waterfowl populations to their historical levels (Minnesota Department of Natural Resources, [MNDNR] 2006). Implicit in this goal is a thorough understanding of statewide populations and an associated historical perspective. However, the majority of Minnesota's forested region is not surveyed regularly for breeding waterfowl (Cordts 2018), despite its importance to commonly harvested species including ring-necked ducks (*Aythya collaris*), wood ducks (*Aix sponsa*), mallards (*Anas platyrhynchos*) and, to a lesser extent, blue-winged teal (*Spatula discors*; Pfannmuller et al. 2017, Soulliere et al. 2007, Zicus et al. 2013). Data on the status of forest-breeding waterfowl in Minnesota are required to achieve goals in the Duck Plan.

Historically, waterfowl hunting heritage and harvest were strong in the forested portion of Minnesota (Kirby et al. 1976, Stoudt 1938). However, more recent estimates indicate a reduction in harvest from the forest (MNDNR 2001). This is likely related to fewer waterfowl hunters, though concern over declining production from forested wetlands has also been a principle issue raised by researchers and wildlife managers (Zicus 2003; *but see* Lawrence 2003). With locally reared ducks contributing as much as 70% of the overall harvest in Great Lakes States (T. Arnold *unpublished data*), duck hunting opportunity in Minnesota's forests relies on an understanding of local population trajectories.

The primary goal of this study is to give context to modern waterfowl populations in the forests of north-central Minnesota by repeating a historical waterfowl production survey. Over 80 years ago, Jerome Stoudt of the United States Forest Service (USFS) established a survey of waterfowl production in the Chippewa National Forest (CNF; Stoudt 1938). The survey was conducted from 1937-1972, providing managers with trends in waterfowl abundance and productivity. By repeating the survey from 2019-2023, we hope to provide wildlife managers

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with important insights into changes, if any, in breeding productivity and species composition of waterfowl in the CNF over the last century. Here, we report on the survey methods and preliminary results from 2019.

## OBJECTIVES

1. Our primary objective is to repeat the CNF waterfowl production survey (hereafter, ‘historical survey’) from 2019-2023 to determine changes in relative abundance, productivity, and species composition of waterfowl from historical to modern time-periods.

## METHODS

The following methods have been summarized from original methodological documents pertaining to the historical survey. These original files have been digitized and archived along with annual data summaries and raw data. We indicate where methods have been modified or where we made assumptions about unknown methodologies during the first year of sampling, 2019.

### Chippewa National Forest Waterfowl Production Survey

The historical survey was conducted on 10 lakes and flowages in the CNF (Figure 1). Each year, the survey was initiated in early-mid July, after most waterfowl broods had hatched, but before the earliest broods had started to fly. Survey dates ranged from 5-26 July, but were most often 8-13 July. Anecdotal reports of broods and pre-survey scouting were utilized to determine when to conduct the survey.

Each study lake was sampled on one morning or evening visit, usually not after 0900 hours or before 1700 hours. Note, in 2019, we surveyed all lakes in the morning hours. Surveys were not conducted when there were high winds or heavy rain. Ideal conditions for sampling were calm, cloudy days. Six of the study lakes cover the entirety of the given lake’s shoreline, while the remainder include only portions of the shoreline (Figure 2). Details regarding survey nuances at each site are beyond the scope of this report and are summarized elsewhere (e.g., lake access points, anticipated vegetative conditions). Generally, the survey was conducted in a similar manner between sites, as described below.

Two observers conducted the survey from a canoe. The canoe was positioned along the shoreline and slowly paddled around the perimeter of the lake. Shoreline context varied greatly, including forested, wetland shrubs (e.g., willow [*Salix* spp.]), sedge mats (*Carex* spp.), aquatic emergent vegetation (e.g., bulrush [*Scirpus* spp.], wild rice [*Zizania* spp.]), un-vegetated (e.g., rocks, sand) and developed (e.g., cabins, docks). Each situation required a slightly different canoe position and speed to detect waterfowl broods. In addition, a third person walking along the shoreline, approximately 50 m ahead of the canoe, was used in locations where broods could escape unnoticed. This method was usually used on hard-bottomed areas with extensive bulrushes. Outboard motors were occasionally used on the canoe for travelling to and from study sites or for surveying sections of barren shoreline where broods could be observed from >100 m.

All waterfowl (ducks, geese, and swans) encountered along designated survey routes were counted and recorded. Note, during the historical survey, no Canada geese (*Branta canadensis*) or trumpeter swans (*Cygnus buccinator*) were observed. However, based on the descriptions of the survey, we believe that these species would have been counted similarly to ducks had they been breeding in these areas during the survey (e.g., the historical survey was consistently referred to as a ‘waterfowl’ survey). Nearly twenty species of waterfowl are potential breeding species in the CNF (Table 1). Historically, only 6 of these were common enough to summarize on an individual basis: American wigeon (*Mareca americana*), blue-

winged teal, common goldeneye (*Bucephala clangula*), mallard, ring-necked duck, and wood duck.

Each observation was categorized by species, age (adult or brood), sex (male or female; adults only) and total count. Broods were further classified by their developmental stage, from fully downy ducklings (class I) to those approaching flight stage (class III; Bellrose and Kortright 1976, Gollop and Marshall 1954). For each brood, surveyors indicated whether the adult hen was present and if they felt the entire brood was accurately counted or not (i.e., if several brood members appeared to have escaped into cover prior to being counted). Surveyors closely observed the behavior of adult hens in order to identify likely broods that were concealed and unavailable to count. Maternal hens will feign injury or lead potential predators away from their broods, often flying short distances or otherwise creating a distraction. These behaviors were used to indicate a concealed brood by recording the hen as a maternal hen.

Incomplete broods and maternal hens were expanded to represent unobserved ducklings by assigning species- and year-specific brood averages for complete broods. Note, in 2019, we used averages from all complete broods for a given species to identify these unobserved ducklings. Historically, the averages were both species- and lake-specific. For example, the average of complete mallard broods for Round Lake in 1953 was applied to each mallard maternal hen or incomplete brood for Round Lake.

### **Data Summary**

We made basic graphical summaries comparing waterfowl counts from the historical survey to 2019. There were adequate historical data to compare: 1) total waterfowl abundance, 2) abundance of adults and juveniles, 3) juvenile-adult ratio, 4) total number of broods, 5) lake-specific waterfowl counts and production, 6) species composition, 7) species-specific abundance, and 8) species-specific production and age of young. Additional data summary and analysis will be included after further sampling, 2020-2023.

## **RESULTS AND DISCUSSION**

In July 2019, we assessed the efficacy of repeating the methodology from the historical survey. We attempted to follow all protocols as documented in the Methods, above. In general, we felt that the survey was repeatable and that it was worthwhile to continue sampling through 2023.

All lakes were surveyed for waterfowl during 8-22 July 2019. We conducted a second survey of one lake (Bowstring) on 2 August 2019 due to updated information we found regarding the sampling route used in historical surveys. Amongst the 10 lakes, approximately 70 miles of shoreline were surveyed for waterfowl. A total of 2,035 waterfowl were observed, which is similar to the historical average count of 1,996 (Figure 3). Past counts fluctuated widely and indicated a long-term decline in total abundance of waterfowl, particularly production of young, from 1937-1972 (Figure 3; Figure 4). The number of juveniles counted in 2019 was similar to those counted in 1960s-1970s, but less than those counted in the 1930s-1940s. However, the number of adults was the third highest counted in the nearly 40 years of surveys. Combining these data points generated a juvenile-adult ratio near the lowest of the historical data (Figure 5). Most individual lakes had total counts that were similar to or above their historical averages, with the exception of Bowstring Lake (Figure 6).

In 2019, we counted more individual waterfowl broods than the historical average (238 versus 200). However, we counted fewer young per brood. Historical averages for complete broods usually ranged from 6-8 ducklings per brood for commonly observed duck species. In 2019, brood averages ranged from 4-6 for these species, including 5.6 for the most common species, mallard (Figure 7). It does not appear that this potential decline in brood-level productivity can

be explained by shifts in age-classes of broods over time; the proportion of mallard broods in 3 standard age-classes was generally similar between 2019 and historical data (Figure 8).

Species composition and abundance of individual species shifted between the historical time-period and samples from 2019 (Figure 9; Figure 10). The total number and percent contribution of ring-necked ducks and wood ducks was far greater in 2019 than 1937-1972. There were fewer blue-winged teal and American wigeon than what was historically counted, though a decline in these species was apparent in the historical data (as well as the aforementioned increase in wood ducks). Common goldeneyes appear to have changed little in abundance. Mallards were consistently the most common species, including in 2019. However, when compared to historical counts, they represented a smaller percentage of all waterfowl. Finally, 'other' waterfowl composed a greater portion of all waterfowl in 2019 versus historical counts. 'Other' was primarily composed of hooded mergansers (*Lophodytes cucullatus*) and Canada geese in 2019, the latter being a species that was never observed during 1937-1972.

With further sampling, 2020-2023, the results of this study will provide state, federal, and tribal managers with new historical context regarding waterfowl populations in the CNF and potentially other forested regions of Minnesota. We plan to use the results of this study, in conjunction with previously identified research needs (e.g., Zicus 2003), to propose research projects aimed at determining management solutions for sustaining or increasing forest waterfowl populations in Minnesota.

## **ACKNOWLEDGMENTS**

We would like to thank Jon Finn, Bruce Davis, and Zachary Jordan for help conducting field work. We would also like to thank Jerome Stoudt and other past biologists from the USFS, United States Fish and Wildlife Service, and Minnesota Department of Conservation (now MNDNR) for initiating the historical survey and developing useful methodological and annual reports. Without this information, we would not be able to repeat the survey. Bruce Davis, Danelle Larson, and John Giudice provided helpful comments on earlier drafts of this report.

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Table 1. List of potential waterfowl species seen during the summer in the Chippewa National Forest. The American Ornithologist's Union acronym is listed for each species. Some species are rare during this season.

<b>Species</b>	<b>AOU abbreviation</b>
Canada goose ( <i>Branta canadensis</i> )	CANG
Trumpeter swan ( <i>Cygnus buccinator</i> )	TRUS
Wood duck ( <i>Aix sponsa</i> )	WODU
Blue-winged teal ( <i>Spatula discors</i> )	BWTE
Northern shoveler ( <i>Spatula clypeata</i> )	NOSH
Gadwall ( <i>Mareca strepera</i> )	GADW
American wigeon ( <i>Mareca americana</i> )	AMWI
Mallard ( <i>Anas platyrhynchos</i> )	MALL
Northern pintail ( <i>Anas acuta</i> )	NOPI
Green-winged teal ( <i>Anas crecca</i> )	AGWT
Canvasback ( <i>Aythya valisineria</i> )	CANV
Redhead ( <i>Aythya americana</i> )	REDH
Ring-necked duck ( <i>Aythya collaris</i> )	RNDU
Lesser scaup ( <i>Aythya affinis</i> )	LESC
Bufflehead ( <i>Bucephala albeola</i> )	BUFF
Common goldeneye ( <i>Bucephala clangula</i> )	COGO
Hooded merganser ( <i>Lophodytes cucullatus</i> )	HOME
Common merganser ( <i>Mergus merganser</i> )	COME
Red-breasted merganser ( <i>Mergus serrator</i> )	RBME

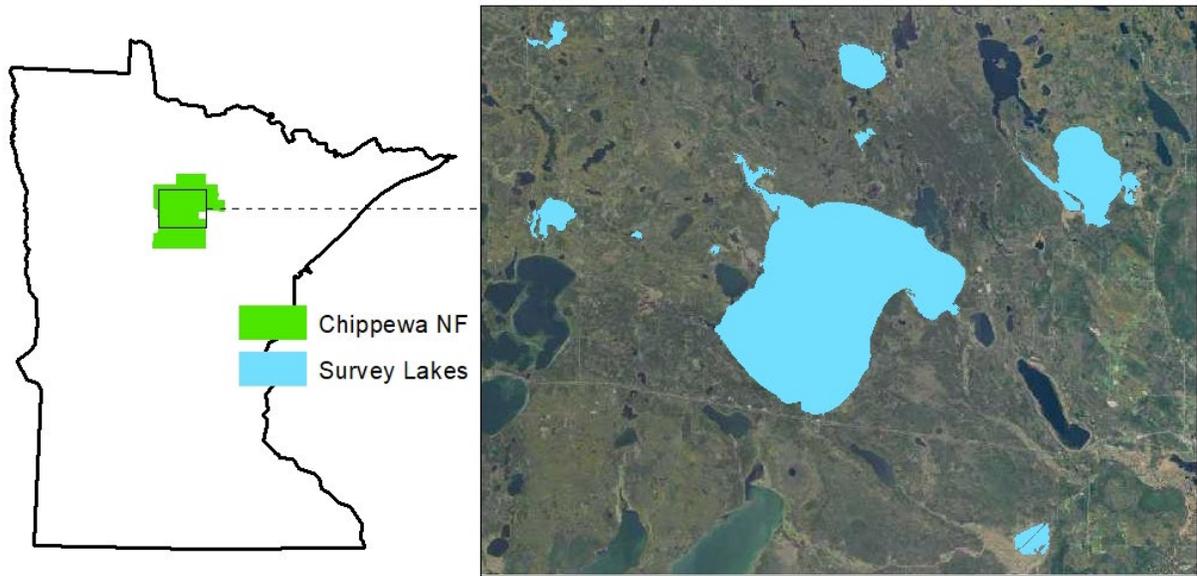


Figure 1. Chippewa National Forest and associated lakes surveyed for waterfowl broods. In the historical survey, 10 lakes and flowages were sampled from 1937-1972 and again in 2019 in Minnesota.

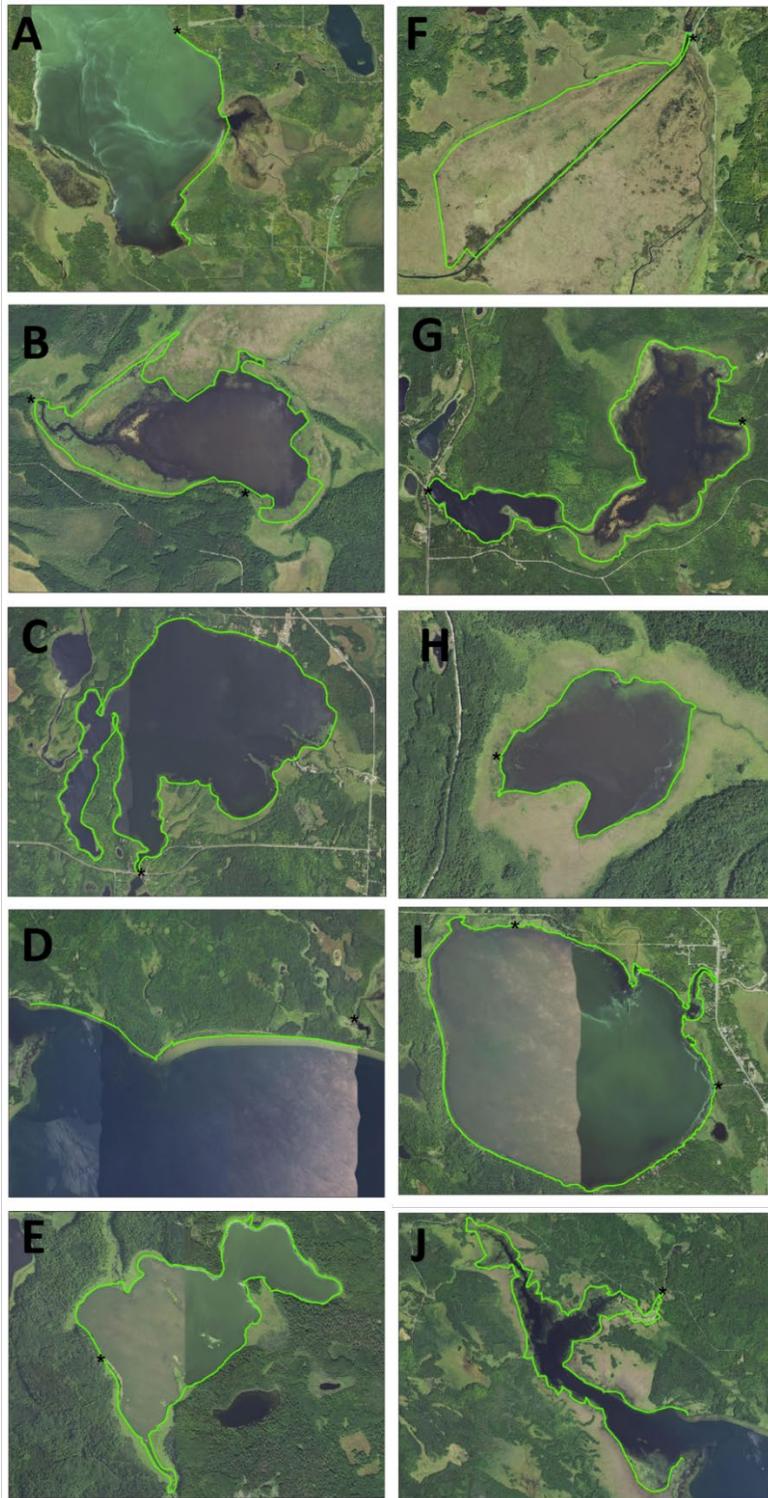


Figure 2. Individual maps of lakes and flowages surveyed for waterfowl broods 1937-1972 and 2019 in Minnesota. The green line indicates the shoreline sampled. A, Bowstring; B, Burns; C, Kitchi; D, Lake Winnibigoshish; E, Lower Pigeon; F, Mud; G, Rabideau; H, Raven; I, Round; J, Third River. Access sites are indicated by an asterisk. Note, the scale is not the same between maps.

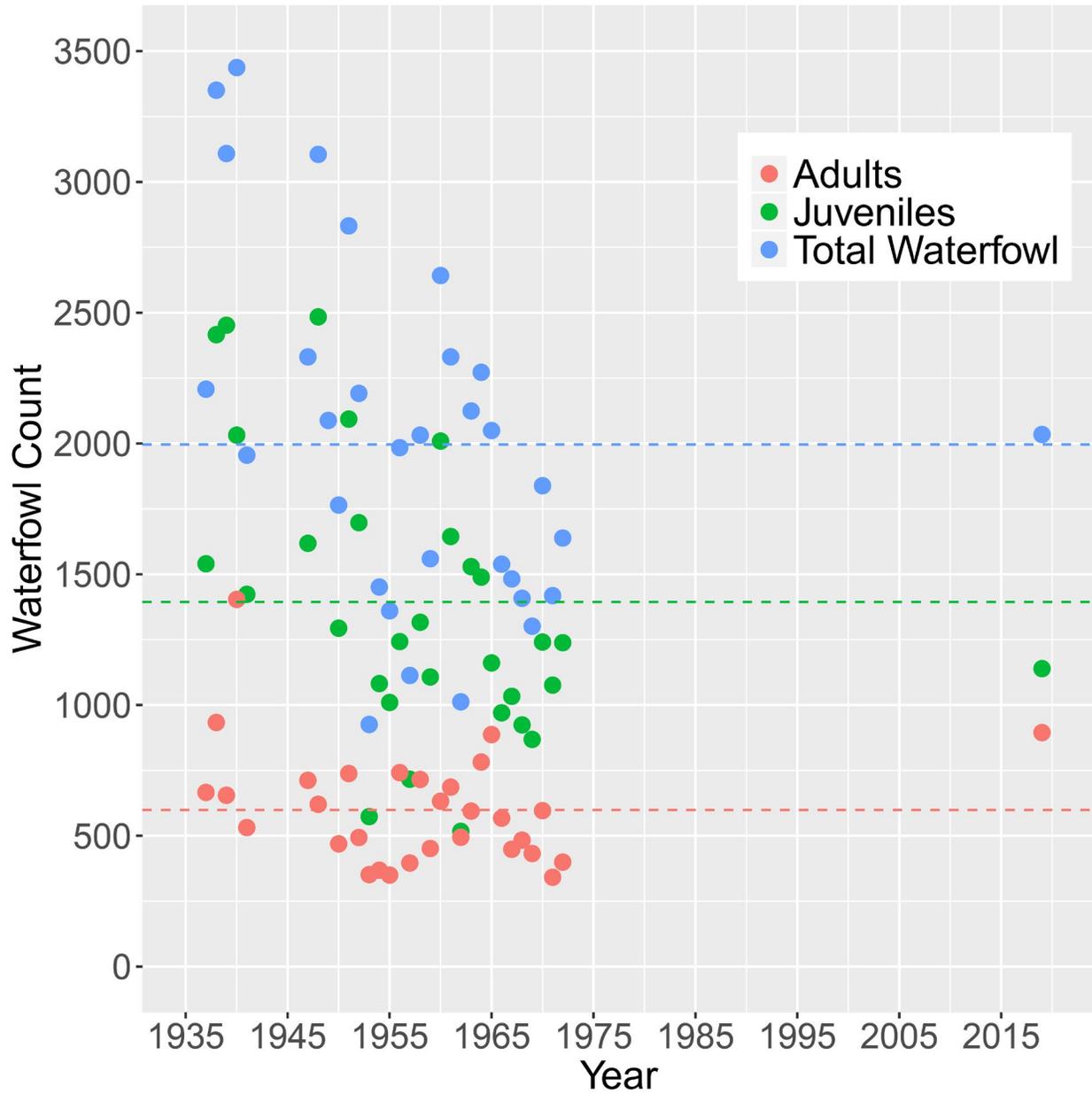


Figure 3. Total number of waterfowl observed on 10 lakes and flowages in the Chippewa National Forest, Minnesota, 1937-1972 and 2019. The total count is split into adults and juveniles. Historical averages (1937-1972) for each total are indicated by a dashed line. The survey was not conducted 1942-1946 due to World War II.

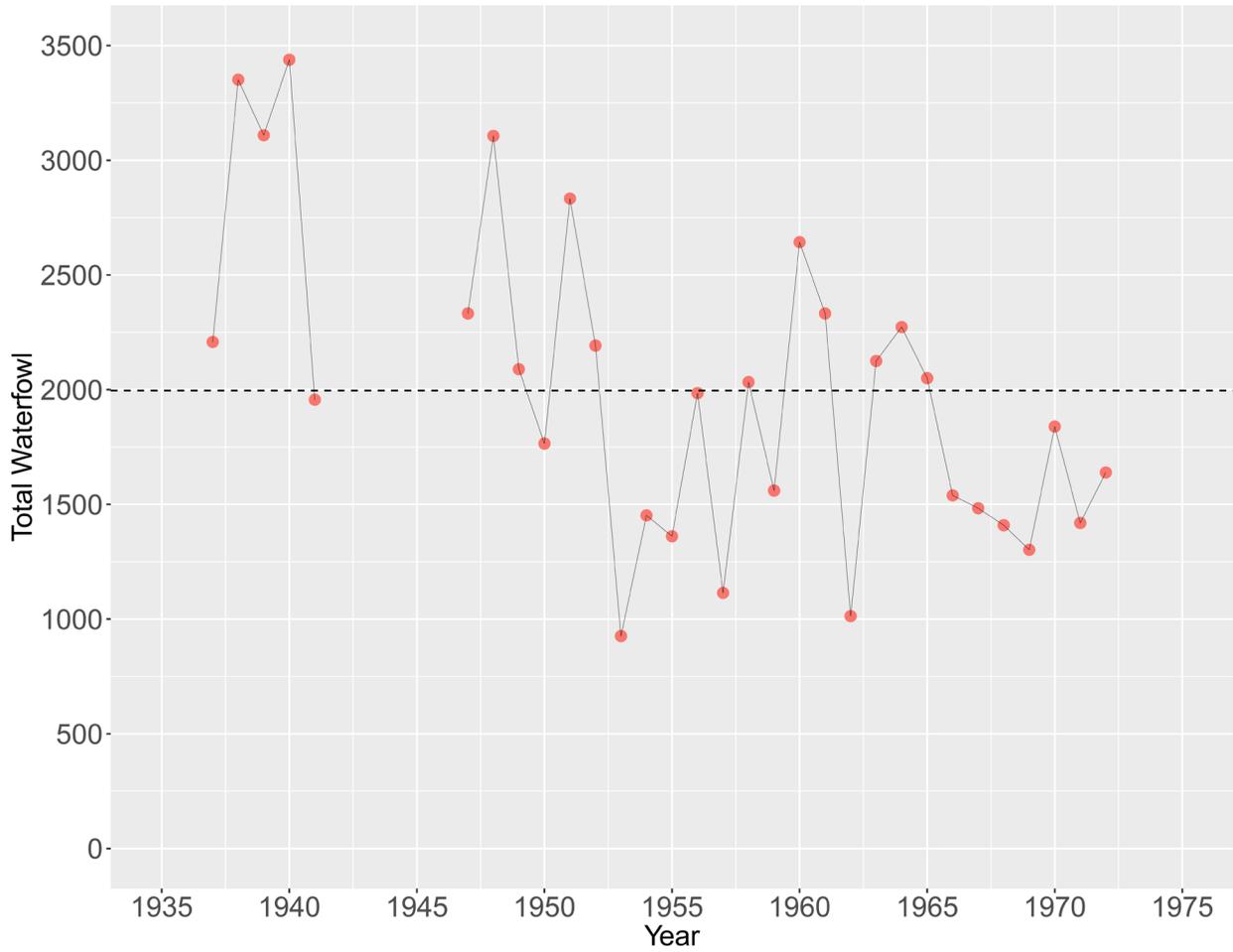


Figure 4. Total number of waterfowl observed on 10 lakes and flowages in the Chippewa National Forest, Minnesota, 1937-1972. Dashed line represents the average total count for these years. The survey was not conducted 1942-1946 due to World War II.

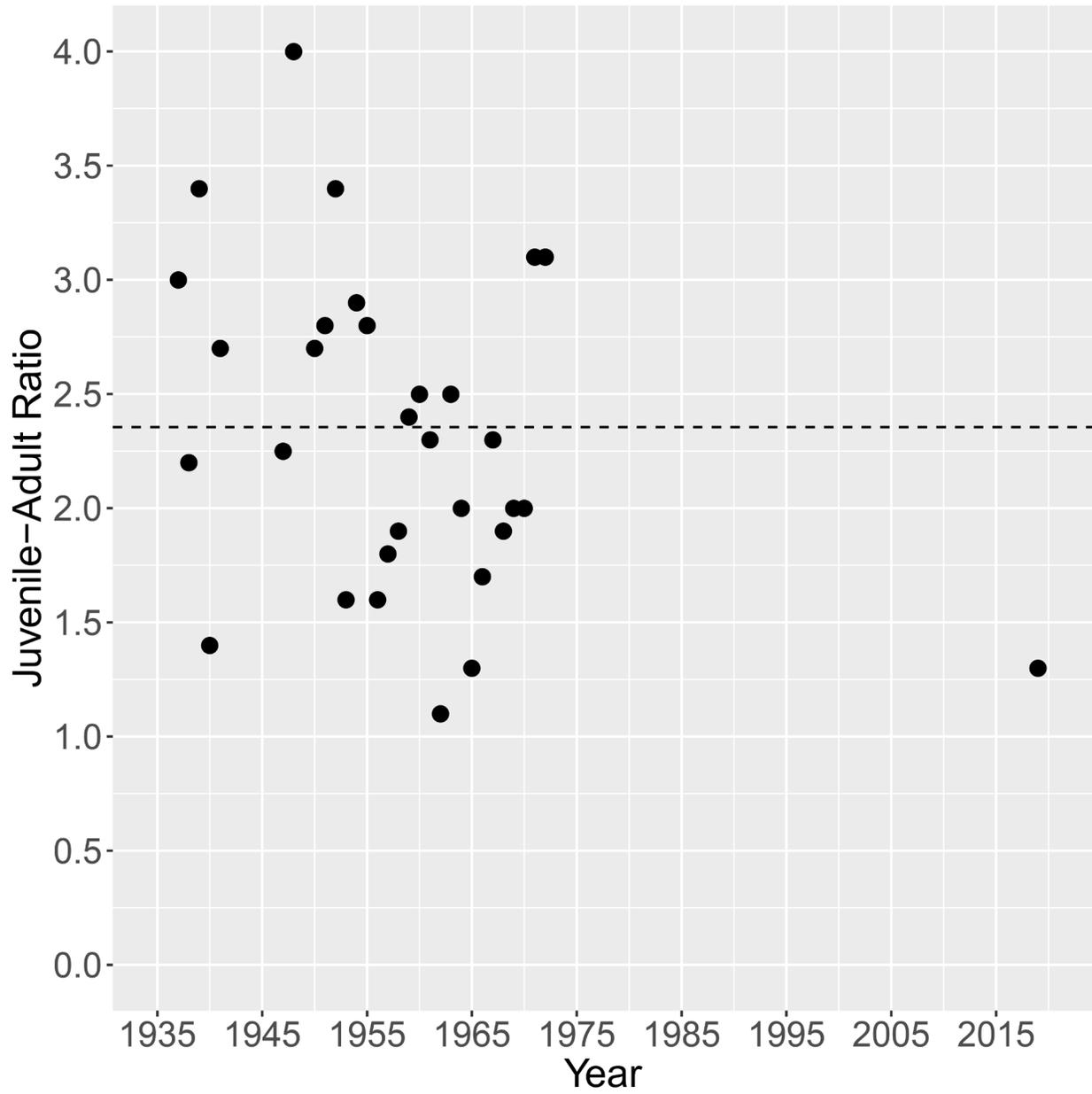


Figure 5. Juvenile to adult ratio of waterfowl observed on 10 lakes in the Chippewa National Forest, Minnesota, 1937-1972 and 2019. The historical average ratio (1937-1972) is indicated by a dashed line.

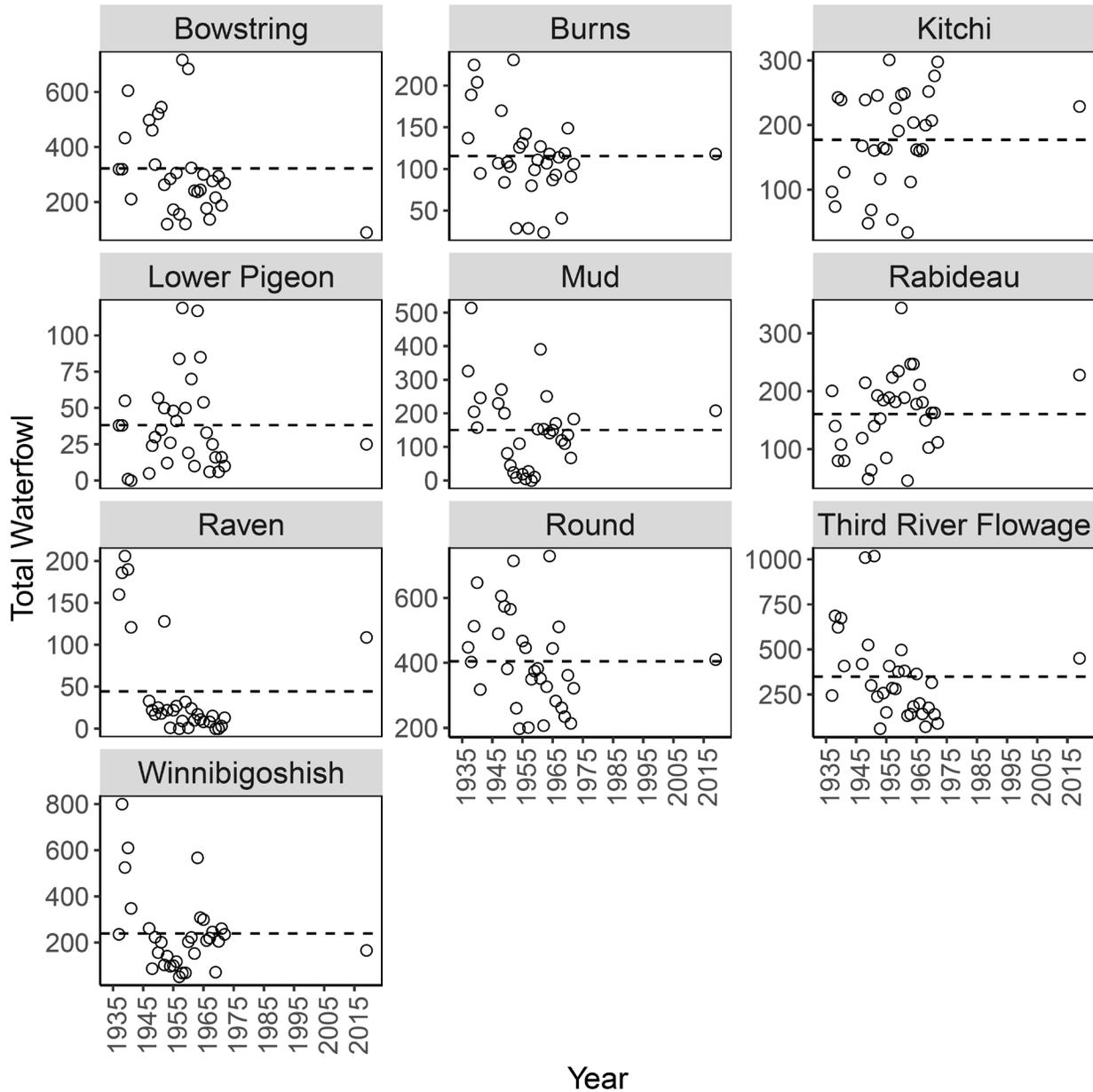


Figure 6. Number of waterfowl observed on 10 lakes and flowages in the Chippewa National Forest, Minnesota, 1937-1972 and 2019. The shoreline of each lake was surveyed for waterfowl broods in July of each year. The shoreline sampled on each lake is indicated in Figure 2. The dashed lines represent the historical average (1937-1972) waterfowl count on the respective lake.

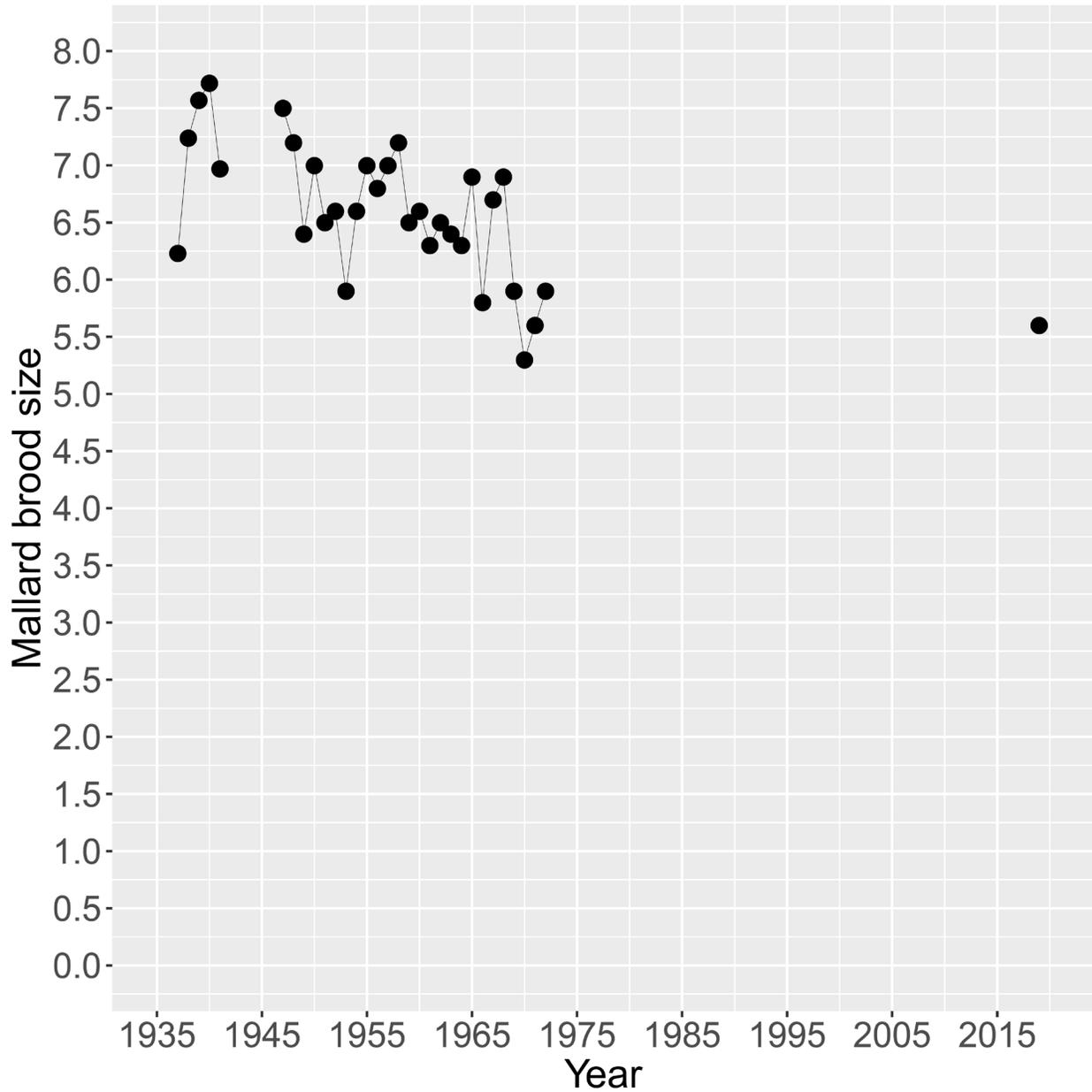


Figure 7. Average number of Mallard ducklings per brood observed on 10 lakes in the Chippewa National Forest, Minnesota, 1937-1972 and 2019. Only broods where the observer could accurately make a complete count of ducklings are included in the average. Hens are not included in the brood number.

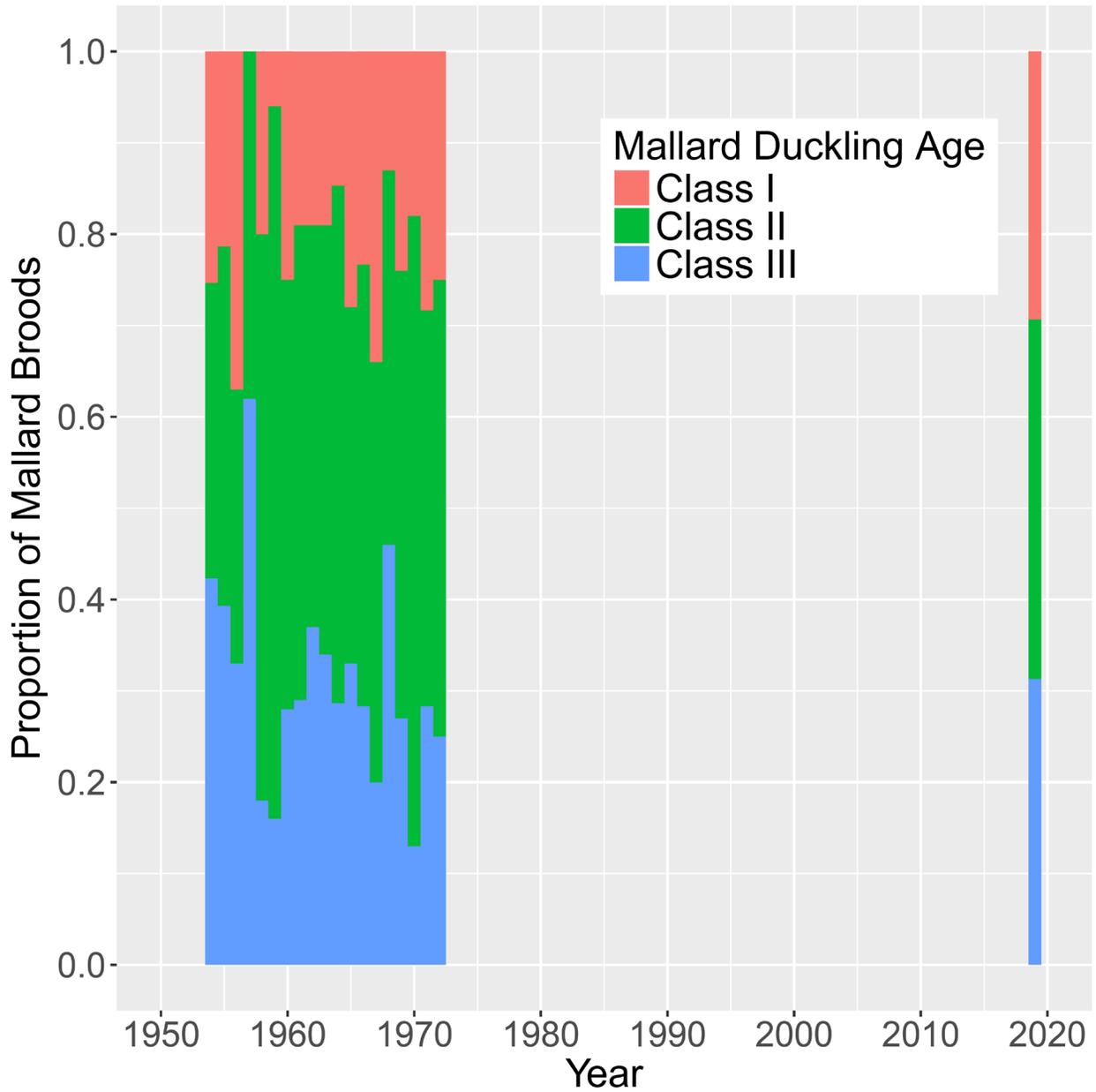


Figure 8. Mallard brood age classes observed on 10 lakes in the Chippewa National Forest, Minnesota, 1937-1972 and 2019. The proportion of broods in each age class is indicated for each year. Class I ducklings are completely down-covered. Class II ducklings have started to develop adult feathers but still have some down. Class III ducklings have little if any down, though are still flightless.

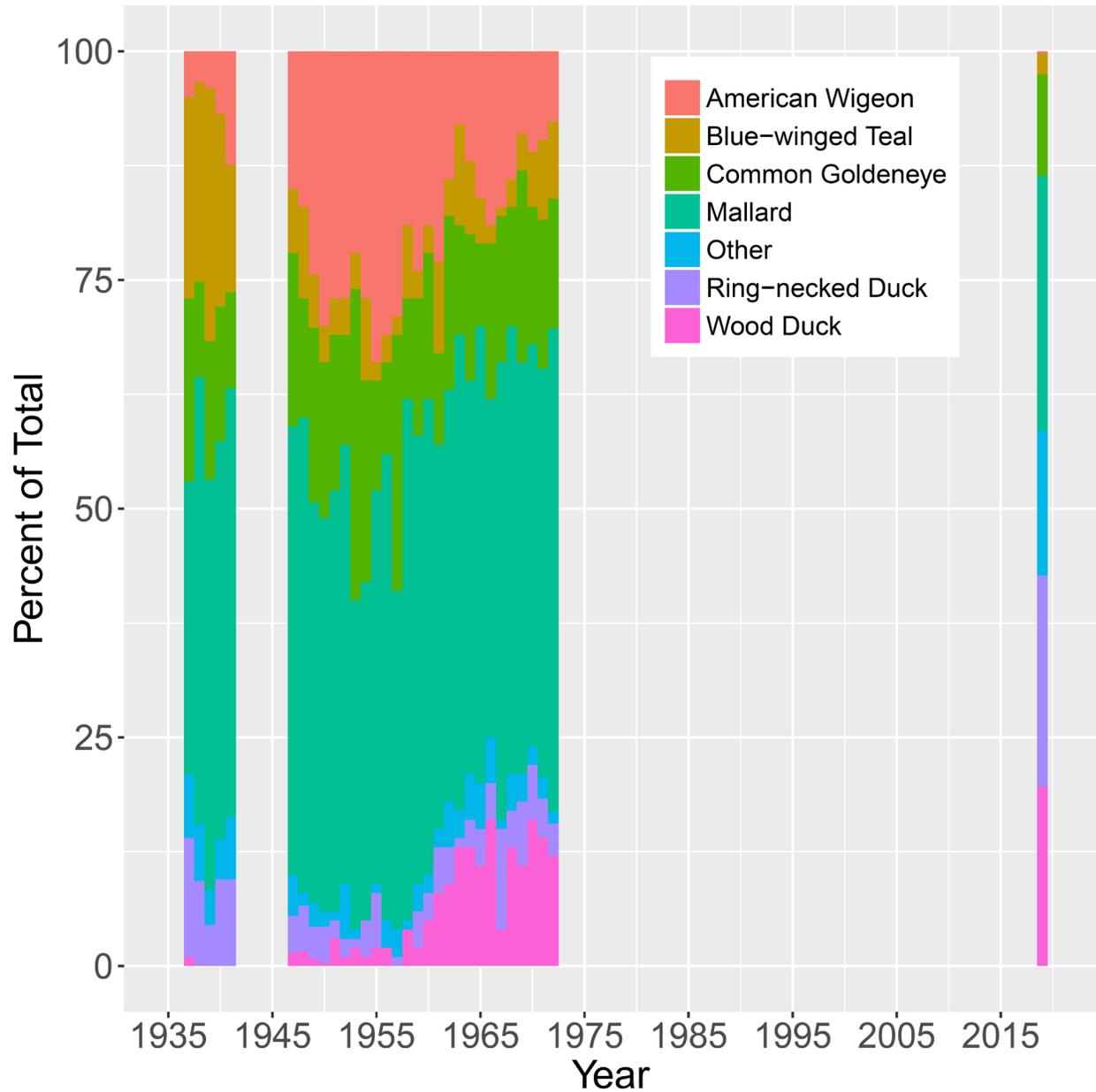


Figure 9. Composition of waterfowl species observed on 10 lakes in the Chippewa National Forest, Minnesota, 1937-1972 and 2019. The percent contribution of the 6 most common species observed historically (1937-1972) is given for each year. All other species are grouped into the 'Other' category. In early years, 'Other' included redhead, ruddy duck, hooded merganser, lesser scaup, northern pintail, green-winged teal and American black duck. In 2019, 'Other' consisted of hooded merganser, Canada goose and trumpeter swan.

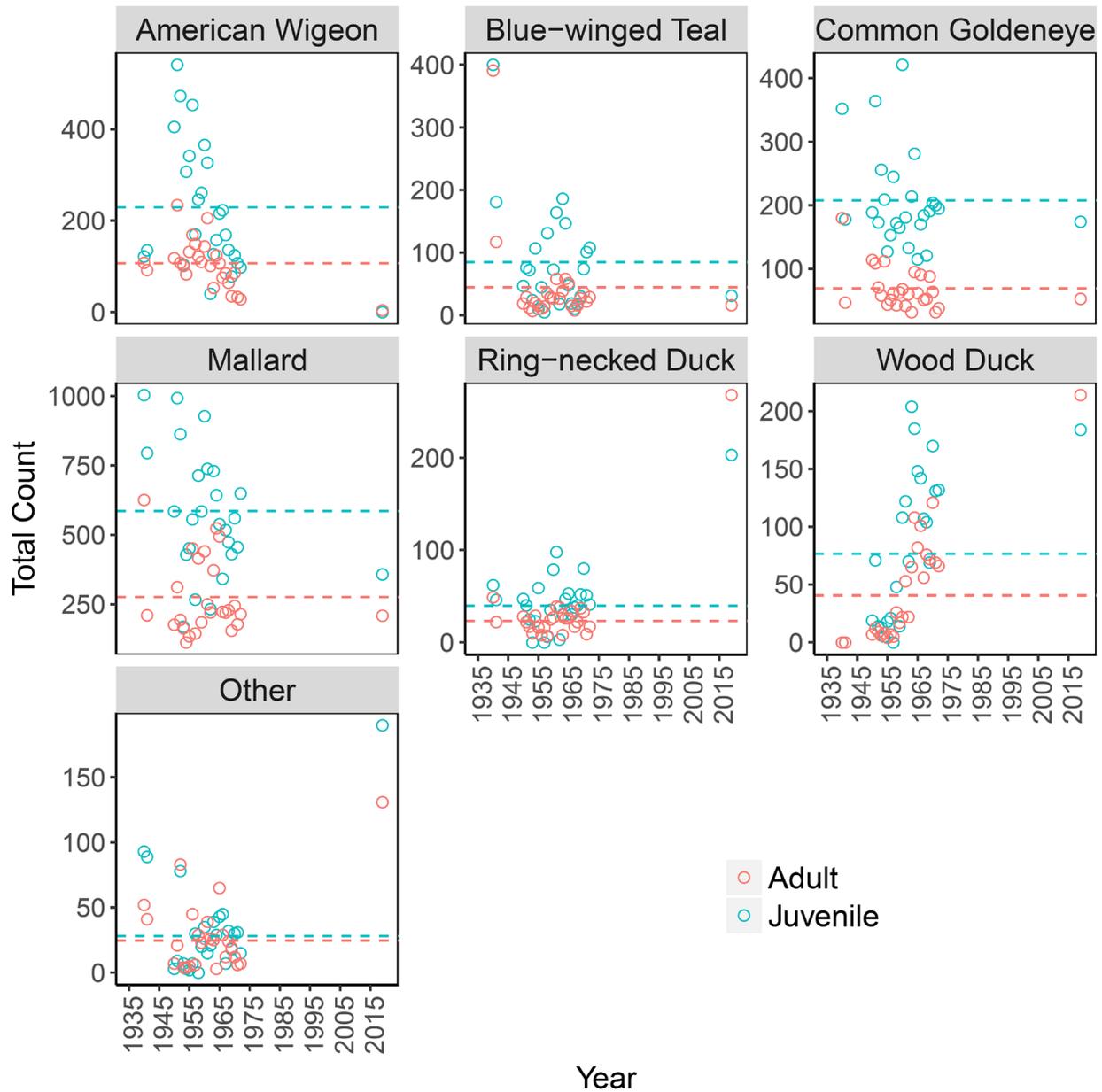


Figure 10. Number of waterfowl observed by species on 10 lakes in the Chippewa National Forest, Minnesota, 1937-1972 and 2019. The 6 most common species in historical counts (1937-1972) are included. For each species, the number of adults and juveniles are indicated. The dashed blue line represents the historical average number of juveniles observed for the given species. The dashed red line represents the historical average number of adults observed for the given species.



## **Publications Lists**



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