Summaries of Wildlife Research Findings 2015



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



SUMMARIES OF WILDLIFE RESEARCH FINDINGS 2015

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ECOLOGY AND POPULATION DYNAMICS OF BLACK BEARS IN MINNESOTA

David L. Garshelis, Andrew Tri, and Brian J. Dirks¹

SUMMARY OF FINDINGS

During April 2015–March 2016, we monitored 22 radiocollared black bears (*Ursus americanus*) at 4 study sites representing contrasting portions of the bear's geographic range in Minnesota: Voyageurs National Park (VNP, northern extreme, poorest food), Chippewa National Forest (CNF; central), Camp Ripley Training Center (southern fringe), and a site at the northwestern (NW) edge of the range. At sites with a higher abundance of foods, bears matured earlier: at NW and Camp Ripley (which has plentiful oaks), >80% of females produced a surviving litter of cubs by age 4, whereas no VNP bears had cubs by age 4. The NW area had the highest reproductive rate, due to an abundance of both agricultural crops and natural foods. Litter sizes of 3 were most common in NW and CNF, whereas litter sizes of 2 were most common in VNP; in Camp Ripley, 3-year-old mothers all had litters of 2, whereas older mothers had an equal proportion of 2- and 3-cub litters. Hunting has been the primary source of mortality in all areas, although vehicle collisions have been a significant source of mortality for bears wandering off Camp Ripley, which is flanked by highways. Camera traps set outside den sites revealed dates of den emergence, and indicated that early, warm springs may prompt bears to leave wet dens.

INTRODUCTION AND STUDY AREAS

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

In 2007, we initiated work in a fourth study site at the northwestern edge of the Minnesota bear range (henceforth NW; Figure 1). This area differs from the other 3 areas in a number of

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

OBJECTIVES

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

METHODS

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

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

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

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

We set remote cameras (camera traps; Reconyx, Inc., Holmen, WI) outside bear dens to gain information about dates and behaviors of bears emerging from dens and departing from the den site (e.g., Do bears just come out and leave, or is it an extended process involving several days, especially when it involves new cubs? Do mothers ever leave cubs unattended and possibly exposed to predation risks during the den emergence period?). Bears that emerged from dens <48 hours after our den visit were excluded from the analysis.

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

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

RESULTS AND DISCUSSION

Radiocollaring and Monitoring

As of April 2015, the start of the current year's work, we were monitoring 19 radiocollared bears: 2 in the CNF, 6 at Camp Ripley, 3 in VNP, and 8 in the NW (Table 1). We trapped 1 new bear this year, and collared 2 female yearlings in dens. Since 1981 we have radiocollared >500 bears, 368 of which were followed until they died (Table 2).

Reproduction

Two collared females in NW produced cubs in 2016. Since all collared females in Camp Ripley produced cubs last year, none produced this year. One VNP bear that was due to have cubs (based on 2-year reproductive cycle) was not checked in the den because we were unable to cross Lake Kabetogama due to poor ice conditions.

Since 1982, within the 4 study areas, we have checked 284 litters with 729 cubs ($\bar{x} = 2.6$ cubs/litter), of which 50.6% were male (Tables 3–6). The sex ratio has become female-biased in all study areas except the CNF, where we have checked an average of only 1 litter per year for the past 10 years. During the most recent decade (2007–2016), the sex ratio of cubs born on all 4 study sites has been female-biased (47%M), whereas it was male-biased (52%M) pre-2007; however, this seeming shift is not statistically significant χ^2 =1.62, *P* = 0.2).

Mortality of cubs during their first year of life averaged 20% (annual range 0–31% for years with at least 10 cubs monitored), with mortality of male cubs (25%) exceeding that of females (16%; χ^2 = 6.28, *P* = 0.01). The timing and causes of cub mortality are unknown.

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

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

Age of first reproduction was dramatically different among areas. By 4 years of age, >80% of bears at Camp Ripley and in the NW had produced surviving cubs (observed in the den at 1 year; Figure 4). Only 36% of bears on the CNF produced surviving cubs by 4 years old and no bears at VNP produced cubs by 4 years of age. Camp Ripley bears sacrificed litter size for earlier age of reproduction (Figures 3 and 4). NW bears had both large litters and early age of first reproduction.

Mortality

Legal hunting has been the predominant cause of mortality among radiocollared bears from all study sites (Table 2). Vehicle collisions are another significant source of mortality at

Camp Ripley, which is flanked by 2 highways.

Despite our request (for the past 15 years) not to shoot collared bears (with large eartags), 2 of 3 collared bears in the CNF were shot by licensed hunters during September 2015 (Table 1). The hunters recognized the bears as being collared, but noted that most of their camera-trap photos were of collared bears, so assumed that most bears in their hunting area were collared; they reasoned that they came from out-of-state and chose to take 2 of them. One other GPS-collared bear was killed in the NW (Thief Lake WMA), also by a hunter who recognized it as being collared.

Camera Trap Photos at Dens

We obtained camera-trap photos of bears that yielded dates of natural emergence and departure from 3 dens in NW, 1 at Camp Ripley, and 2 others outside of our 4 study sites (1 near Park Rapids, 1 near Cloquet). One camera trap outside a den in a culvert under a road at Camp Ripley yielded information on cub survival: we could not handle this bear during the winter because it denned too far into the culvert, but learned from the photographs that this bear's 2-cub litter was reduced to 1 yearling.

In March 2015, 3 bears that we monitored with camera traps all emerged during 13–18 March, whereas in 2016, all but 1 bear emerged earlier (Table 7). In 2015, 1 bear left the den site prematurely after disturbance by people; the other 2 left on 31 March and 1 April. In 2016, the range of dates for departing the den site was wider (11 March–12 April), but the median dates (30 March–2 April) were virtually the same as in 2015. We found no consistent pattern relating den departure date or time loitering at the den site and whether the mother had new cubs or yearlings. All bears remained at the den site (going in and out of the den, except 1 den that flooded) for at least 1 week, and up to 5 weeks (Table 7).

Early spring thaw likely influenced the dates of emergence and departure. In the NW, most of the area is flat lowland, so wet dens may be common. One excavated den that we monitored became completely filled with water, forcing the mother to remove her cubs and tend to them for over a week on a relatively dry mound of soil and brush near the den entrance. She also drank from the pooled water in the den (Figure 5). Another bear raised 3 yearlings in a den under the roots of a fallen tree; early rising water filled the lower half of her den, and she left with her 3 yearlings (Figure 6). Most bears raked additional bedding material into the den (Figure 7); sometimes the yearlings joined in this activity (Figure 8). This seemed like an odd behavior given that the temperatures were warming and the bears were nearing the end of the denning period, but possibly indicated that the dens were getting wet. It has regularly been reported that bears leave den sites earlier with warmer springs, and Miller (2014) observed, using camera traps at dens in Utah, that bears also emerged earlier with warmer springs, but wet dens was not identified as the reason for the earlier emergence.

During the period between first emergence and departing the den site, bears often nursed or played with their offspring (no nursing of yearlings), were vigilant as to their whereabouts, and carried young cubs by their mouth to move them as necessary (Figure 9). One cub of a 2-cub litter died. It was a healthy 6 pounds when we handled it in February but did not leave the den with the mother. We searched the den and found no carcass or bones, so she must have eaten it and defecated when outside the den. Miller (2014) also documented, with camera traps, females consuming offspring at den sites. Absent the camera, we would not have known when this cub had died, as normally we would only observe the litter again the following year in the den. We did not detect any predators at this den, although we did photograph a bobcat (*Lynx rufus*) visiting a den still occupied by bears at Camp Ripley, and last year filmed a wolf (*Canis lupis*) pack investigating an occupied den, also at Camp Ripley.

After the bears left, many other species of wildlife visited the den sites (Figure 10), including raccoons (*Procyon lotor*), porcupine (*Erethizon dorsatum*), white-tailed deer (*Odocoileus virginianus*), snowshoe hare (*Lepus americanus*), red squirrel (*Tamiasciurus*)

hudsonicus), feral cats (*Felis catus*), and ruffed grouse (*Bonasa umbellus*). One bear with yearlings returned to the den area 9 days after departure. The yearlings entered and exited the den for 3 minutes and the family moved on. A wolf (*Canis lupis*) came by 12 days later, but stayed only a few minutes (Figure 10).

Other bears also visited den sites after the original occupants left. We documented 2 different bears visiting a den in the NW. One was a 2-year-old offspring of the mother that left the den site 3 days before (Figure 11). This was the den that had flooded, so the mother's scent on the soil and trees at the den entrance may have attracted the 2-year-old. A second untagged bear investigated the den site 1 month later.

Some bears, after emergence, investigated the camera traps and the heart-monitoring equipment that we left near the den site. One mother opened the box containing the heart monitoring equipment, and its yearling removed the insulation and took it into the den (Figure 12). The family continued to explore the contents of the box for a few days. We plan to continue and expand this monitoring of dens to gauge the effects of changing spring weather conditions on den emergence.

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	CNF	Camp Ripley	VNP	NW
Collared sample April 2015	2	6	3	8
Killed as nuisance				
Killed in vehicle collision		1		
Killed by Minnesota hunter ^a	2			1 ^b
Natural mortality				
Dropped collar				
Failed radiocollar				
Lost contact ^c				2
Collared in den		1		1
Trapped	1			
Collared sample April 2016	1	6	3	6

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 2015-March 2016.

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

^b With a GPS collar that failed prematurely. ^c Due to radiocollar failure, unreported kill, or long-distance movement (the 2 lost collared bears in NW were, oddly, a mother and her yearling)

Table 2. Causes of mortality of radiocollared black bears ≥1 year old in 4 Minnesota study sites, 1981–2016. Bears did not necessarily die in the area where they usually lived (e.g., hunting was not permitted within Camp Ripley or VNP, but bears were killed by hunters when they traveled outside these areas).

	CNF	Camp Ripley	VNP	NW	All combined
Shot by hunter ^a	227	12	15	13	267
Likely shot by hunter ^b	8	1	0	4	13
Shot as nuisance	22	2	1	3	28
Vehicle collision	12	9	1	3	25
Other human-caused death	9	1	0	0	10
Natural mortality	8 ^c	3	5	0	16 ^c
Died from unknown causes	4	2	0	3	9
Total deaths	290	30	22	26	368

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

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

^cOnly 1 bear died of "old age".

Year	Litters	Number of	Mean	% Male	Mortality
real	checked	cubs	cubs/litter	cubs	after 1 year ^a
1982	4	12	3.0	67%	25%
1983	7	17	2.4	65%	15%
1984	6	16	2.7	80%	0%
1985	9	22	2.4	38%	31%
1986	11	27	2.5	48%	17%
1987	5	15	3.0	40%	8%
1988	15	37	2.5	65%	10%
1989	9	22	2.4	59%	0%
1990	10	23	2.3	52%	20%
1991	8	20	2.5	45%	25%
1992	10	25	2.5	48%	25%
1993	9	23	2.6	57%	19%
1994	7	17	2.4	41%	29%
1995	13	38	2.9	47%	14%
1996	5	12	2.4	25%	25%
1997	9	27	3.0	48%	23%
1998	2	6	3.0	67%	0%
1999	7	15	2.1	47%	9%
2000	2	6	3.0	50%	17%
2001	5	17	3.4	76%	15%
2002	0	0	_	_	_
2003	4	9	2.3	22%	0%
2004	5	13	2.6	46%	33%
2005	6	18	3.0	33%	28%
2006	2	6	3.0	83%	33%
2007	2	6	3.0	67%	17%
2008	1	3	3.0	100%	33%
2009	1	3	3.0	33%	33%
2010	1	4	4.0	100%	50%
2011	1	4	4.0	25%	50%
2012	1	3	3.0	67%	33%
2013	1	3	3.0	67%	0%
2014	1	3	3.0	67%	b
2015	0	0 0	_	_	_
2016	õ	Ő	_	_	_
Overall	179	472	2.6	53%	19%
Overall	1/9	412	2.0	JJ 70	13%

Table 3. Black bear cubs examined in dens of radiocollared mothers in or near the Chippewa National Forest during March,1982–2016. High hunting mortality of radiocollared bears severely reduced the sample size in recent years.

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

Table 4. Black bear cubs examined in dens in northwestern Minnesota during March, 2007–20

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%	
Overall	30	83	2.8	45%	18% ª

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

Year	Litters	Number of	Mean	% Male	Mortality
	checked	cubs	cubs/litter	cubs	after 1 year
1992	1	3	3.0	67%	0%
1993	3	7	2.3	57%	43%
1994	1	1	1.0	100%	_
1995	1	2	2.0	50%	0%
1996	0	0	_	—	—
1997	1	3	3.0	100%	33%
1998	0	0	_	—	_
1999	2	5	2.5	60%	20%
2000	1	2	2.0	0%	0%
2001	1	3	3.0	0%	33%
2002	0	0	_	—	_
2003	3	8	2.7	63%	33%
2004	1	2	2.0	50%	—
2005	3	6	2.0	33%	33%
2006	2	5	2.5	60%	_
2007	3	7	2.3	43%	0%
2008	2	5	2.5	60%	0%
2009	3	7	2.3	29%	29%
2010	2	4	2.0	75%	25%
2011	3	8	2.7	50%	25%
2012	1	2	2.0	100%	0%
2013	6	14	2.3	50%	21%
2014	1 ^b	b	—	—	—
2015	6	15	2.5	20%	10%
2016	0	0	—	—	—
Overall	46	109	2.4	48%	20%

Table 5. Black bear cubs examined in dens in or near Camp Ripley Training Center during March, 1992–2016.

^a Blanks indicate no cubs were born to collared females or collared mothers with cubs died before the subsequent den visit to assess cub survival.

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

Table 6. Black bear cubs examined in dens in Voyageurs National Park during March, 1999–2016. All adult collared females were killed by hunters in fall 2007, so no reproductive data were obtained during 2008–2009.

Year	Litters checked	Number of cubs	Mean cubs/litter	% Male cubs	Mortality after 1 year ^a
1999	5	8	1.6	63%	20%
2000	2	5	2.5	60%	80%
2001	3	4	1.3	50%	75%
2002	0		_	_	_
2003	5	13	2.6	54%	8%
2004	0		_	_	_
2005	5	13	2.6	46%	20%
2006	1	2	2.0	50%	0%
2007	3	9	3.0	44%	_
2008	0		_		_
2009	0		_		_
2010	1	2	2.0	50%	0%
2011	1	2	2.0	0%	0%
2012	1	2	2.0	0%	50%
2013	1	2	2.0	50%	_
2014	1	3	3.0	33%	0%
2015	0	0	_	_	_
2016	0 ^b	0	_	_	_
Overall	29	65	2.2	48%	25%

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

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

Table 7. Timing of den emergence and departure recorded by camera traps at dens of 6 adult female bears in Minnesota, February-March 2016.

	Bear ID					
Parameter	4128	2130	4064	4061	4087	5005
Area	Two Inlets	Camp Ripley	NW	NW	NW	Cloquet
Offspring	1 cub	3 yearlings	3 yearlings	3 cubs	3 cubs	3 yearlings
Den type	excavated	excavated	root mass	excavated	excavated	excavated
Date of research handling	22 Feb	16 Dec 15	3 Mar	4 Mar	5 Mar	12 Mar
Date of first emergence	28 Feb	25 Feb	7 Mar	10 Mar ^a	12 Mar	24 Mar
Date of first offspring emergence	30 Mar	25 Feb	3 Mar	12 Mar ^a	26 Mar	24 Mar
Date of departure from site	3 Apr	30 Mar	11 Mar	21 Mar	2 Apr	12 Apr
Order of den departure	5	3	1	2	4	6
Days between emergence and departure from site	35	34	8	11	7	19

^a Den flooded 12 March.

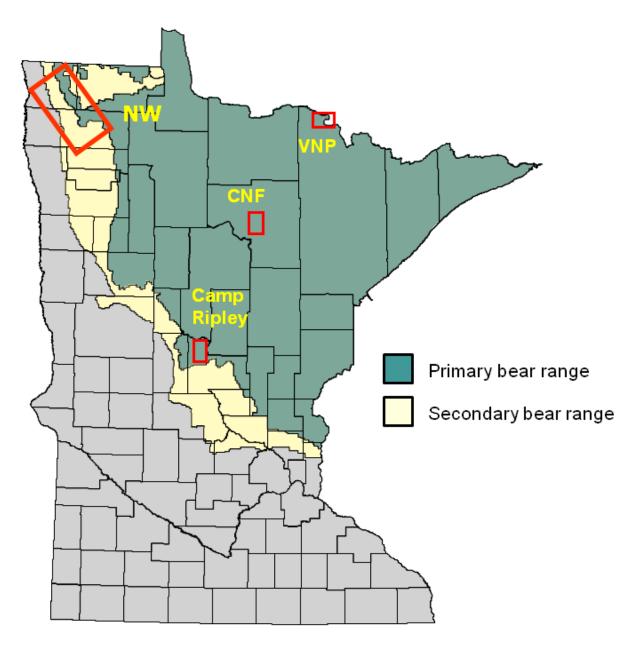


Figure 1. Location of 4 study sites within Minnesota's bear range: CNF (Chippewa National Forest, central bear range; 1981–2016); VNP (Voyageurs National Park, northern fringe of range; 1997–2016); Camp Ripley Military Reserve (near southern edge of range; 1991–2016); NW (northwestern fringe of range; 2007–2016).

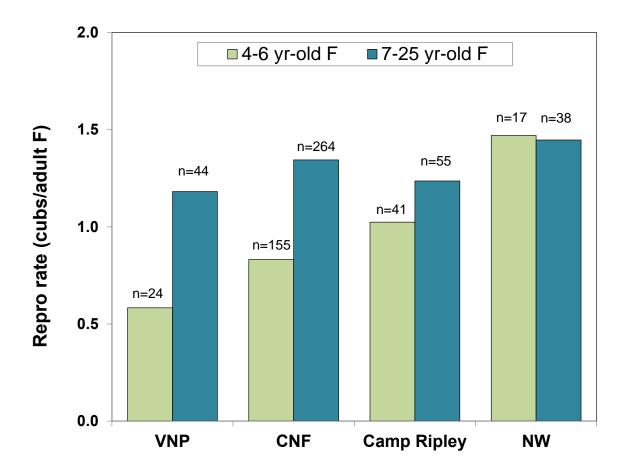


Figure 2. Reproductive rates of radiocollared bears within 4 study sites (see Figure 1) through March 2016 (VNP since 1997, CNF since 1981, Camp Ripley since 1991, NW since 2007). Data include only litters that survived 1 year (even if some cubs in the litter died). Sample sizes refer to the number of female bear-years of monitoring in each area for each age group. Some bears in CNF, Camp Ripley, and NW produced cubs at 3 years old, but are not included here.

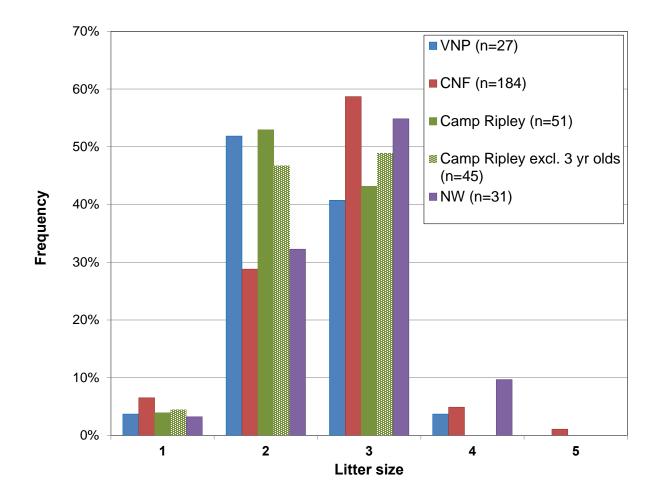


Figure 3. Frequency of cub litter sizes (examined in natal dens in March) within 4 study sites (see Figure 1) through March 2016. Data include only litters that survived 1 year (even if some cubs in the litter died). Camp Ripley data are shown for mothers of all ages, as well as excluding 3-year-old mothers. For the other sites, elimination of 3-year-olds did not make a difference.

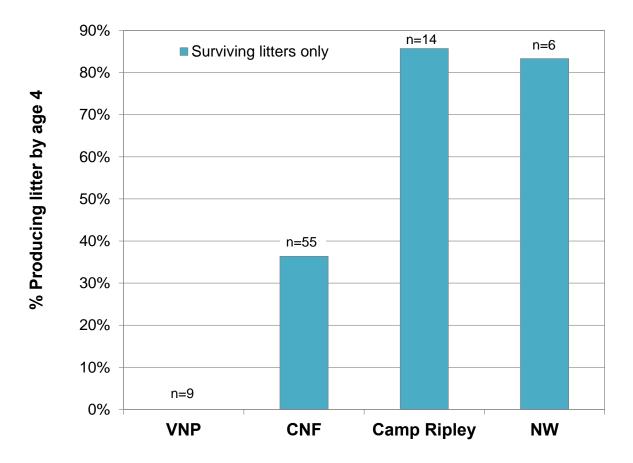


Figure 4. Percent of radiocollared females on each study site that produced a surviving litter of cubs by 4 years old. Births of cubs were detected in natal dens in March each year (through March 2016). A surviving litter was one in which at least one yearling was present in the mother's den the next winter. Note that no females in VNP produced cubs by 4 years of age.



Figure 5. A camera-trap photo captured a female bear drinking water from her former den that was flooded by warm temperatures during spring 2016.



Figure 6. A female and her 3 yearlings departed their wet root den during spring 2016.



Figure 7. In spring, a female bear amassed a sizeable mound of additional grass for bedding in the den with her 3 cubs. The solar panel was used to power heart-monitoring equipment.



Figure 8. A female bear and yearling raked additional grass for bedding in the den.



Figure 9. Camera traps revealed when bears first emerged from dens, when they left the den site, and their activities between emergence and departure. Mothers spent considerable time nursing and watching their cubs climb. Sometimes they picked up cubs with their mouth to bring them back to the den if they started wandering away (upper right). Mothers also played with their yearlings (bottom left).



Figure 10. A variety of species visited the den sites after the bears left. Wildlife smelled the dens, but used the dens for multiple purposes: climbing grounds, foraging browse, dust bathing, or breeding grounds.



Figure 11. A 2-year-old bear (bottom, identified by its eartags) visited the den site of her mother 3 days after the mother left. It sniffed the same tree (at the same spot) that the mother sniffed after she emerged from the den, 2 weeks prior.



Figure 12. A mother and her yearlings dismantled the box of heart monitoring equipment outside their den. This equipment relays the heart data from the mother to a station in Minneapolis, via a cell phone relay mounted on the tripod with the solar panel.



HAIR-SNARING POPULATION ESTIMATE OF BLACK BEARS IN THE CHIPPEWA NATIONAL FOREST: SUBSAMPLING FOR COST EFFICIENCY WITH SPATIALLY EXPLICIT CAPTURE-RECAPTURE MODELS

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SUMMARY OF FINDINGS

Genetic mark-recapture studies estimate animal abundance using non-invasive DNA identification methods to "capture" and subsequently "recapture" individuals that leave genetic material at trap sites. Due to the cost of genotypic analysis, researchers often choose to process only a subsample of this genetic material. Traditional (nonspatial) mark-recapture estimators of abundance have been shown to be biased in this case, especially when the study population displays a behavioral trap response that varies at the individual level. Less is known about the influence of subsampling genetic mark-recapture data, randomly or non-randomly, when using spatially explicit capture-recapture (SECR) models to estimate abundance. We analyzed hairsnare data obtained from a 2012 genetic mark-recapture study of black bears (Ursus americanus). We simulated the process of subsampling hair samples either randomly or nonrandomly (i.e., ensuring that at least 1 sample was chosen from all sites where bears left hair each session). Similar to non-spatial mark-recapture estimators, subsampling produced density estimates that were lower, on average, than the full data estimate; however, non-random subsampling had much less of an effect on estimator performance, particularly at small sample sizes. Thus, non-random subsampling may be preferable to random sampling, despite the inherent violations of SECR assumptions that may result.

To obtain the hair samples, we erected corrals consisting of 2 strands of barbed wire encircling bait and scent in 121 1-mi² cells in the Chippewa National Forest of north-central Minnesota. Hairs were collected from barbs in 6 sampling sessions at intervals of 10 days, from 25 May to 19 July, 2012. We obtained 2,784 samples (barbs) of hair, of which 1,019 were genotyped. We identified 43 bears (26 M, 17 F), with individuals detected at 1–22 hair traps. SECR models that included sex and behavior as covariates influencing detection rates yielded a density estimate of 14 bears/100 mi². This compares to 1986–1989, when 53–72 bears (excluding cubs) occupied the same area, yielding an average density of 52 bears (excluding cubs)/100 mi² (derived by Petersen mark–recapture, weighting each radiocollared bear by the amount of time it resided in the defined study area; Garshelis 1992).

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The purpose of the hair-snaring in 2012 was to assess the extent of population decline. Several differences, though, between the 1980s and 2012 estimates, namely the use of physical capture in the 1980s versus hair-captures in 2012, and the use of telemetry-based adjustments to calculate density in the 1980s versus SECR in 2012, may account for some difference in the estimates. Nonetheless, it is apparent that the population was considerably lower in 2012.

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QUANTIFYING THE RELATIONSHIP BETWEEN GRASSLANDS, CONSERVATION RESERVE PROGRAM (CRP) ENROLLMENTS AND GREATER PRAIRIE-CHICKEN POPULATIONS (*Tympanuchus cupido pinnatus*) IN MINNESOTA

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SUMMARY OF FINDINGS

The Conservation Reserve Program (CRP) has multiple objectives, one of which is to provide habitat for wildlife. We aim to quantify greater prairie-chicken (*Tympanuchus cupido pinnatus*) population responses to CRP enrollments using population indices (males/lek and leks/km²) derived through annual monitoring efforts in Minnesota. We will quantify land cover during the period 2004–2014 in survey blocks where systematic greater prairie-chicken surveys were conducted during the same period to evaluate the contribution of CRP enrollments to available grassland habitat and estimate changes through time. In addition, we will evaluate existing vegetation characteristics of grassland CRP conservation program practices to assess how different CRP management strategies are related to greater prairie-chicken abundance and lek persistence. To date, we have quantified grassland CRP contract data in each survey block for the study period and developed a land cover map that will be ground-truthed during Jun-Aug 2016. We will also measure vegetation composition and structure of CRP plantings during summer 2016. Spatial analyses will begin in fall 2016. Information resulting from this study will aid the Farm Service Agency (FSA) and other organizations in targeting conservation programs in areas where they will be most effective for greater prairie-chickens.

INTRODUCTION

Agriculture programs such as the Conservation Reserve Program (CRP) have the potential to influence the cover-type composition in agriculturally dominated landscapes. Established in 1985, the CRP was authorized to remove up to 18 million ha of highly erodible land from crop production. It has the stated objectives to reduce soil erosion, improve water quality, and restore and protect wildlife habitats. These objectives are accomplished by providing financial incentives to convert agricultural land to a more natural state by reseeding to sod-forming or ecologically native vegetation for 10–15 years (Riley 2004, Stubbs 2014). In the Upper Midwest, the CRP emphasizes grassland cover types, and although the protection and restoration of wildlife habitat is a stated objective of the CRP, the impacts of the CRP on grassland birds are not always clear.

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Intuitively, if conversion of grasslands and intensification of agriculture are leading threats to grassland birds, then areas enrolled in the CRP should contribute to the conservation of these species. Many studies have confirmed that areas enrolled in the CRP help provide critical breeding habitat for grassland birds, in particular grassland obligate birds (Ryan et al. 1998a, Ryan et al. 1998b, Heard et al. 2000, Riley 2004, Herkert 2009, Evans and Potts 2015). However, population-level impacts are less studied (Ryan 2000, Haroldson et al. 2006, Nielson et al. 2008) and not always demonstrated (Rodgers 1999, Roseberry and David 1994, Giudice and Haroldson 2007). Furthermore, McCoy et al. (1999) concluded that the CRP likely contributed to conservation of many grassland bird species in Missouri, notably several of high conservation concern, but also served as sink habitat for other species of grassland birds. The relationship between greater prairie-chickens and CRP enrollments has been previously studied in Minnesota; Toepfer (1988) observed greater prairie-chickens nesting in CRP grasslands and non-native grasslands in Minnesota. Use of CRP grasslands and other grasslands by greater prairie-chickens fluctuated in relation to the habitat quality determined by both the type of grass-forb mix planted and management techniques employed (Rosenquist 1996, Svedarsky et al. 1997). Merrill et al. (1999) reported that areas enrolled in the CRP likely had a role in providing greater prairie-chicken habitat, based on observation of a significantly larger proportion of the landscape enrolled in the CRP surrounding leks than random non-lek points in northwestern Minnesota. Merrill et al. (1999) also reported that smaller amounts of residential areas, farmsteads, and forests and greater amounts of area enrolled in the CRP were associated most strongly with presence of greater prairie-chicken leks. Niemuth (2003) suggested that spatially explicit models based on landscape characteristics of leks could be used to target CRP grassland establishment in Wisconsin to increase suitable habitat. Conversely, Klute et al. (1997) observed that although overall abundance and species richness of grassland birds were greater in CRP grasslands than pasture, greater prairie-chicken mean abundance was not significantly different between CRP grasslands and pasture in Kansas. Toepfer (University of Wisconsin Stevens Point, unpublished data) also reported that nesting success was lower in CRP grasslands than native grasslands.

Area enrolled in the CRP has declined nationwide since its peak enrollment of approximately 15 million ha in 2007 (Stubbs 2014). This decrease will continue because the 2014 Farm Bill reduced the enrollment cap from approximately 13 million ha to <10 million ha by 2018 (Stubbs 2014). These reductions have raised the concern of partners in annual monitoring efforts for greater prairie-chickens in Minnesota. Because greater prairie-chickens are a Species of Special Concern in Minnesota and an indicator species for upland prairie and grassland (Minnesota Prairie Plan Working Group 2011), conservation programs have been specifically designated to protect and restore their habitat. Greater prairie-chickens have been listed as one of the high priority species identified in the State Acres for Wildlife Enhancement (SAFE) Program in Minnesota and Back Forty Pheasant Habitat CRP-SAFE practice (USDA 2008). To make informed decisions about how to target these conservation programs, the Farm Service Agency (FSA), which administers the CRP, and other conservation organizations require information about how greater prairie-chickens respond to grassland in agricultural landscapes, and where program activities are likely to have the most impact.

Greater prairie-chickens are considered grassland obligate birds, but some available grassland cover types may not meet habitat requirements (Jones 1963, Niemuth 2000, McNew et al. 2015). Nationwide, millions of ha of area enrolled in the CRP may be available, but most are not considered high-quality habitat for greater prairie-chickens because the seeding mix planted or subsequent management plan for the planting does not result in high-quality habitat

conditions (Niemuth 2003, Burger et al. 2006, McNew et al. 2015). Greater prairie-chickens have multiple habitat requirements including areas for day resting, night roosting, courtship, nesting, and brood rearing requiring a mosaic of vegetation composition and structural components (Jones 1963, McNew et al. 2015). In addition, the spatial configuration of these components influences their impact on prairie-chickens (Ryan et al. 1998, Merrill et al. 1999, Winter et al. 2006, Nielson et al. 2008). Because vegetation composition, structure, management, and spatial configuration influence survival and population growth rate of greater prairie-chickens, it is necessary to understand how these factors are related to greater prairie-chickens in specific landscapes (Jones 1963, Niemuth 2000, McNew et al. 2015).

OBJECTIVES

- 1. Quantify the relationship between greater prairie-chicken population indices (i.e., males/lek and leks/km²) during 2004–2014 and landscape composition and configuration, with a focus on the amount and distribution of grassland and CRP enrollments.
- 2. Develop a model linking compositional and structural diversity of vegetation in grassland CRP categories within the greater prairie-chicken range in western Minnesota to population indices (i.e., males/lek and leks/km²) during 2004–2014.
- 3. Identify areas, including CRP expirations within the greater prairie-chicken range in western Minnesota, that can be prioritized for greater prairie-chicken conservation.

STUDY AREA

Our study focuses on the greater prairie-chicken survey area in western Minnesota. Greater prairie-chicken surveys have been coordinated by the Minnesota Department of Natural Resources (MNDNR) and executed annually in collaboration with Minnesota Prairie-chicken Society (MPCS), The Nature Conservancy (TNC), U. S. Fish and Wildlife Service (USFWS), and other volunteers since 2004. Survey biologists use a standardized protocol in 7 western Minnesota counties including Red Lake, Polk, Norman, Mahnomen, Clay, Becker, Wilkin, and Otter Tail counties (Giudice 2004). Within these counties, 17 41-km² survey blocks (Fig. 1) were non-randomly selected to include prairie-chicken habitat across the greater prairie-chicken range (Giudice 2004). Two of the 17 blocks are composed of a majority of state and federally managed lands, 5 blocks are areas that were mostly under CRP contract in 1997, and 10 blocks are areas that have a mixture of CRP enrollment, state, federal, and TNC lands.

METHODS

Land Cover Data

Shapefiles for CRP enrollments and corresponding conservation practice codes were obtained from FSA for 2006–2011, 2013, and 2014 through a Memorandum of Understanding between the MNDNR and FSA. Shapefiles were missing for the years 2004, 2005, and 2012. We reconstructed data for missing years in ArcGIS (ERSI 2015) by examining contract expiration dates provided in the available shapefiles and aerial photography. Because the shapefiles obtained from FSA included all CRP practice codes within the survey blocks, the CRP practice codes that provide suitable grassland habitat were distinguished using the classification categories of Nielson et al. (2008) and Drum et al. (2015). These practice codes include CP 1, 2, 4D, 8A, 10, 12, 18, 18B, 18C, 21, 23, 23A, 25, 27, 28, 30, 38E, and 42.

Because CRP grasslands may not be the only land cover type that provides suitable greater prairie-chicken habitat, non-CRP grassland cover will also need to be identified and quantified within the study area over the period of interest. To evaluate cover types, we will use the Minnesota land cover classification (MLCC) and impervious surface area based on LANDSAT and LiDAR: 2013 update (UMN-MLCC 2013), NASS Cropscape Cropland Data Layer (CDL; USDA-NASS 2015), National Land Cover Database (NLCD), infrared imagery, and LiDAR data layers in ArcGIS. We will also use site histories of state, federal, and TNC managed areas. We reclassified the CDL data layer and MLCC layer in each prairie-chicken survey block into 7 vegetation classes (i.e., grassland, forest, developed/barren, shrubland, open water, cropland, wetland) based on the literature on land cover classification of different grassland types (Merrill et al. 1999, Neimuth 2000, Poiani et al. 2001, Neimuth 2003, Giudice and Haroldson 2007, Nielson et al. 2008, Drum et al. 2015, Hovick et al. 2015b). We will ground truth this reclassification during Jun–Aug 2016.

We will use FRAGSTATS (McGarigal et al. 2012), a spatial pattern analysis program, to calculate number of patches, mean patch size, patch edge density, and contiguity of patches of grassland cover types within each survey block and track the changes in these metrics over the period of interest. In addition, we will determine the number and size of natural land-use (i.e., wetland, forest, grassland, CRP) patches within a 2-km buffer around leks to represent the life-cycle habitat radius of greater prairie-chickens and to determine the distance of leks to the patch edge (Merrill et al. 1999, Niemuth 2003, Winter et al. 2006, Nielson et al. 2008, Niemuth 2011, Hovick et al. 2015a).

Greater Prairie-chicken Survey Data

Data from the greater prairie-chicken spring survey consist of count and location information for leks during 2004–2014 within established survey blocks (Fig. 1). We have locations for 58–114 leks per year within these survey blocks that are recorded to the level of quarter-section or GPS point coordinates. We will use the metrics of males/lek and number of leks/km² as indices of greater prairie-chicken abundance, stability, and persistence in the study area. These metrics have been used previously as indices of greater prairie-chicken population size and habitat quality (Hamerstrom and Hamerstrom 1973, Niemuth 2011). Using lek shapefiles in ArcGIS we will assess stability by calculating the number of consecutive years that a lek has had >1 displaying males (Schroeder and Braun 1993) and persistence of a lek by calculating the number of years during the study period (not necessarily consecutive) that >1 male displayed (Merrill et al. 1999).

Vegetation Measurements

We will sample vegetation in the context of factors that can be assessed remotely and that may influence habitat suitability of CRP enrollments for greater prairie-chickens, including CRP grassland conservation practice (CP) code, time since planting (0–4, 5–9, 10–14, 15–19, ≥20 years), soil type (sand, loam, clay, muck, silt), type of management (prescription burn, mowing, haying, woody removal, chemical, other), seed mix prescribed (introduced grass, introduced grass and legumes, native grasses, native grasses and forbs/legumes), years since management (0–4, 5–9, 10–14, 15–19, ≥20), previous land cover (crop, CRP), elevation, and slope. The categories of CRP grassland CP code have been determined by analyzing which CRP grassland practices constitute \geq 2% of each survey block during the 2016 field season. If there are no CRP

grassland practices that constitute \geq 2% of a survey block, no vegetation surveys will occur in that survey block.

We have randomly selected 200 survey points across the study area, stratified by the occurrence of primary variables of grassland CRP category, soil type, and time since planting. Each survey point will serve as the middle of a transect extending 12 m in each cardinal direction, which will be restricted to lie entirely within the same patch of grassland cover. At the center of the transect and at 6-m intervals along each 12-m arm of the transect, we will measure vegetation in 1-m² plots, for a total of 9 1-m² plots per transect (Daubenmire 1959, McNew et al. 2015). We will estimate the frequency of occurrence and percent cover of vegetation categories following the approach of Nack and Andersen (2006) and using a 1-m x 1-m frame divided into 16 cells of 0.0625 m² each. In addition, we will take photographic images from 2 m above ground level at each of the 1-m² plots (Booth et al. 2008). These photographs will serve as a source of data verification for vegetation measurements and allow future possible image analysis as a second means of quantifying cover types. We will describe vegetation structural composition by taking 2 visual obstruction readings at opposite directions along the contour at a distance of 2 m and height of 0.5 m in the center plot and the plots at the end of the 4 12-m arms of each transect to determine vegetation vertical density (Robel et al. 1970, McNew et al. 2015). We will use the mean of the 10 measurements from each transect as a measure of vegetation density for that transect. Vegetation surveys to estimate percent cover will occur once and surveys to estimate visual obstruction and vegetation height will occur twice during Jun-Aug 2016.

Data Analysis

We will use mixed-effects models to analyze the relationship between greater prairiechicken population indices and landscape metrics (Haroldson et al. 2006). We will also develop a habitat suitability model relating vegetation characteristics to remotely measured variables (e.g., soil type) and characteristics of CRP enrollments (e.g., time since planting). We will use the output of that habitat suitability model to predict areas of high quality greater prairie-chicken habitat and use metrics of greater prairie-chicken abundance, lek persistence, and lek stability to assess how well the habitat suitability model predicts those metrics. Based on this habitat suitability model and validation using greater prairie-chicken lek data, we will use remotely measured characteristics discussed above to predict habitat suitability for greater prairie-chickens across the western Minnesota landscape. Finally, we will simulate expirations of grassland CRP contracts to measure the impact of various gain and loss scenarios on greater prairie-chicken habitat.

RESULTS AND DISCUSSION

To date, we have reconstructed survey block grassland CRP contract data for 2004, 2005, and 2012 and will update that classification based on recently released CRP data. All survey blocks (where data are complete at this time) experienced a decline in area enrolled in grassland CRP categories during the period 2004–2014 (Fig. 2). The greatest proportional decline from peak enrollment in 2007 to 2014 was in the Becker 2 block with 72.5% of the former acres lost (789-ha). The survey block with the smallest proportional decline was Norman 1 with a 11.6% decline (106 ha). When grassland CRP data are combined for all survey blocks, there was a 58.8% decrease from 2007 to 2014, and recently obtained, more complete data

show a 65.6% decrease over the same period (Table 1). Preliminary assessment of the relationship between grassland CRP enrollment and greater prairie-chicken abundance data from survey blocks suggests a negative relationship for both males/lek (Fig. 3) and lek/km² (Fig. 4). We have developed a land cover map based on the 7 cover-type classification classes, which will be ground-truthed during Jun–Aug 2016 before spatial analysis begins. Data will be collected during Jun–Aug 2016 on vegetation composition and structure.

Results from this study will be provided to FSA to help target conservation programs in areas where they will be most effective for greater prairie-chicken conservation. Strategic prioritization of enrollments is necessary because more landowners apply than can be enrolled in the CRP. Our results will also provide guidance on the most effective grassland CRP practices, seedings, and management techniques across the landscape for greater prairie-chicken conservation.

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We thank MPCS for initiating greater prairie-chicken surveys in 1974, coordinating surveys through 2003, and their continued participation as survey block coordinators and surveyors. In 2004, John Giudice designed the standardized survey approach with 17 survey blocks and analyzed survey data from 1976 to 2004. Mike Larson coordinated prairie-chicken surveys from 2005 to 2012. Numerous cooperators and coordinators have contributed to annual monitoring efforts over this period, with large contributions by MNDNR staff, MPCS, TNC, USFWS, and many other volunteers including Steve Bommersbach and Dan Svedarsky, who have had a long-term involvement in surveys and MPCS. We also thank David Andersen, James Forester, and John Fieberg for providing guidance and support for quantitative analysis and the University of Minnesota U-spatial help desk for geospatial support. Finally, we would like to thank the staff at Rydell and Glacial Ridge National Wildlife Refuges for providing field housing for northern field sites during our 2016 field season. Surveys and this project are funded in part through the Wildlife Restoration (Pittman-Robertson) Program W-69-S-13 Project #16. This research is being conducted through the Minnesota Cooperative Fish and Wildlife Research Unit (cooperators include the Minnesota Department of Natural Resources, U.S. Geological Survey, University of Minnesota, Wildlife Management Institute, and the U.S. Fish and Wildlife Service), housed in the Department of Fisheries, Wildlife, and Conservation Biology at the Univesity of Minnesota. Mike Larson provided comments that improved this report.

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Year	Hectares of CRP grassland	Percent change from previous year
2004	12,296	N/A
2005	12,537	+1.96
2006	12,677	+1.12
2007	12,746	+0.55
2008	11,612	-8.90
2009	10,067	-12.45
2010	9,549	-6.07
2011	9,280	-2.81
2012	8,262	-10.96
2013	5,771	-30.16
2014	5,251	-9.00

Table 1. Hectares of Conservation Reserve Program (CRP) grassland (practice codes 1, 2, 4D, 8A, 10, 12, 18, 18B, 18C, 21, 23, 23A, 25, 27, 28, 30, 38E, 42) enrolled in greater prairie-chicken survey blocks in Minnesota from 2004 to 2014. Note that data have been reconstructed for 2004, 2005, and 2012 from contract enrollments in other years and are subject to change as updated information on CRP contracts is analyzed.

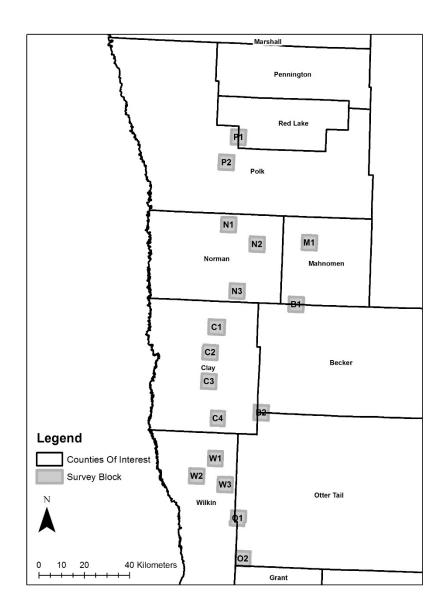


Figure 1. Location of the 17 greater prairie-chicken survey blocks (gray labeled squares, 41 km² each) in western Minnesota. Alpha-numeric codes simply enumerate the blocks by county.

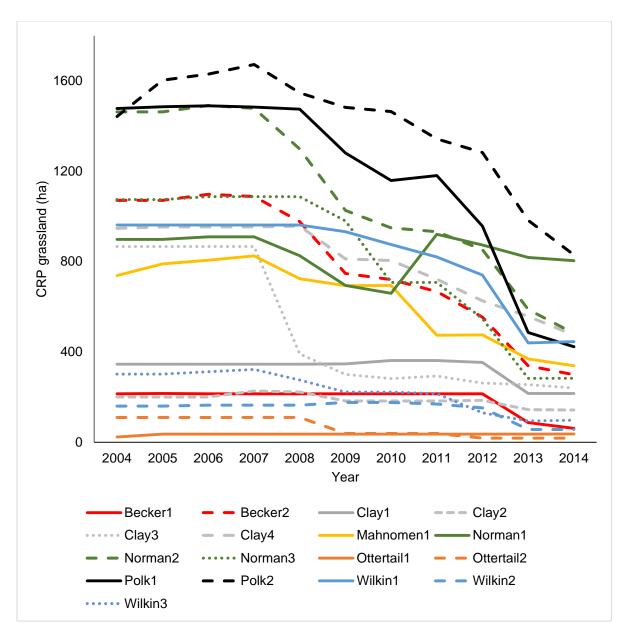


Figure 2. Hectares enrolled in Conservation Reserve Program (CRP) grassland (practice codes 1, 2, 4D, 8A, 10, 12, 18, 18B, 18C, 21, 23, 23A, 25, 27, 28, 30, 38E, 42) in greater prairiechicken survey blocks in Minnesota. Note that data for 2004, 2005, and 2012 have been reconstructed from contract enrollments in other years and are subject to change as updated information on CRP contracts is analyzed.

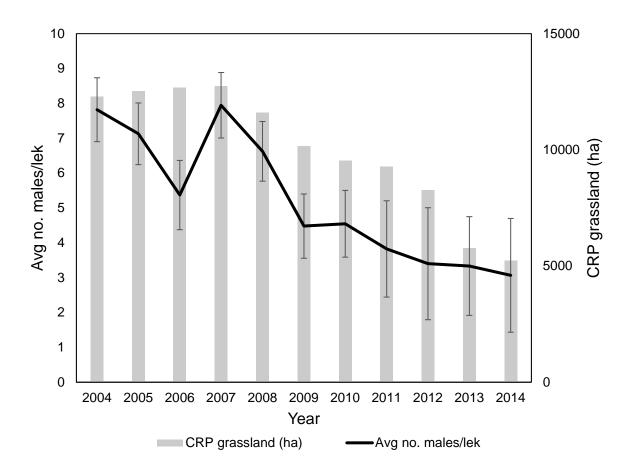


Figure 3. Average number of greater prairie-chicken males/lek in survey blocks in Minnesota with 95% confidence intervals and hectares of grassland Conservation Reserve Program (CRP) enrollments (practice codes 1, 2, 4D, 8A, 10, 12, 18, 18B, 18C, 21, 23, 23A, 25, 27, 28, 30, 38E, 42) in greater prairie-chicken survey blocks in Minnesota.

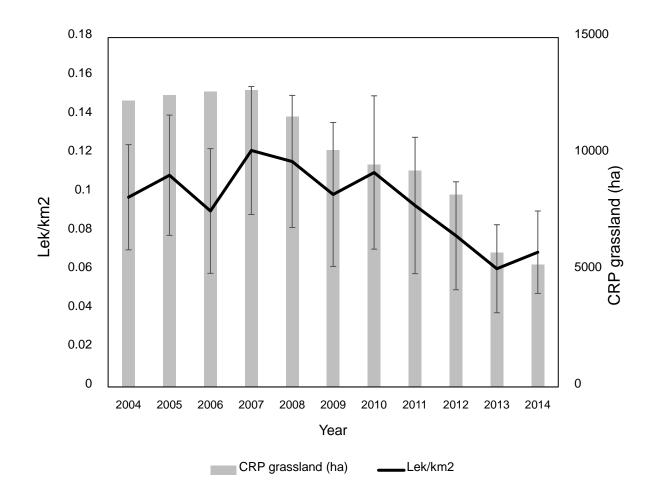


Figure 4. Lek/km² in greater prairie-chicken survey blocks in Minnesota with 95% confidence intervals and hectares of grassland Conservation Reserve Program (CRP) enrollments (practice codes 1, 2, 4D, 8A, 10, 12, 18, 18B, 18C, 21, 23, 23A, 25, 27, 28, 30, 38E, 42) in survey blocks in Minnesota.



SHARP-TAILED GROUSE RESPONSES TO FALL PRESCRIBED FIRE AND MOWING

Charlotte Roy and Lindsey Shartell

SUMMARY OF FINDINGS

We began a 2-year pilot study in August 2015 to examine sharp-tailed grouse (i.e., grouse, *Tympanuchus phasianellus*) responses to habitat management in the fall (mid-August through November). Our study area included the northwest (NW) and east-central (EC) sharp-tailed grouse populations in Minnesota. We studied responses to prescribed fire and mechanical treatment (i.e., mowing) using a Before-After-Control-Impact (BACI) design. In fall 2015, we measured sharp-tailed grouse use and vegetation of 10 managed and 9 control sites prior to and following management. Managed areas included 6 mowing treatments and 4 prescribed burns, ranging in size from 12 to 664 ac (4.9–269 ha) and totaling 1,153 ac (467 ha). We also conducted surveys of sharp-tailed grouse use and vegetation at an additional 10 control sites and 13 sites that were planned to be managed, but for which management could not be completed in 2015 because of unfavorable weather and site conditions. These included 10 planned burns and 3 planned mows, including 2 mows that were attempted but could not be completed due to a high water table.

We conducted surveys of sharp-tailed grouse use 0–28 (mean 9.9) days before management (PRE), 1 week after (1WK), 1 month after (1MO), and the following spring (SP) by conducting fecal pellet transects and documenting grouse observed at the site. We detected sharp-tailed grouse pellets at 2 of the 10 treatment sites and 2 of the 9 control sites prior to treatment. Following treatment, sharp-tailed grouse pellets were detected in \geq 1 survey (1WK, 1MO, or SP) at 6 treatment sites and 3 control sites. Grouse were observed at only 1 treatment site prior to treatment and at 4 treatment sites and 2 control sites in \geq 1 survey post-treatment.

During fall 2016, additional sites will be added to the study. We anticipate that 5-10 years of data collection will be necessary to understand the variables that influence sharp-tailed grouse responses to these types of management actions.

INTRODUCTION

Sharp-tailed grouse rely on early successional habitats of open grass and brushland. Historically, these habitats were created and maintained through periodic wildfire. More recently, fire suppression has played a role in reducing habitat for sharp-tailed grouse (Berg 1997). Prescribed fire has become an important management tool for maintaining open grass and brushlands habitats but can be difficult to implement effectively or safely under many conditions (e.g., too wet, windy, 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.

Historically, fires occurred throughout the year and maintained early successional habitats, such as 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 in grass and shrub vegetation types and understory fires in forest and woodland types occurred at 0- to 10-year intervals (Brown and Smith 2000).

Native Americans referred to the sharp-tailed grouse as the "fire grouse," or "fire bird," because of their association with fire that keeps their habitat open. Sharp-tailed grouse respond to spring prescribed fire treatments; numbers of broods hatched per 100 acres was higher in 2 burned areas compared to an unburned control area the following spring (Kirsch and Kruse 1973). Furthermore, 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 away. Sharp-tailed grouse also return to leks the day after a burn to dance (J. Provost, MNDNR, pers. comm.).

Currently, most prescribed burns on DNR lands in sharp-tailed grouse range occur in the spring (Roy and Shartell, unpubl. data from DNR Wildlife Managers). However, fall may be an important period for management because juvenile sharp-tailed grouse disperse and settle new areas during this time. Fall burns might attract dispersing juveniles. 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 (Hanser et al. 2011), density of red grouse (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 design a longer term study (perhaps 5 or more years) to compare sharp-tailed grouse use within burn, mow, and control treatments before and after fall management.

METHODS

Study Areas

Our study area included both the NW and EC populations of sharp-tailed grouse in Minnesota. Differences in landscape composition, management history, and sharp-tailed grouse population trajectories exist between these populations, and thus, we might expect sharp-tailed grouse responses to management to differ. We included DNR-managed public and private land, as well as sites owned and managed by The Nature Conservancy. We conducted pre-treatment surveys at 23 sites that were planned to be managed and 19 controls. Of these, 10 sites were managed in the fall of 2015 (Table 1), including 6 mowing treatments and 4 prescribed burns. Ten planned burns and 3 planned mows could not be completed because moisture and wind conditions were not favorable, although 2 of the planned mows were attempted and terminated due to the high water table. In the EC region, the fall of 2015 was extremely wet, which posed an impediment to planned brushland management. Consequently, all of the completed management occurred in the NW region.

Data Collection & Experimental Design

Treatment sites varied in size, date of management action, vegetative composition, surrounding landscape, and local sharp-tailed grouse population density. We attempted to pair each treatment site with a control site of similar size and vegetative composition *a priori* as determined by inspection of aerial imagery, conversations with managers (e.g., crude habitat classification, visual assessment of percent cover of shrubs and herbaceous vegetation, average shrub height), and site visits. Control sites were identified ≤ 6 km from treatment sites (based on dispersal distances of young males in the fall; Gratson 1988). Control sites help 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.

We surveyed control sites within 14 days of treatment sites both before and after treatment in a Before and After Control Impact (BACI) design (Smith 2002, also see Morrison et al. 2001:118-130) with one exception; the Red Lake WMA control site could not be surveyed during the spring thaw (i.e., surveyed 15 days after the treatment site survey). Most treatment and control sites were surveyed within a few days of each other, but larger sites required more time to complete surveys at both sites (i.e., <4 days per site). We walked systematically spaced parallel transects with a starting point placed on the site boundary and counted grouse pellet piles ≤ 0.5 m from the transect (Evans et al. 2007, Schroeder and Vander Haegen 2014). We standardized the sampling rate to 10 m of transect/ac (1 ac is 0.4 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). Transects were placed to traverse the entire length of the treatment or control area, capturing both edge and interior portions. We sampled transects 4 times at each site; once before treatment (mean = 9.9 days, range = 0–28 days; PRE) and 3 times after treatment; 1 week after treatment (1WK), 1 month after treatment (1MO), and the spring after treatment (after snow melt, SP). When mowing took numerous days to complete at a site (Table 1), we sampled 1 week and 1 month after the treatment end date. Controls were sampled 4 times in a similar temporal framework. We walked transects and removed (or crushed) all pellet piles ≤ 0.5 m from transects (Evans et al. 2007, Schroeder and Vander Haegen 2014), and recorded pellet freshness and the vegetation category (i.e., grass, shrub, forb, grass-shrub mix, grass-forb mix, etc.) where pellets were found. We conducted detection accuracy assessments by conducting surveys with pellets placed in known locations (but unknown to observers) to estimate detection probabilities for each vegetation category. We also recorded all sharp-tailed grouse observed at the site while walking transects.

Vegetation was sampled within treatments and controls using point intercept transects (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. Vegetation transects began at points along the pellet transect and extended 20 m perpendicular to the pellet transect. The number of transects was determined by the size of the site (generally sites <40 ac (<16.2 ha) had 5 transects and sites >40 ac had 10 transects, but changes in actual treatment area affected the final sample size). Vegetation class, status (live or dead), and height were recorded for all classes of vegetation present at points every 0.5 m along the transect. Percent cover was determined as the number of hits for each vegetation class divided by the total number of points sampled (40 per transect), and transects were averaged for each site. Vegetation height was averaged by class for each site. Other metrics, such as variation in percent cover and height across transects, will be used in future analyses.

We asked managers to search for leks in the vicinity (≤ 6 km) of control and treatment sites the spring before treatment when possible, but lek history was not always known in advance of management activities. We listened for sharp-tailed grouse leks in open areas within 1.6 km (1 mile) of treatment and control site boundaries. We stopped every 0.8 km or in open areas to listen for leks. Sometimes, leks were detected while within this buffer that were just outside the buffer and we documented these observations up to 2 km. We surveyed these leks to be as inclusive as possible of birds that might respond to the management treatments, given that the buffer distance was selected somewhat arbitrarily based on the available literature. Surveys were conducted before 0900 hr on mornings with little or no wind or precipitation. Each site was surveyed 1–3 times depending on the quality (i.e., survey conditions, time) of each survey. We recorded the number of males, females, and birds of unknown sex whenever possible, but in some cases leks were heard but not seen. Flush counts were conducted when lek visibility did not allow for a good visual count whenever possible.

RESULTS

Sharp-tailed grouse pellets were detected at 2 of 10 treatment sites (20%) and 2 of 9 control sites (22%) prior to treatment. Following treatment, sharp-tailed grouse pellets were detected in \geq 1 survey (1WK, 1MO, or SP) at 6 of 10 treatment sites (60%) and 3 of 9 control sites (33%, Tables 2 and 3). However, if each survey is considered individually (to keep survey effort constant), then 20–30% of managed sites and 11–22% of controls had pellet detections in later surveys. Grouse observations exhibited similar patterns with detections at 1 treatment site (10%) before management, and at 4 treatment sites (40%) and 2 control sites (22%) in \geq 1 survey post-treatment. Considering surveys individually, 10–40% of treatment sites and 0–11% of control sites had grouse observations post-treatment.

Spring lek survey data in consecutive years were available for 6 treatment sites and 5 control sites (Table 4). For those sites surveyed in both years, 1 lek disappeared near sites that were mowed and burned at Thief Lake Wildlife Management Area (WMA) and 1 new lek was detected in 2016 at Thief Lake WMA, although it was nearer to a control site than a treatment site. Variability in lek counts within 2016 are apparent and make interpretation of year-to-year changes in lek counts difficult.

Because of natural plant senescence during late fall and early spring sampling periods, it was unclear whether vegetation was dormant or dead. Thus for this analysis live and dead/dormant vegetation were combined. Vegetation sampling at treatment and control sites showed some seasonal changes in vegetation cover and height as well as treatment effects (Tables 5 and 6). At control sites, mean cover and height for graminoids, forbs, and shrubs were lowest during spring measurements and highest during pre-treatment measurements. Management obscured measurement of seasonal vegetation changes at treated sites.

Mow treatments decreased vegetative cover and height more than prescribed fire treatments and controls (Table 5 and 6). Change in the mean proportion of shrub cover one month after treatment was greatest at mow treatments (-0.35) compared to mow controls (-0.08), burn treatments (-0.04), and burn controls (-0.04). Change in mean shrub height was also greatest at mow sites (-1.04 m), and relatively unchanged at mow controls (-0.13 m), burn sites (-0.01 m), and burn controls (-0.06 m). The proportion of forbs was reduced at mow treatments (-0.30) but also showed decreases in other sites due to seasonal changes (-0.21 at burn treatments, -0.12 at mow controls, and -0.17 at burn controls). The proportion of graminoids one month after, however, was reduced by both mow (-0.37) and burn (-0.43) treatments and remained the same for mow controls (no change) and burn controls (-0.06).

Mean vegetation cover and height at sites with sharp-tailed grouse pellet detections were similar to measures at sites without detections both pre- (detected during PRE surveys) and post-treatment (detected during the 1WK or 1MO surveys, Table 7), however statistical analysis will be necessary to detect potential significant differences in these data.

DISCUSSION

Detection of fecal pellets and sharp-tailed grouse appeared to increase modestly at treated sites following treatment. However, the number of sites is still very small, and it is too early to draw any reliable conclusions. An increase in pellet detection after management might occur for several reasons unrelated to changes in grouse use. Mowing and burning treatments would be expected to reduce vegetation height, and if pellets are easier to find in low vegetation, then more pellets likely would be detected after management. To account for this we estimated detection of pellets of known density along transects under controlled conditions. Preliminary data from pellet detection accuracy assessments indicated that 0.5–1.0 of known pellet groups were detected, and that detection may differ among vegetation types, observers, and between roost piles and single pellets.

We also observed pellets and birds off-transect during surveys at sites where grouse were otherwise undetected. This may indicate that our sampling is insufficient to capture site use perfectly and that detection probability needs to be incorporated into site use. Another possible explanation for changes in grouse detections after management is that grouse use of sites might change seasonally. Control sites should help account for this possibility, but support for a seasonal increase in use during the fall is currently lacking.

Vegetation measurements helped quantify the effect of management on vegetative structure and composition and will help account for seasonal changes in vegetation at all sites. We expect that sharp-tailed grouse will respond more quickly to burn treatments due to the availability of visual cues associated with fire, but that responses related to structural changes in vegetation will be more immediate for mow treatments which reduce vegetation cover and height immediately. These differing vegetation conditions would serve different functions for sharp-tailed grouse and thus, we might expect grouse to use the two treatment types during different seasons corresponding to different stages of their life history, at least initially. For example, open areas with less cover and shorter vegetation are preferred for spring lek sites, but areas with more cover and taller vegetation are more commonly used for summer nesting and brood rearing. We also expected that treatments might be applied to sites at different successional stages and with different vegetation; for example, mow sites might possess more vertical structure and have more graminoid and forb cover by comparison. These 2 successional stages would serve different functions for grouse and thus may be used at different times of year. We plan to add an additional sampling period in August, which will allow assessment of use during brood rearing that would not be evident in fall or spring sampling. Preliminary vegetation analyses show a change in vegetation structure following treatment, particularly in mow sites; however there was little difference in cover and height between sites where grouse pellets were detected and sites without detections during both pre- and posttreatment (1WK or 1MO) surveys. Further analysis is necessary to determine statistical significance and to tease apart seasonal changes and treatment effects.

This was the first year of data collection, and we anticipate 5–10 years will be necessary to understand the complex responses of sharp-tailed grouse to fall management treatments and associated vegetation changes. However, this pilot study will help inform the feasibility and design of a longer study. Managers throughout the sharp-tailed grouse range have expressed a need for this type of information to more effectively manage for grouse.

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

Site name	Work area	Treatment	Treatment date	Treatment ac	Control ac
Site name	WORK area	Treatment	Treatment date	(ha)	(ha)
Roseau River	Roseau River	Mow	28 Aug–16 Sep	31 (12.5)	28 (11.3)
Skull Lake	Karlstad	Burn	1 Sep	90 (36.4)	70 (28.3)
Halma	Karlstad	Mow	16–23 Sep	41 (16.6)	39 (15.8)
Red Lake Mow	Red Lake	Mow	22 Sep	12 (4.9)	22 (8.9)
Spooner	Baudette	Mow	28 Sep	22 (8.9)	26 (10.5)
Caribou	Karlstad	Burn	28 Sep	664 (268.7)	No control
Thief Lake Burn	Thief Lake	Burn	28 Sep	58 (23.5)	31 (12.5)
Red Lake Burn	Red Lake	Burn	19 Oct	152 (61.5)	176 (71.2)
Prosper	Baudette	Mow	19–30 Oct	63 (25.5)	201 (81.3)
Thief Lake Mow	Thief Lake	Mow	30 Oct	20 (8.1)	19 (7.7)

Table 2. Sharp-tailed grouse pellet detections at treatment and control sites in order of increasing treatment size. Surveys were conducted before (PRE), 1 week after (1WK), 1 month after (1MO), and the spring (SP) following treatment. Data have not been corrected for detection. Detection categories are indicated as 0 = no pellets, + = pellets, **OT** = pellets observed off-transect, indicative of site use not captured in sampling, * = snow impeded detection of pellets, **T** = grouse tracks detected in snow. Gray highlighting is used to draw attention to surveys with confirmed grouse use through any source of sign at the site during a survey.

Fecal pellets		Treat	tment			Cor	ntrol	
Site	PRE	1WK	1MO	SP	PRE	1WK	1MO	SP
Red Lake mow	0	0	0	0	0	0	0	0
Thief Lake mow	0	0	0*	0	0	0	0*	0
Spooner mow	0	0	+	+	0	0	0	0
Roseau mow	+	ОТ	+	0	0	0	0	0
Halma mow	0	0	0	0	+	+	+	0
Thief Lake burn	ОТ	0	+	0	0	0	0	0
Skull Lake burn	0	+	0	0	0	0	0	+
Red Lake burn	0	0	0	0	0	0	0*	0
Prosper mow	0	+	0*	0	+	+	Τ*	+
Caribou burn	+	+	ОТ	+	· ·			

Table 3. Sharp-tailed grouse observed at treatment and control sites in order of increasing treatment size. Surveys were conducted before (PRE), 1 week after (1WK), 1 month after (1MO), and the spring (SP) following treatment. Data have not been corrected for detection. Detection categories are indicated as 0 = no grouse, + = grouse, OT = grouse observed while off-transect, indicative of site use not captured in sampling. Gray highlighting is used to draw attention to surveys with confirmed grouse use through observations of any birds at the site during a survey.

Grouse		Treat	ment		Control					
Site	PRE	1WK	1MO	SP	PRE	1WK	1MO	SP		
Red Lake mow	0	0	0	0	0	0	0	0		
Thief Lake mow	0	0	0	0	0	0	0	0		
Spooner mow	0	0	+	+	0	0	0	0		
Roseau mow	ОТ	+	+	ОТ	0	0	0	0		
Halma mow	0	0	+	0	0	+	0	0		
Thief Lake burn	+	0	0	0	0	0	0	0		
Skull Lake burn	0	0	0	0	0	0	0	0		
Red Lake burn	0	0	0	0	0	0	0	0		
Prosper mow	0	0	0	0	0	0	0	+		
Caribou burn	0	+	+	0						

Table 4. Males and birds of unknown sex counted during sharp-tailed grouse lek surveys <1.6 km from treatment and control sites that were surveyed during 2015, 2016, or both years, in order of increasing treatment size. Leks as far as 2 km from sites are included, but they were detected by observers that were within the 1.6-km search area. Surveys conducted by managers (M) and researchers (R) are both provided because sometimes manager counts are higher, and the highest count is retained in the annual state survey data. NS indicates No Survey. Methods used are heard (H), visual count (V), and flush count (F) and are reported for 2016 only. Areas surveyed in both years are shaded for emphasis and new leks are bolded. Leks appearing on the same line for control and treatment sites are the same.

			Treatm	nent				Cont	rol	
Site	2015	2016	2016	2016	Distance	2015	2016	2016	2016	Distance
	м	М	R	Method	(m)	М	М	R	Method	(m)
Red Lake mow	0	NS	0	-	-	0	NS	0	-	-
Thief Lake mow	16	0	0	-	715					
						NS	NS	0	-	-
Spooner mow	NS	9	20	F	0					
	NS	7	11	V	650					
	NS	NS	3	V	1480					
						16	14	11	F	600
Roseau mow	NS	13	14	F	170					
	20	20	12	F	1250					
						NS	0	0	-	-
Halma mow	NS	NS	8	F	475	NS	NS	8	F	170
	NS	NS	32	V	1475	NS	NS	32	V	690
	NS	NS	9	V	1950					
Thief Lake burn	0	13	8	F	1450	0	13	8	F	250
	16	0	0	-	210	16	0	0		1030
	12	15	9	F/V	1940	12	15	9	F/V	2000
Skull Lake burn	NS	NS	0	-	-	NS	NS	0	-	-
Red Lake burn	0	NS	0	-	-				-	-
						NS	NS	0	-	-
Prosper mow	12	7	7	V	1050					
·	NS	NS	12	V	1485					
						NS	NS	0	-	-
Caribou burn	NS	NS	6	V	0					
	NS	NS	н	н	0					
	NS	NS	н	н	510					
	NS	NS	н	н	<400					

Table 5. Mean proportion of graminoid, forb, and shrub vegetation at treatment and control sites pre-treatment (PRE), 1 month post-treatment (1MO), and the spring following treatment (SP). Sites are grouped by burn and mow treatments (and associated controls) and ordered by size from smallest to largest. Shaded/bolded values indicate site surveys where sharp-tailed grouse activity was detected by pellets or observations during transect surveys.

		Graminoid			Forb			Shrub	
Site	PRE	1MO	SP	PRE	1MO	SP	PRE	1MO	SP
Thief Lake Burn	1.00	0.53	0.67	0.15	0.07	0.04	0.03	0.04	0.02
Thief Lake Control	0.99	0.99	0.92	0.13	0.11	0.03	0.38	0.21	0.31
Skull Lake Burn	0.95	0.50	0.33	0.39	0.04	0.04	0.41	0.36	0.42
Skull Lake Control	0.96	0.92	0.91	0.41	0.18	0.16	0.29	0.21	0.23
Red Lake Burn	0.86	0.53	0.55	0.13	0.01	0.06	0.18	0.10	0.09
Red Lake Control	0.87	0.75	0.83	0.09	0.03	0.04	0.34	0.39	0.49
Caribou Burn	0.99	0.63	0.62	0.33	0.10	0.03	0.16	0.09	0.12
Red Lake Mow	0.90	0.77	0.76	0.78	0.19	0.23	0.16	0.02	0.04
Red Lake Control	0.75	0.79	0.68	0.80	0.49	0.46	0.34	0.20	0.32
Thief Lake Mow	1.00	0.54	0.93	0.20	0.03	0.09	0.24	0.06	0.10
Thief Lake Control	1.00	0.93	1.00	0.28	0.30	0.26	0.03	0.06	0.04
Spooner Mow	0.58	0.25	0.35	0.96	0.45	0.58	0.30	0.07	0.12
Spooner Control	0.92	0.96	0.89	0.41	0.21	0.25	0.82	0.61	0.80
Roseau River Mow	0.70	0.33	0.26	0.16	0	0	0.70	0.04	0.10
Roseau R. Control	0.85	0.88	0.69	0.59	0.32	0.05	0.72	0.45	0.47
Halma Mow	0.97	0.60	0.65	0.25	0.03	0.05	0.56	0.09	0.31
Halma Control	1.00	0.99	0.96	0.21	0.08	0.08	0.19	0.20	0.18
Prosper Mow	1.00	0.44	0.86	0.17	0	0	0.46	0.13	0.18
Prosper Control	0.94	0.90	0.90	0.20	0.04	0.05	0.53	0.50	0.52

Table 6. Mean height (m) of graminoid, forb, and shrub vegetation at treatment and control sites pre-treatment (PRE), 1 month post-treatment (1MO), and the spring following treatment (SP). Sites are grouped by burn and mow treatments (and associated controls) and ordered by size from smallest to largest. Shaded/bolded values indicate site surveys where sharp-tailed grouse activity was detected by pellets or observations during transect surveys.

		Graminoid			Forb			Shrub	
Site	PRE	1MO	SP	PRE	1MO	SP	PRE	1MO	SP
Thief Lake Burn	0.66	0.36	0.19	0.41	0.37	0.42	0.71	0.65	0.70
Thief Lake Control	0.50	0.38	0.22	0.32	0.26	0.17	0.66	0.59	0.62
Skull Lake Burn	0.51	0.28	0.17	0.37	0.47	0.38	0.80	0.90	0.69
Skull Lake Control	0.53	0.48	0.20	0.39	0.35	0.33	0.77	0.80	0.58
Red Lake Burn	0.58	0.42	0.20	0.40	1.10	0.10	0.96	0.61	1.03
Red Lake Control	0.50	0.32	0.26	0.30	0.27	0.33	0.93	0.78	0.85
Caribou Burn	0.56	0.38	0.20	0.27	0.23	0.27	0.66	0.64	0.62
Red Lake Mow	0.28	0.11	0.10	0.25	0.11	0.11	0.66	0.15	0.20
Red Lake Control	0.35	0.29	0.18	0.33	0.24	0.22	0.96	0.80	0.86
Thief Lake Mow	0.54	0.16	0.13	0.45	0.18	0.16	0.83	0.18	0.22
Thief Lake Control	0.47	0.23	0.16	0.42	0.31	0.20	0.64	0.55	0.58
Spooner Mow	0.48	0.17	0.14	0.41	0.23	0.20	1.12	0.28	0.35
Spooner Control	0.58	0.46	0.28	0.31	0.37	0.33	1.29	1.09	1.17
Roseau River Mow	0.61	0.11	0.10	0.25	0	0	2.52	0.27	0.17
Roseau River Control	0.57	0.49	0.17	0.37	0.32	0.25	1.85	1.85	1.75
Halma Mow	0.43	0.17	0.13	0.43	0.21	0.25	1.55	0.43	0.48
Halma Control	0.51	0.44	0.20	0.39	0.39	0.45	0.92	0.98	1.00
Prosper Mow	0.78	0.16	0.14	0.48	0	0	1.51	0.30	0.31
Prosper Control	0.69	0.43	0.26	0.39	0.36	0.26	1.92	1.67	1.69

Table 7. Mean proportion cover and mean height (m) of vegetation at sites with and without sharp-tailed grouse pellet detections. Vegetation data are from the pre-treatment sampling period for detections pre-treatment and from the 1 month post-treatment sampling period for detections post-treatment. Pellet detections for post-treatment sites included detections in the 1 week or 1 month post-treatment sampling periods.

	Mean p	proportion co	over	Mean height (m)				
	Graminoid	Forb	Shrub	Graminoid	Forb	Shrub		
Detected pre-treatment	0.94	0.23	0.35	0.59	0.34	1.39		
Not detected pre-treatment	0.92	0.36	0.35	0.52	0.38	1.04		
Detected post-treatment	0.67	0.10	0.20	0.35	0.35	0.84		
Not detected post-treatment	0.77	0.15	0.23	0.29	0.27	0.75		



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

Charlotte Roy, Eric Nelson¹, and Andrew Gregory²

SUMMARY OF FINDINGS

Landscape genetics is an emerging field that examines both structural and functional landscape connectivity by combining geographic information with information about genetic variation within and among spatially structured samples or populations. This study aims to identify landscape features that pose barriers to prairie grouse movement and those that enable movements among areas of suitable habitat, using genetic information from feather samples in a landscape genetic approach. During the spring of 2014, cooperators and staff collected 174 sharp-tailed grouse (*Tympanuchus phasianellus*) and 162 greater prairie-chicken (*Tympanuchus cupido*) feather samples from leks. During the fall of 2014, hunters also submitted wings from 30 sharp-tailed grouse and 22 prairie-chickens. From these samples, we identified 123 unique sharp-tailed grouse and 107 prairie-chicken individuals. In spring 2015, 657 sharp-tailed grouse and 347 prairie-chicken feather samples were collected from leks. Hunters again submitted 52 additional sharp-tailed grouse and 30 additional prairie-chicken wings for the study in 2015. From samples collected in 2015, 347 unique sharp-tailed grouse (341 new) and 192 greater-prairie chicken (188 new) were identified. Thus, 464 unique sharp-tailed grouse and 295 unique prairie-chicken samples will be included in our landscape genetic analysis to be completed during fall 2016.

INTRODUCTION

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

Landscape genetics is an emerging field that provides methods to examine connectivity on the landscape by combining geographic place-based information with information about genetic variation within or among populations (Braunisch et al. 2010, Lowe and Allendorf 2010, Sork and Waits 2010, Haig et al. 2011). This tool can be used to examine effective dispersal (gene flow) on the landscape, without having to rely on telemetry techniques, which can be expensive and may require large numbers of marked animals if successful dispersal events are infrequent (Coulon et al. 2004, Spear et al. 2010). Landscape genetic methods have been used

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in recent years to identify barriers to dispersal, including human development, non-habitat land cover types, and distance in species like capercaillie (*Tetrao urogallus*, Braunisch et al. 2010), northern bobwhite (*Colinus virginianus*, Berkman et al. 2013a,b), and prairie-chickens (Gregory 2011). Thus, landscape genetics can be used to examine the movements of birds in a spatially explicit manner.

OBJECTIVES

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

METHODS

Wildlife managers, cooperators, and seasonal technicians surveyed prairie-chickens and sharp-tailed grouse at leks throughout Minnesota in the springs of 2014 and 2015. Feathers lost during male contests, copulations, and as a result of other activities were collected from leks. To maximize the probability of sampling many different individuals, staff and technicians were instructed to spread out the sampling at each lek, sample feathers from discrete locations on the lek, and only collect one sample per location or cluster of feathers encountered. Each sample of feathers, or single feather when necessary to ensure that only one individual was represented, was placed in an envelope and labeled with the lek location (coordinates or Township, Range, Section, and quarter-section information), date, collector name, contents, and species. Information from each envelope was recorded in a database and assigned a unique sample number. Areas underrepresented in 2014 were given greater effort in the spring of 2015. Feather samples from leks were supplemented with samples from hunter-harvested birds in both 2014 and 2015. Wings from harvested birds were aged based on plumage characteristics (Bihrle 1993).

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

In the fall of 2016, genetic information will be linked to spatial information in a GIS to examine the connectivity of the landscape. Areas that share greater connectivity will contain birds that are similar genetically, whereas areas with restricted connectivity will be more dissimilar genetically. Analytical methods will be revisited for the most recent advances prior to initiating data analysis.

RESULTS/DISCUSSION

We collected 174 sharp-tailed grouse and 162 greater prairie-chicken feather samples from leks during the spring of 2014 (Figures 1 and 2). We anticipated that some of these feathers would be duplicates from the same individual, and that in some cases the DNA would be too degraded to be useful. Yet, we obtained DNA of sufficient quantity and quality to amplify DNA from 111 (64%) sharptail and 115 (71%) prairie-chicken lek samples. Duplicate samples were detected as expected, but 95 unique sharp-tailed grouse and 85 greater prairie-chicken individuals were identified from the feather samples submitted in 2014. Thirty sharp-tailed grouse and 22 greater prairie-chicken wings were submitted by hunters during fall 2014 (also shown in

Figures 1 and 2), and DNA from all these samples were of good quality and produced unique samples.

In spring 2015, 657 sharp-tailed grouse samples and 347 greater prairie-chicken samples were collected from leks (Figures 1 and 2). Hunters also submitted 52 sharp-tailed grouse and 30 greater prairie-chicken wings for the study in 2015 (Figures 1 and 2). Amplification success for the combined sample types was 79% and 85% for sharptails and prairie-chickens, respectively. Analysis of 2015 samples identified 347 (341 new in 2015) unique sharp-tailed grouse and 192 (188 new) unique prairie-chicken individuals. Landscape genetic analysis will be completed for the 464 unique sharp-tailed grouse and 295 unique greater prairie-chicken samples during fall 2016.

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

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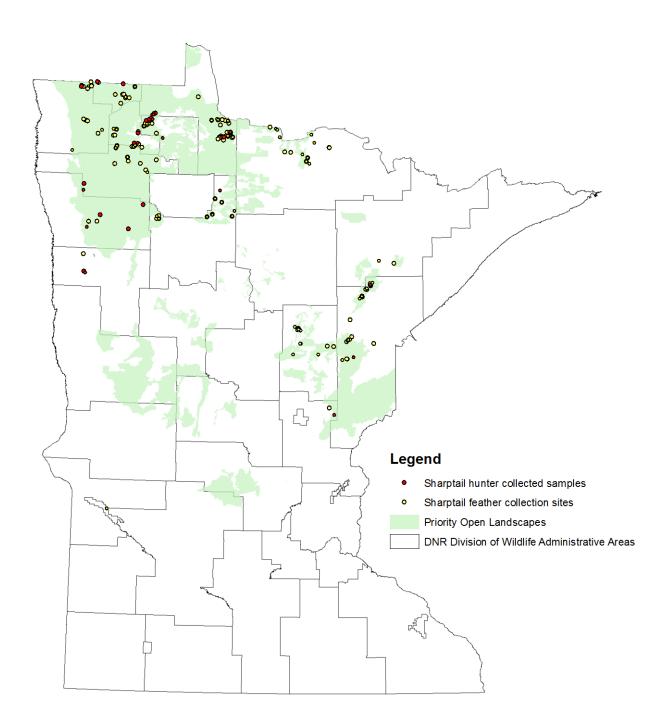


Figure 1. Locations where sharp-tailed grouse feather samples (n = 831) were collected at leks or by hunters (n = 82) in Minnesota during 2014 and 2015.

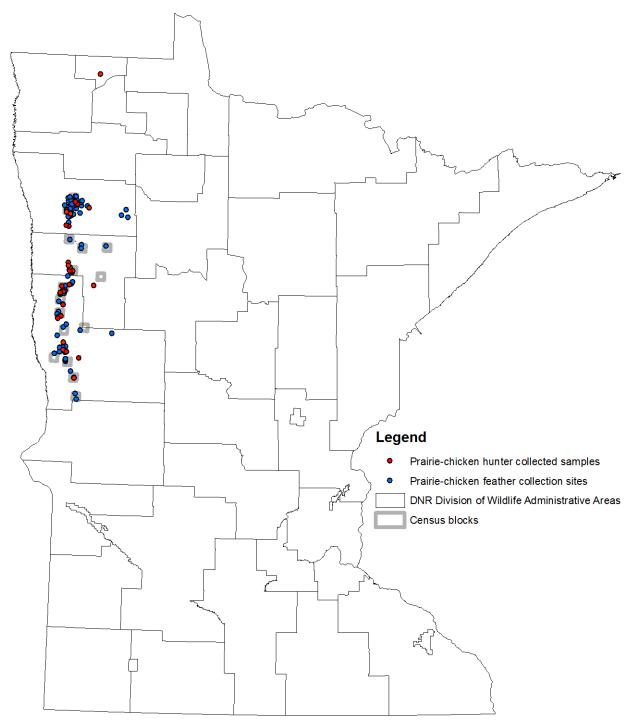


Figure 2. Locations where greater prairie-chicken feather samples (n = 509) were collected from leks and hunter-sample collection sites (n = 52) during 2014 and 2015.



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

Charlotte Roy, Da Chen¹, Julia Ponder², Mark Jankowski³

SUMMARY OF FINDINGS

Neonicotinoid pesticides (e.g., imidacloprid, thiamethoxam, thiacloprid, clothianidin) are commonly applied to agricultural seeds (e.g., corn, soybean, wheat, sunflower), and are known to cause lethal and sub-lethal effects in birds. Neonicotinoid-treated seeds could be available to wildlife through spillage or exposure to treated seeds near or at the soil surface after planting (de Leeuw et al. 1995, Pascual et al. 1999, Lopez-Antia et al. 2016). We are examining sublethal exposure of wild birds to these pesticides in agricultural landscapes of Minnesota. We are quantifying seed availability at the soil surface in recently planted fields and the rate of seed spills during planting, as well as documenting birds eating treated seeds through field studies with trail cameras and harvested birds. Thus far, we have documented ring-necked pheasants (Phasianus colchicus), Canada geese (Branta canadensis), American crows (Corvus brachyrhynchos), various species of sparrows (Emberizidae) and blackbirds (Icteridae), blue jays (Cyanocitta cristata), and white-tailed deer (Odocoileus virginianus) consuming seeds but have viewed <1% of images taken near seed spills. We documented 216 seed spills in 38 townships during planting but missed the peak of planting in many of the townships we surveyed, so our current estimates are conservative and not yet corrected for planting status of fields. We documented exposed seeds at the surface in plots at 25% of 48 fields sampled after planting. We are still conducting analyses to determine the length of time that neonicotinoids persist on seeds exposed at the soil surface, and whether the seeds are consumed before the chemicals have degraded.

We are also conducting laboratory experiments to try to identify non-lethal sampling methods that could lead to methods for measurement of individual and population-level exposure, including residues in excreta and blood. Early results suggest that residues are highest (geometric mean) in the brain, followed by liver, spleen, muscle, blood, kidney, then feces in birds dosed in the lab. Residues were detected in 90.9% of chicken fecal samples collected in the lab, the highest detection frequency of all tissues tested. Twelve of 16 liver samples collected from hunter-harvested sharp-tailed grouse (*Tympanuchus phasianellus*) and 9 of 11 greater prairie-chickens (*Tympanuchus cupido*) from hunter-submitted samples contained detectable concentrations of at least one neonicotinoid. Similarly, 18 of 29 fresh

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prairie grouse fecal pellets collected from leks had detectable concentrations of at least one neonicotinoid. Results from this study will be used to inform a larger study to begin in spring 2017.

INTRODUCTION

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

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

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

Thirty bird species were observed picking up treated seeds from cereal fields in Spain and 3.1% of partridge gut contents collected by hunters tested positive for imidacloprid after planting of winter cereal crops (Lopez-Antia et al. 2016). Dead and poisoned partridges have been found in agricultural fields in France following use of imidacloprid-treated seed (Berny et al. 1999). The EPA estimated that ~1% of seeds remain accessible to granivores after planting (as reported by Goulson 2013, Lopez-Antia et al. 2015). Unfortunately, neonicotinoid use of "treated articles," such as seed, is not currently tracked by the government due to the exemption in 40CFR §152.25(a). Yet, almost all corn planted in the Midwest has been treated with these pesticides (Stokstad 2013), as well as most soybean, wheat, and sunflower seeds, and they are widely used with other application methods for other crop types.

Studies of neonicotinoid effects on vertebrates are overwhelmingly laboratory-based (91% of studies), which limits our ability to interpret the significance of findings in more natural settings (Gibbons et al. 2014). Higher densities of exposed seeds result in greater attraction of birds to fields (Murton et al. 1963, Feare et al. 1974). Bednarska et al. (2013) identified a need for feeding rate information in the field to allow extrapolation of lab data to the field. Lopez-Antia et al. (2013) pointed to a "need for evaluation of real exposure to coated seed ingestion by wild birds, including feeding behavior analyses and estimation of food intake rates." We are therefore conducting a study to develop tools with which we are ascertaining whether birds are at risk for exposure to neonicotinoid-treated seeds in agricultural landscapes.

OBJECTIVES

The overarching objective is to ascertain whether birds are at risk for exposure to neonicotinoid-treated seeds in agricultural landscapes. Specifically, we will:

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

METHODS

Documenting Consumption of Treated Seeds

We selected 12 Wildlife Management Areas (WMAs) to place trail cameras from the 1,707 WMAs in Minnesota, of which a subset have food plots or Cooperative Farming Agreements (CFAs). The most recently available data on CFAs on DNR-managed land indicate 7,420 acres (3,003 ha) of row crops in 341 CFAs in Region 4 (southern region) and 2,431 acres (984 ha) of row crops in 66 CFAs in Region 1 (northwest region; M. Benage and J. Williams, respectively, pers. comm.). We selected WMAs with a landcover composition similar to that of the surrounding landscape using the 2014 National Cropland Data Layer (USDA-NASS 2015) in ArcGIS 10.2 (ESRI 2015), but required them to have food plots or Cooperative Farming Agreements (CFAs) after they met the first criterion. Working on WMAs minimized bias in farming activities that might result from prior knowledge of the study. Furthermore, neonicotinoid seed has been commonly used by farmers on WMAs to date and many of the managers have reported difficulty finding seeds that have not been treated. Because neonicotinoids will be banned from WMAs in Minnesota beginning in 2017, we prioritized this portion of the study in 2016.

Cameras were placed to minimize risk of theft and to view a recently planted field to document foraging at a simulated seed spill and exposed or submerged seeds or seedlings. Spills were simulated with 1000 corn or soybean seeds to allow determination of the time for

birds to discover spills and the number of seeds consumed in each foraging bout/bird. Additionally, we placed cameras at 2 privately owned fields. Cameras were deployed in each location for 3–6 weeks after planting. At each field, 2 cameras were deployed; one that captured 1 image/sec in still photos and a second that captured 60 sec of video when triggered by motion. The camera set for still photos also took field scans at 5 min intervals between 0600–0800 hr and 1830–2030 hr to document birds foraging in fields during sunrise and sunset periods during the planting season. Images will be examined to identify species, number of birds consuming seeds, and number of seeds consumed per foraging bout, or in broader views, to document birds using crop fields after planting.

Quantifying Spills and Seed Surface Exposure

All chemically treated seeds (e.g., neonicotinoids, fungicides, other pesticides) are unnaturally colored, as mandated by the Federal Seed Act. These seeds are highly visible and easily identified by their unusual color (e.g., pink, blue, green, purple), which is used to prevent accidental feeding to livestock. We are quantifying the frequency of seed spills on the landscape by inspecting fields with visual access from roads, field access points, and roadsides in agricultural areas. We hope to avoid bias in spill rates that might result from obtaining permission to access privately owned fields on foot, but this method makes the implicit assumption that spill rates associated with refilling hoppers and overfilling is similar for fields adjacent to roads and fields that are not adjacent to roads.

We identified 211 townships in the western third and southeastern part of the state with at least 50 miles of roads and 50% of the area in corn, soybeans, and/or wheat production using the 2014 Cropland Data Layer (USDA-NASS 2015) and the DOT Roads Layer (DOT 2008) in ArcGIS. These criteria were used to select townships with visual access to fields from roads, while also not becoming so restrictive that the spatial distribution of the sample was constrained. We drew a spatially balanced sample of 50 townships and surveyed the 38 most western townships selected during the spring of 2016. We began in the southern counties and worked north beginning in late April as crops were planted.

We recorded locations and approximate number of seeds in spills near *recently planted* fields with the DNRSurvey mobile computer application. Documenting only *recently planted* fields allowed for control in temporal variation in the timing of planting. For example, a field that has not been planted yet will not have a spill at the time of sampling, which is different from a spill not occurring during planting. Thus, by only including recently planted fields in our estimates, we measured spills during planting. We defined a "field" as a quarter quarter-section (i.e., 40 acres). We recorded each quarter quarter-section in agricultural production, whether any part of it was recently planted (i.e., \leq early seedling stage), documented the amount (number of seeds) of spilled seed on the road, field edge, or visible in the field, and crop type (when possible). To determine the proportion of seed spills that contain neonicotinoid-treated seed, we collected seeds from 109 spills and will quantify 7 neonicotinoids (Chen et al. 2014).

To estimate the amount of seed at the soil surface after planting, we used a 1-m² frame to define plots in 48 recently planted fields and counted all treated seeds visible within the frame after planting (Lopez-Antia et al. 2016). We sampled 5 plots in a field corner and 5 plots in the field center as estimated visually from field boundaries while standing in the field. For corner locations, we randomly selected 1 field corner per field by flipping a coin twice, and paced 15 m and 30 m along each edge in an L-shape that had the field corner for a vertex for a total of 5

measurements. This approach incorporated sampling parallel and perpendicular to planting rows, and we suspected that seed exposure would be greater at the end of rows at turning points than within rows. For field centers, we paced 15 m in each cardinal direction to sample for a total of 5 measurements including the center.

Linking Exposure to Concentrations in the Lab

We are quantitatively linking field sample concentrations to lab exposure concentrations through work with UMN-CVM and SIUC. We are determining how many days post-exposure imidacloprid (i.e., the most common seed treatment in Minnesota, J. Zachmann, MDA, pers. comm.) is detectable in both non-lethally and lethally collected samples. A non-lethal method to determine sub-lethal exposure would facilitate data collection during spring planting when spills would be expected to be most numerous.

At UMN, domestic chickens (Gallus gallus domesticus) were orally exposed to imidacloprid (IMI) for 7 days and serially sampled during and after the course of exposure to simulate repeated sub-lethal exposures. Chickens served as our model species given their suitability to captivity and close taxonomic relationship with wild grouse (Family Phasianidae). Small sample sizes are commonly used in dosing studies because the differences among treatment groups are expected to be very large and variability within groups low (e.g., Berny et al. 1999, Bednarska et al. 2013). We exposed animals (n = 5) to 1, 5, and 20% of the LD₅₀ (104.1 mg/kg IMI, Kammon et al. 2010) daily for 7 days by giving ~1.5 kg birds a daily IMI bolus of 1.04 mg/kg/day ("low"), 5.20 mg/kg/day ("medium"), and 20.80 mg/kg/day ("high"). The LD₅₀ is the single dose that is expected to be lethal to 50% of test subjects. The LD₅₀ could be obtained if chickens ingested ~260-946 corn seeds (depending on application rate to seeds, which varies among seed companies), or stated differently, 3-10 seeds is comparable to the 1% LD₅₀ dose. Thus, these were realistic doses. Prairie grouse are smaller (0.6–1.2 kg) and thus a smaller dose (104-780 seeds depending on bird weight) would be expected to produce similar results. Other neonicotinoids have a lower LD₅₀ than IMI so lethality would be expected at much lower seed ingestion levels than for IMI.

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

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

Detecting Neonicotinoids in Free-ranging Birds

We also collected samples from wild birds through both invasive and non-invasive methods to try to identify ways to assess exposure to neonicotinoids in the field. Fresh fecal pellets and blood samples from trapped prairie grouse were collected during lek visits for a genetic study in spring 2015. Samples were stored frozen until shipped to the lab at SIUC. Hunters also voluntarily submitted harvested prairie grouse in fall 2015. Tissues and fecal pellets are being tested for thiacloprid, acetamiprid, thiamethoxam, imidacloprid, clothianidin, dinotefuran and nitenpyram.

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

RESULTS

Documenting Consumption of Treated Seeds

Thus far, we have viewed <1% of images collected by trail cameras at simulated spills during spring 2016 (n >100,000 images). In the images viewed to date, we have documented ring-necked pheasants, Canada geese, American crows, blue jays, various species of sparrows and blackbirds, and deer consuming treated seeds. We will view images during fall 2016 and winter 2016–17 and summarize results in future research summaries.

Quantifying Spills and Seed Surface Exposure

Preliminary examination of data indicates 216 spills in 38 townships surveyed during planting. However, we missed the peak of planting in many of the townships surveyed because the spring of 2016 was very wet and crops were planted later than usual. Our current estimates are conservative and not corrected yet for planting status of fields.

We documented exposed seeds in 25% of the fields sampled. Seeds were exposed in \geq 1 centrally located plot in 14.6% of fields measured. Exposed seeds were detected in \geq 1 corner plot of 18.8% of fields measured. Most (79%) of the fields we measured were planted to corn, 17% were planted to soybeans, and 4% were planted to wheat. Most (96%) sampled fields were on public land but 79% of the sampled fields on public land were planted by private cooperating farmers with their own equipment. We suspect that spill rates are influenced by the type of equipment used for sowing (Lopez-Antia et al. 2016) and possibly the seed type.

Linking Exposure to Concentrations in the Lab

We collected 72 blood samples, 100 fecal samples, 15 muscle, brain, liver, and kidney samples, and 103 eggs during experiments for neonicotinoid analysis. Imidacloprid (IMI) was detected more frequently and for a longer duration post-exposure in fecal samples (90.9%, ≤21 days post exposure) than blood (32.9%, ≤7 days post exposure). Blood concentrations increased from the first samples taken at the start of the experiment (hr 0), increased at hr 8 and declined again at hr 24 (Figure 1); after this time, samples did not contain detectable IMI except for one sample taken on day 8. Fecal IMI concentrations followed a 3rd order polynomial pattern, increasing from the start of the experiment (day 0) until approximately day 6, decreasing until day 18 and holding steady or slightly increasing by day 21 (Figure 2). The low dose group tended to exhibit lower IMI fecal concentrations than birds in the medium dose

group, as expected. IMI was rapidly removed from blood, but the change in concentrations varied 17,234-fold (c.f., 279-fold in feces; fold change is maximum detected concentration/minimum detected concentration across all groups and times), and thus blood may provide a more sensitive indicator of an acute exposure than feces. By contrast, fecal samples provided a more integrated, longer, and more consistent detection in exposed birds (Figure 2) and thus may be more applicable to field applications where time from chemical exposure will be more variable.

IMI was measured in internal organs (Figure 3) collected on the final day of the experiment, depending on when birds were euthanized. Low- and medium-dosed birds were euthanized on day 21, whereas high-dosed birds were euthanized after showing clinical signs of distress on day 1. Detection frequency of IMI was highest in kidney, liver, and spleen (73.3%), although muscle and brain also exhibited similar detection frequencies (66.7%). Geometric mean tissue concentrations were highest in brain and lowest in the kidney (Table 2).

Detecting Neonicotinoids in Free-ranging Birds

Field-collected prairie grouse samples sent for neonicotinoid analysis included 61 sharptailed grouse fecal pellet groups and 34 greater prairie-chicken fecal pellet groups. We also collected 5 blood samples from trapped sharp-tailed grouse, as well as 2 brains and 3 breast muscles from sharp-tailed grouse for which we had whole carcasses and sent them for neonicotinoid analysis. Hunters submitted 11 prairie-chicken livers, 22 sharp-tailed grouse livers, and 3 livers from prairie-chicken/sharptail hybrids.

A subset of field samples from wild prairie grouse have been analyzed for neonicotinoids thus far. Twelve of 16 liver samples collected from hunter-harvested sharp-tailed grouse, 9 of 11 greater prairie-chicken livers, and 3 of 3 sharptail-chicken hybrids from hunter-submitted samples had detectable concentrations of at least one neonicotinoid. Dinotefuran and nitenpyram were not detected in any samples. The most commonly detected neonicotinoids in livers of harvested prairie grouse were imidacloprid (66%), acetamiprid (20%), clothianidin (17%), thiacloprid (10%) and thiamethoxam (7%). Maximum concentrations detected in livers of harvested sharp-tailed grouse were 7.89 ng/g, 0.71 ng/g, 3.58 ng/g, 1.18 ng/g, and 0.5 ng/g, respectively. Maximum concentrations of neonicotinoids in prairie-chicken livers were 6.21 ng/g, 0.21 ng/g, 0.42 ng/g, 0 ng/g and 0.43 ng/g, respectively. Similarly, 18 of 29 fresh prairiechicken fecal pellets collected from leks during spring contained detectable concentrations of at least one neonicotinoid. The most commonly detected neonicotinoid in fecal pellets was imidacloprid (62%), followed by clothianidin (10%), and thiacloprid (10%). Acetamiprid and thiamethoxam were not detected in feces, perhaps due to differences in the way they are metabolized or excreted. Maximum concentrations of imidacloprid, clothianidin, and thiacloprid in feces were 6.12 ng/g, 0.90 ng/g, and 1.05 ng/g, respectively. Samples which contained multiple neonicotinoids (8 livers and 4 fecal samples) generally contained imidacloprid, except for 1 liver which contained thiacloprid, thiamethoxam, and clothianidin only.

Birds collected while foraging on treated seeds included 1 ring-necked pheasant, 5 redwinged blackbirds (*Agelaius phoeniceus*), 2 yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), 3 brown-headed cowbirds (*Molothrus ater*), and 5 common grackles (*Quiscalus quiscula*). These samples have not been analyzed yet.

DISCUSSION

Fecal samples appear to provide a possible non-invasive means to detect exposure in birds based on our findings and the potential to refine analytical methods. Previous studies have demonstrated that neonicotinoids (e.g., thiamethoxam) are excreted primarily through the kidneys in mammals (Bednarska et al. 2013, Tomizawa and Casida 2005). Ongoing analytical work to measure metabolites of IMI in feces is expected to provide a more sensitive (i.e., higher fold concentration change) assay than current parent compound (i.e., IMI unmodified by metabolic processes) data. Further work will be required to quantify how the potential environmental IMI exposure scenarios (concentration, duration, and frequency) influence the detection of parent compound and metabolites in feces and the uric acid wash. However, fecal samples could be collected from the GI tract of hunter-killed birds, from live birds, or non-invasively from the environment. Further work is necessary to refine non-invasive collection because UV light can and microbial degradation may degrade neonicotinoids (Lu et al. 2015; Lu et al. 2016; Ma et al. 2014), so pellet freshness would be an important consideration.

Our data provide evidence that internal organs can serve as an indicator of IMI exposure in lethal collections including hunter-killed birds. However, based on detection frequencies in organs and feces, fecal samples may provide a more reliable index of exposure than organs. Berny et al. (1999) reported that liver and kidney had the most consistent imidacloprid concentrations in fatally exposed wild birds, whereas crop and gizzard provided inconsistent concentrations. However, Lopez-Antia et al. (2015) reported that imidacloprid could be consistently detected in crops and livers of dosed partridges (*Alectoris rufa*).

The highest concentration of imidacloprid detected in livers of harvested prairie grouse was higher than that of chickens in the low and medium dose group at the end of the experiment. However, it was lower than the high LD_{50} group after early euthanization. Similarly, the highest concentration of imidacloprid detected in field collected feces was lower than both the 1% and 5% dose groups shortly after exposure, and was more similar to both of these groups a few weeks post-exposure. We cannot know if this indicates a lower initial exposure, the passage of time since exposure, or both; but, given that 1% LD_{50} (1.04 mg/kg) is comparable to the dose received after consuming 3–10 corn seeds and that IMI can be detected in tissues at least 21 days post-exposure, we consider it likely that this finding reflects an exposure to imidacloprid that occurred a few weeks prior to sample collection. Winter wheat is planted in September and October in Minnesota, so grouse might be newly exposed to treated seeds in the fall, although it is not clear how long spring exposure would be detectable in organs. At a minimum, detection of IMI in tissues of wild birds provides us with a qualitative index of exposure, which is one step closer to understanding the effects of IMI in wild birds in Minnesota.

The high detection frequencies of IMI in internal organs on experimental day 21 after 7 consecutive days of exposure indicates a persistence of IMI that is notable but not easily comparable to other acute studies. Most studies have suggested a rapid metabolism and elimination (~48 hours) of parent (i.e., unchanged) compound in the urine after *single* oral doses (Bednarska et al. 2013; Tomlin 2004). Our findings demonstrated a relatively high persistence of parent compound in feces and organs and may therefore indicate an appreciable toxicological risk for birds.

The locations of the compounds in the tissues provide insight into which systemic effects warrant examination. Based on the high splenic concentrations, we hypothesize IMI will cause

immune system changes in birds. The detection of IMI in neurological tissues (brain) indicates a potential for behavioral changes as well. If immune system or behavioral effects impact survival and reproduction, then population–level impacts are plausible. Our laboratory data will be useful in understanding the absorption, distribution, excretion, and effects of IMI, as well as in the design of future laboratory and field studies in birds. We will also contribute some of the first information on exposure of wild birds in the United States to neonicotinoids.

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Table 1. Summary of imidacloprid (IMI) detections in domestic chicken blood and feces in each of 3 dose groups (low, medium, and high with 1.04 mg/kg/day, 5.02 mg/kg/day, and 20.80 mg/kg/day doses, respectively). Note that birds in the high dose group were euthanized early, which may have limited the ability to eliminate IMI in feces.

	Dose (mg/kg/day)	Percent detects	Fold change	Median	Geometric Mean	Minimum	Maximum	Ν
	1.04	20	4.2	1.7	1.4	0.5	2.1	6
Blood	5.02	33.3	9.8	2.6	2.2	0.7	6.9	10
(ng/ml) 20.80	20.80	61.5	2051.7	3270	805.6	4.2	8617	8
Faaaa	1.04	81.3	91.8	14.6	10.1	0.8	73.4	26
Feces (ng/g wet	5.02	97.5	278.9	19.1	14.1	0.7	195.2	39
weight)	20.80	100	2.8	3.2	3.7	2.3	6.5	5

Table 2. Summary of tissue concentrations of imidacloprid (IMI; ng/g wet weight in tissues and ng/ml for blood) in all laboratory-exposed chickens for all dose groups combined.

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

¹NA = Not applicable because tissues were collected when chickens were killed the last day.

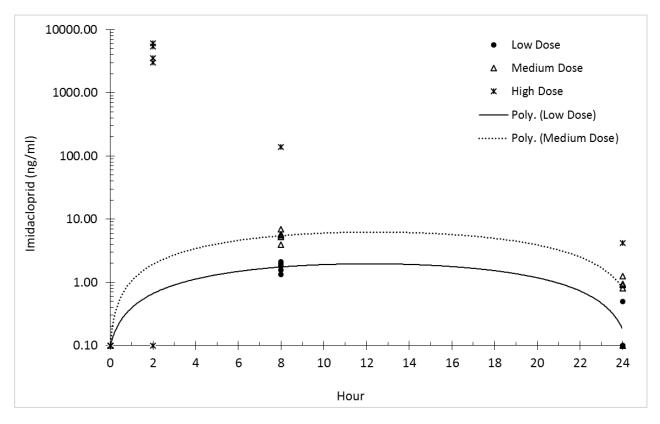


Figure 1. Changes in imidacloprid (IMI) concentrations in blood of dosed chickens over time after one dose. IMI doses were 1%, 5%, and 20% of a reported IMI LD_{50} for chickens (i.e., low, medium, and high dose groups, respectively). IMI detection limit is 0.10 or -1.0 log₁₀ ng/ml in blood. Data points overlap when plotted on x-axis minimum value. A trend line could not be fit to the data from high-dosed birds because chickens in this dose group were euthanized within 24 hours due to animal welfare concerns. Thus, the high dose group is not directly comparable to the other dose groups.

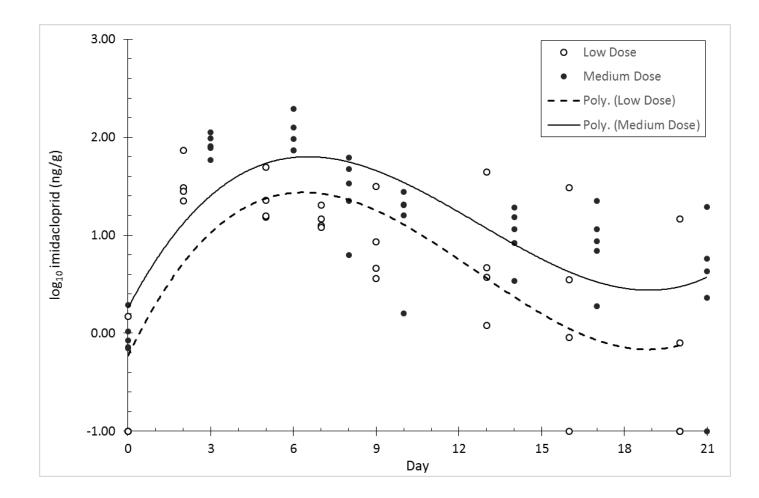


Figure 2. Changes in imidacloprid (IMI) concentrations in feces of dosed chickens over time. Samples collected on day 0 were baseline samples, prior to exposure. Daily IMI dose for 7 days of 1% (low dose) and 5% (medium dose) of a reported IMI LD₅₀. The last day of gavage exposure occurred on day 7 of the 21 day experiment. IMI detection limit is 0.10 or -1.0 log₁₀ ng/g in feces. The high dose group is not included because samples were collected only on day 0 so no temporal trends could be determined. Chickens in the high dose group were euthanized within 24 hrs after dosing due to animal welfare concerns. Thus, the high dose group is not directly comparable to the other dose groups.

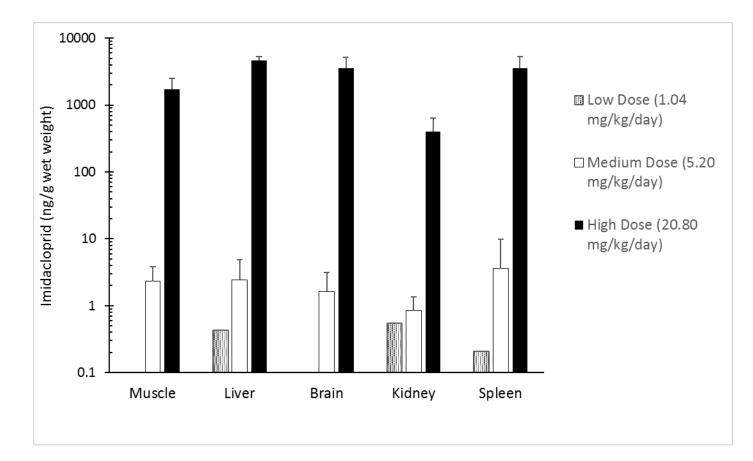


Figure 3. Concentrations of imidacloprid (geometric mean + SD ng/g wet tissue weight) in tissues of laboratory-exposed chickens on experimental day 1 (high dose) or 21 (low and medium dose). Data at the detection limit of 0.10 ng/g are not visible. Error bars represent the standard deviation of observations for a given group.



MONITORING SPRUCE GROUSE IN MINNESOTA: A PILOT STUDY (2014–2016)

Charlotte Roy, John Giudice, and Chris Scharenbroich

SUMMARY OF FINDINGS

Data collection began in 2014 to develop survey methodology for a large-scale survey of spruce grouse (Falcipennis canadensis; SPGR) in Minnesota. During 2014 and 2015, we examined 2 primary methods of spruce grouse detection: a cantus-call survey and a pellet survey. Based on field work conducted in 2014 and 2015, we determined that spruce grouse responses to the cantus call were more frequently detected earlier in the day, earlier in the 15-min or 9-min broadcast period, earlier in the season (April > May), and when habitat occurred on both sides of the road. Pellet surveys along circular transects centered on call survey points had 5 times the apparent detection rate of call surveys (20% and 4%, respectively) in 2014, and after slight improvements to the call survey in 2015, still produced 3-4 times more detections than the call survey. During 2015, pellet and call surveys at paired points on and off roads allowed examination of the effects of roads on survey counts. These paired surveys indicated that detections at roadbased points were lower than at points located off roads at 1 of 2 study areas, but this effect was nearly eliminated in forest types preferred by spruce grouse. In 2016, we piloted a road-based pellet survey throughout the probable spruce grouse range in Minnesota. Results were consistent with anecdotal accounts of spruce grouse observations from wildlife managers and indicated that spruce grouse are relatively rare in the Northern Minnesota Drift and Lake Plains Ecological Classification System (ECS) section and more abundant in the Northern Superior Uplands and Northern Minnesota and Ontario Peatlands sections, with relative abundance increasing along a southwest to northeast gradient. Methods will be further refined in 2017, with the goal of having an operational survey developed for regular implementation beginning in 2018.

INTRODUCTION

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

Developing large-scale monitoring programs that are both reliable and cost effective is challenging, especially when the species is relatively rare and occupies habitats that are not easily accessible. New York (Fritz 1979) and Wisconsin (Worland et al. 2009) have conducted statewide Page 69

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

As part of a pilot study, we evaluated survey methods that might be useful for monitoring spruce grouse populations in Minnesota or investigating questions related to habitat use and metapopulation dynamics. We evaluated an auditory survey using playback of female cantus calls, which is the most common approach to survey spruce grouse (Fritz 1979, Boag and McKinnon 1982, Whitcomb et al. 1996, Lycke et al. 2011, among others). We also conducted pellet surveys and used pointing dogs to locate birds on survey plots following completion of a cantus-call survey (Roy et al. 2013b, 2014).

OBJECTIVES

The primary objectives of the pilot study were to:

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

STUDY AREAS

In 2014, we focused on the Red Lake Wildlife Management Area (RLWMA) and Beltrami Island State Forest (BISF; Roy et al. 2013b, 2014). This study area is on the southwestern edge of the presumed spruce grouse range, where changes (range contraction or negative trends in abundance, density, or patch occupancy) might occur earlier than in more central portions of the range. In 2015, we focused on portions of RLWMA and BISF where spruce grouse detections occurred in 2014, so survey methods would be evaluated in areas where birds were known to occur (Figure 1). We also added a second study site near Isabella (Figure 2), which is more centrally located within Minnesota SPGR range. This study site offered insights into survey methods where populations might be more robust to initial habitat changes. Hereafter, we refer to this study site as the NE study site and the one at RLWMA and BISF as the NW study site.

In 2016, we expanded the survey area to include all or most of spruce grouse range in Minnesota (Figure 3). The current limits of spruce grouse range are unknown, so we focused on forest types used by spruce grouse within 3 ECS sections (Northern Minnesota and Ontario Peatlands, Northern Superior Uplands, and Northern Minnesota Drift and Lake Plains) to delineate an area to be surveyed for spruce grouse in 2016. We also referenced harvest data reported in the Small Game Hunter Mail Survey (Dexter 2015) to incorporate county-level harvest information for spruce grouse.

METHODS

Identifying Spruce Grouse Habitat

The literature is conflicting with respect to forest ages of importance for spruce grouse; earlier successional stages have been reported to be important in the western U.S. (Boag and Schroeder 1992), but mature forest was important in Wisconsin (Anich et al. 2013). In 2014, we included forest types reported to be preferred by spruce grouse in our region, including jack pine

(*Pinus backsiana*), black spruce (*Picea mariana*), and tamarack (*Larix lariana;* Robinson 1969, Pietz and Tester 1982, Anich et al. 2013). We included all stand ages because of the lack of clarity in the literature but focused on preferred habitat types rather than all used habitat types. We also included white cedar (*Thuja occidentalis*), which was reported to be used but not a preferred habitat type (Anich et al. 2013), because managers were specifically interested in surveying this forest type.

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

In 2016, we continued with the use of forest types used in 2015—black spruce, jack pine, balsam fir, red pine, white spruce, tamarack and white cedar \geq 6 years old. Sources of forest inventory data expanded to include lands administered by the U.S. Forest Service Chippewa National Forest as well as by county land departments, including Aitkin, Beltrami, Carlton, Cass, Clearwater, Crow Wing, Hubbard, Itasca, Koochiching, Lake and St. Louis Counties. Since harvest and stand replacement disturbance information was not readily available for all forest inventory sources, a satellite-interpreted forest loss data layer (Hansen et al. 2013) was used to identify areas of forest stands \geq 6 years old. Forest stands meeting the cover type and age requirements were further dissolved into patches to determine sites that had a sufficient amount of habitat to support spruce grouse. Habitat patches \geq 8 ha were used to identify potential survey route corridors when they overlapped accessible roads based on patch occupancy rates and home range information from the literature (Fritz 1979, Whitcomb et al. 1996).

Survey Routes and Listening Points

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

In 2015, we used the same GIS layers to select survey points, but also used current data for U.S. Forest Service roads, forest harvest, and fire data for the NE study site (U.S. Forest Service 2013, National Interagency Fire Center 2013, U.S. Forest Service 2015a,b). However, our focus in the second season was a comparison of off-road and on-road survey points to examine the impact of roads on survey detections. We selected paired points that had at least 30% spruce grouse habitat (based on selected forest types) within 150 m of each point, but limited our selection to areas where habitat occurred on both sides of the road. Off- and on-road points were separated by 300 m, and we alternated the side of the road where off-road points were selected, except when creeks limited access on foot.

In 2016, we used a GIS to identify 1,862 potential survey points in probable spruce grouse range that were located on accessible roads, permitted access off road without limitations by water barriers, had \geq 30% spruce grouse habitat within 150 m of each point on both sides of the road, and were associated with spruce grouse habitat patches \geq 8 ha. We spaced points \geq 400 m to obtain the greatest spatial coverage of focal stands throughout the probable spruce grouse range. We used a Generalized Random Tessellation Stratified Sampling approach (Stevens and Olsen 2004) to select seed points for approximately 80 routes and attempted to identify groups of 8–10 points that were in spatial proximity to construct survey routes. We also considered proximity to potential lodging centers (travel time), local expertise on accessibility, and the distribution of routes by ECS subsections and sections when selecting the final sample of routes and points. Our final sample consisted of 65 routes with 2–13 survey points/route (median = 10). Forty-three routes (400 survey points) were located in the N. Superior Uplands (core of probable spruce grouse range in MN), 11 routes (120 points) were in the N. Minnesota & Ontario Peatlands (containing the RLWMA and BISF), and 11 routes (93 points) were in the N. Minnesota Drift & Lake Plains (southern edge of probable spruce grouse range in MN).

Cantus Call Surveys

We used a playback of female cantus calls to conduct point-count surveys of spruce grouse (Fritz 1979, Boag and McKinnon 1982, Schroeder and Boag 1989, Whitcomb et al. 1996, Lycke et al. 2011). In 2014, we surveyed as many points as possible to provide information on survey duration (1–15 min), time needed to complete multiple surveys, habitat associations, and the responsiveness of spruce grouse to cantus calls (i.e., time of day and season). Surveys were conducted during April–May, beginning at sunrise, when winds were <10 mph and precipitation was absent or light. Each point count lasted 15 min (Lycke et al. 2011, Anich et al. unpubl. data) and was divided into 5 consecutive 3-min listening intervals. The 8-sec cantus call was broadcast once per min throughout the 15-min listening period. Observers recorded initial and subsequent detections of each spruce grouse by listening interval, which allowed us to construct individual detection histories for a time-of-detection analysis (TOD, Alldredge et al. 2007). We also recorded type of initial detection (flutter flight, approach, etc.), survey date, arrival time, wind speed, temperature, dominant tree species (as classified from the roadway: jack pine, black spruce, tamarack, white cedar, red and white pine, balsam fir, deciduous, other), and background noise (none, low, medium, high).

We used the function 'occup' in R package 'unmarked' (Fiske and Chandler 2011; R Core Team 2016) to fit some exploratory hierarchical occupancy models to the cantus call survey data from 2014. We included detection covariates for survey date and arrival time, and occupancy covariates for relative amount of spruce grouse habitat around each listening point (habitat sides = 0, 1, 2) and survey date. We also included a time covariate that allowed probability of detection to vary by listening interval. Continuous covariates (survey date and arrival time) were standardized prior to analysis. We used AIC (Burnham and Anderson 2002) to select a best approximating model for the detection process and then used that structure to examine occupancy covariates. We considered models with Δ AIC values <2.0 to have similar levels of fit and in such cases we used the simplest model for inference (Arnold 2010). For simplicity, we restricted our exploratory analysis to initial visits and excluded surveys with background noise = 3 (high) or missing data. The final dataset consisted of 459 cantus-call surveys at unique listening stops.

In 2015, we modified call survey methods to incorporate findings from 2014. Specifically, we reduced the survey length from 15 to 9 min, began surveys 30 min earlier, and ended call surveys by 0930 hr. For analysis, we used a dynamic occupancy modeling approach (MacKenzie et al. 2006:183–224) to look at TOD and revisits in the same analysis. We used the 'colext' function in the R package 'unmarked' (Fiske and Chandler 2011, R Core Team 2016) to fit models. We used visits as the primary sampling unit and TOD as secondary sampling occasions, and we allowed the true occupancy status to change between visits (i.e., via transition probabilities). We examined 4 site-level covariates (study area, year, location [road vs. off-road], forest type), 5 visit-level covariates (observer, survey date, start time, start temperature, and wind speed), and 2

observation-level covariates (TOD interval and previous detection). We first fit and examined covariates for the detection process and then carried the best approximating model forward to examine covariates for the state variable (occupancy). Transition probabilities (γ and ϵ) were viewed as nuisance parameters in this analysis and we had no *a priori* reason to model these parameters using covariates. Therefore, we used constant transition probabilities in all models. We used a bootstrap Goodness of Fit to evaluate the fit of our full model (model with largest number of parameters) and AIC to select among competing models. We considered models with Δ AIC values <2.0 to have similar levels of fit and in such cases we used the simplest model for inference (Arnold 2010).

Call survey methods were discontinued in 2016. Data collected in 2014 and 2015 indicated that pellet surveys had substantially higher detection probabilities and would be easier to implement in a large-scale survey of spruce grouse in northern Minnesota.

Pellet Surveys

We counted grouse pellets and roost piles <1 m on either side of transects. We distinguished ruffed grouse pellets from spruce grouse pellets on the basis of length, thickness, uric acid wash, and color (N. Anich, A. Ross, M. Schroeder, pers. comm.). Ruffed grouse pellets tend to be shorter, thicker, and usually have a uric acid wash, whereas spruce grouse pellets are longer, thinner, and infrequently have a uric acid wash. Spruce grouse pellets are also darker green in color when spruce grouse are consuming conifer needles (during winter), but color changes depending on diet (pers. observ.); spruce grouse pellets can have a similar color to ruffed grouse pellets later in the spring. Finally, we recorded dominant and subdominant tree species along each circular path to compare forest-type classification based on GIS, roadside observations, and pellet surveys.

In 2014, we surveyed circular transects of 75-m and 100-m radii centered on call survey points on roads. In 2015, we surveyed circular transects of 100-m radius centered on paired points on and off roads, because the larger radius improved detection. Surveys were repeated up to 3 times to allow for modeling of detection using function 'occu' in R package 'unmarked' (Fiske and Chandler 2011). In 2016, pellet surveys were conducted at each transect once to maximize spatial coverage, and all pellet transects were centered on roads.

In 2016, we fit generalized linear mixed-effect models to the data to compute 2 monitoring metrics: an occupancy index (using presence-absence of pellets and a binomial link function) and a pellet-count index (using a Poisson link function). In each case we treated 'route' as a random effect to account for the clustered sampling design, and evaluated potential covariates affecting the response metric (e.g., amount of spruce grouse habitat, spatial location) as well as covariates that might serve as a surrogate for probability of detection (i.e., snow coverage [none, partial, complete] and days since last snow; based on results from 2014 and 2015). We used the function 'glmer' in the R package 'lme4' (Bates et al. 2015, R Core Team 2016) to fit the models, and we used AIC to select among competing models. Because our sample of points was not proportionally allocated (we sampled more heavily in core areas), we used our best approximating models, with the surrogate variable for detection held constant, to predict mean naïve occupancy and pellet abundance for each point in the sampling frame (1,862 points). We then computed a simple arithmetic mean prediction by ECS section and rangewide to generate 2 monitoring metrics. We used a bootstrap of routes (200-300 replicates, with replacement) to compute percentile confidence intervals that included uncertainty in model-fitting and prediction. We also used the R package 'akima' (Akima and Gebhardt 2015) to implement a bivariate interpolation of our irregularly spaced prediction surface, which we used to qualitatively assess how model predictions varied over Minnesota's probable spruce grouse range as a function of spatial location and the relative abundance of jack pine and black spruce cover types.

Capture and Radiomarking

We attempted noosing a few spruce grouse in 2014 (Zwickel and Bendell 1967), but the primary obstacle to using this technique was locating birds. We examined the efficacy of using

trained dogs to find birds in spring 2014. We also utilized the cantus call to locate birds while they were responsive.

We tested other capture methods at RLWMA in 2015. We deployed 8 lily-pad traps and 7 mirror traps in late March and trapped for 58 days (Guillon 1961, 1965). We dyed corn red (Guillon 1961) with hummingbird food to appear like berries and placed the seed inside traps as a lure. We used the cantus call as a lure in traps a few times, but batteries needed to be replaced in playback devices >1/day, which was a lower priority than call and pellet surveys in 2015. During 3–6 September, we enlisted the help of 4 volunteers with 6 trained dogs to see if success finding spruce grouse was higher when birds would be expected to spend more time on the ground (i.e., more foods on the ground in the late summer, as opposed to conifer needles in trees during winter and early spring).

We deployed 10 necklace-style transmitters (12 g, model A-3950 from ATS- Isanti, MN) on spruce grouse at RLWMA in spring 2015 to gather pilot data on movements and tracking constraints in areas with limited road access. Marked birds were tracked by Area Wildlife Staff at monthly intervals throughout the year. Tracking will continue while transmitters are active (estimated battery life is 253–445 days), but we expect transmitters to expire by the end of summer 2016.

RESULTS

2014 Abridged

We surveyed 56 roadside routes for a total of 530 unique listening points that were surveyed up to 3 times (see Roy et al. 2014 for more detailed results). We detected spruce grouse at 26 call-survey points (4%). Birds were detected in all 5 listening intervals, although 78% of birds were detected in the first 3 intervals.

Our best approximating hierarchical occupancy model included detection covariates for survey date, arrival time, whether the bird was detected in a previous listening interval, and an occupancy covariate describing the relative amount of spruce grouse habitat surrounding the listening point (habitat sides = 0, 1, 2). Mean probability of detection was negatively associated with survey date and arrival time (Figures 4 and 5). Not surprisingly, probability of detection increased dramatically if a bird was detected in a previous listening interval. The mean probability of occupancy for a listening stop with spruce grouse habitat on both sides of the road was 0.23 (95% CI = 0.02-0.78; Figure 6), and the overall probability of detection for the entire 15-min survey, given mean covariate values for survey date and arrival time, was 0.25 (95% CI = 0.02-0.93).

We conducted pellet surveys at 230 listening points and detected pellets at 45 (20%) points. Pellet surveys and cantus-call surveys had 82% concordance for presence-absence of spruce grouse. However, we detected pellets at 36 points (16%) where we failed to detect a bird during cantus-call surveys. This contrasts with failure to detect pellets at 5 (2%) points where we detected spruce grouse during cantus-call surveys. The 100-m radius survey path resulted in 28 detections (39%) compared to 18 (11%) detections with a 75-m radius path.

Nineteen additional spruce grouse were located while walking transects around survey points with dogs. We spent 33 additional hrs walking trails with dogs and their handlers and located 6 more spruce grouse.

2015

We surveyed 200 paired points in the NW study area and 190 points in the NE study area 1–3 times. Our findings for the cantus call survey in 2015 were qualitatively similar to those in 2014, with higher detection probabilities earlier in the day, earlier in the season, earlier in the listening period or in a former listening period (Figures 4–5). The call detection rate was 3-fold higher (compared to 2014), but still 3–4 times lower than that for pellet surveys. Comparisons between study areas indicated similar detection rates with the call survey and pellet survey on road-based points, but slightly higher detection rates at off-road points in the NE study area (Table 1). However, this effect was much smaller in stands that are preferred by spruce grouse based

on the literature (Figure 7). Based on these findings, a pellet survey was deemed the better approach for a large-scale survey.

We captured 3 spruce grouse in 870 trap-days; 1 female spruce grouse in a lily-pad trap with the cantus call in late March and a male and female in mirror traps in late April-early May. We also noosed 7 birds that responded to cantus call surveys or were encountered by chance. Two additional spruce grouse were noosed after location by a trained dog, but a consistent effort with dogs was not pursued in spring 2015. During fall, we spent 20 hrs searching for spruce grouse with volunteers and trained dogs and located 11 spruce grouse. We attempted to noose 6 and caught 4 (67%).

We placed 10 transmitters on 12 different birds (5 males, 7 females). Three birds died; 2 females and 1 male 4-10 months after marking. One male dropped his collar due to improper crimping, and the collar was redeployed on a female. We believe 1 transmitter malfunctioned during fall 2015 because a telemetry flight could not find the signal within 5 miles of Norris Camp (near its last location). One transmitter was not in mortality mode when the bird was found dead. Thus far, birds have remained close enough to roads to locate them from the ground. Minimum convex polygons encompassing all points for a particular grouse were 1–64 ha. Eight transmitters were still deployed and active as of May 2016.

2016

We removed 38 (6%) pellet survey points that were not surveyed due to access issues (private land, roads closed or impassable, etc.) and 8 (1%) survey points with missing data (e.g., no spruce grouse habitat to survey) or duplicate information (>1 visit). Thus, our final sample consisted of 567 survey points organized into 65 survey routes. However, 77 (14%) of the 567 survey points would probably need to be removed or replaced in an operational survey due to significant access challenges (e.g., water crossings, long walks, difficult terrain, etc.). Eighty-two percent of the points were located on dirt roads and traffic was light to none during most (86%) surveys. Based on GIS data, spruce grouse habitat at the 567 survey points comprised, on average, 80% of the cover, with jack pine and black spruce cover types accounting for 38% (range = 0–100%). Upon inspection, 8 plots (1%) were dominated by deciduous or open cover types, but they contained at least some marginal spruce grouse habitat on 1 side of the road (habitat = 1). Fifty-two percent of the points contained \geq 30% jack pine or black spruce cover types. Thus, the GIS data performed reasonably well in identifying potential survey points.

Pellet surveys were conducted over 42 survey days from 2 March to 29 April 2016. Spruce grouse pellets were detected at 24% of the survey points, but it varied by ECS section (Table 2). On points where spruce grouse pellets were detected, we counted a mean of 5.3 pellet groups (SD = 6.2); 87% of these points contained roost piles and 24% contained fresh pellets (Table 3). Ruffed grouse pellets were detected at 56% of the survey points where spruce grouse pellets were detected at 56% of the survey points where spruce grouse pellets were detected at 56% of the survey points where spruce grouse pellets were detected on 8% of "occupied" points but only 2% of "unoccupied" points.

The probability of detecting spruce grouse pellets was positively correlated with percent cover of jack pine and black spruce habitat (based on GIS data), negatively correlated with complete snow cover (a surrogate for detection probability), and positively correlated with a southwest to northeast spatial gradient (Figure 8). The same model structure best explained variation in pellet-group counts, but uncertainty associated with the mean functions was much greater (e.g., Figure 9). Consistent with anecdotal information, both monitoring metrics suggested spruce grouse were relatively rare in the N. Minnesota Drift & Lake Plains eco-section and more abundant in the Northern Superior Uplands and N. Minnesota & Ontario Peatlands eco-sections (Figure 10). Likewise, when viewed over a smoothed prediction surface, both metrics suggested the relative abundance of spruce grouse increased on a southwest to northeast gradient (Figure 11).

DISCUSSION

We will continue to refine our survey methodologies with the goal of designing a largescale pellet survey that can be conducted regularly. Pellets are numerous, more easily detected, and identification can be easily learned by field biologists with limited instruction. In 2016, we piloted a large-scale survey and found that many points were difficult to access, clearly indicating the need for further refinements before making the survey operational by staff. However, staff stationed closer to survey locations will have the benefit of waiting until survey conditions improve (i.e., roads clear, snow not accumulating) before conducting surveys, which should make data collection easier and more consistent over time. We also plan to explore more sophisticated analytical approaches for single-visit surveys where both the detection and state process vary as a function of covariates (e.g, Lele et al. 2011, Solymos et al. 2012). We hope to conduct a simulation study to examine sample size needs to be able to detect population-level changes in our proposed monitoring metrics, as well as to evaluate the representativeness of our roadside sample in terms of point attributes (e.g., cover types).

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LITERATURE CITED

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Year	Study area	Location ¹	Method ²	n (total)	n (used)	Prop. with detection ³	95% LCL⁴	95% UCL⁵
2014	RLWMA	R	Call	530	26	0.05	0.03	0.07
2014	RLWMA	R	Pellet	230	45	0.20	0.15	0.25
2015	RLWMA	R	Call	100	13	0.13	0.06	0.20
2015	RLWMA	OR	Call	100	19	0.19	0.11	0.27
2015	Ely	R	Call	95	13	0.14	0.07	0.21
2015	Ely	OR	Call	95	24	0.25	0.16	0.34
2015	RLWMA	R	Pellet	100	64	0.64	0.55	0.73
2015	RLWMA	OR	Pellet	100	63	0.63	0.53	0.73
2015	Ely	R	Pellet	95	59	0.62	0.52	0.72
2015	Ely	OR	Pellet	95	76	0.80	0.72	0.88
2015	RLWMA	R	P+Add	100	68	0.68	0.59	0.77
2015	RLWMA	OR	P+Add	100	67	0.67	0.58	0.76
2015	Ely	R	P+Add	95	60	0.63	0.53	0.73
2015	Ely	OR	P+Add	95	76	0.80	0.72	0.88
2015	RLWMA	R	C+P+Add	100	70	0.70	0.61	0.79
2015	RLWMA	OR	C+P+Add	100	69	0.69	0.60	0.78
2015	Ely	R	C+P+Add	95	62	0.65	0.55	0.75
2015	Ely	OR	C+P+Add	95	77	0.81	0.73	0.89

Table 1. Naïve detection rates for spruce grouse using 2 survey methods in northern Minnesota during springs 2014 and 2015.

¹Location of survey points: R = road, OR = off-road.

²Survey method: C or call = call survey, P or Pellet = pellet survey, Add = additional sightings of spruce grouse at survey points.

³Proportion of survey points where spruce grouse or spruce grouse sign were detected.

⁴95% lower confidence limit of proportion.

⁵95% upper confidence limit of proportion.

Table 2. Sample statistics and occurrence indices for a survey of spruce grouse pellets at points (pts) in northern Minnesota during spring 2016.

ECSS ¹	No. possible sample pts	Prop. sample	No. pts surveyed	Sample fraction	No. survey routes	Prop. pts pellets detected	Prob. pellets post- adjust ²	85% LCL ³	85% UCL⁴
NSU	865	0.46	364	0.42	43	0.297	0.243	0.205	0.294
NMOP	407	0.22	115	0.28	11	0.209	0.173	0.080	0.308
NMDLP	590	0.32	88	0.15	11	0.034	0.036	0.019	0.055
All	1,862	1.00	567	0.31	65	0.238	0.166	0.129	0.207

¹ECSS = Ecological Classification System Section (NSU = Northern Superior Uplands; NMOP = Northern Minnesota & Ontario Peatlands; NMDLP = Northern Minnesota Drift & Lake Plains).

²Mean predicted probability of observing \geq 1 pellet after adjusting for snow coverage (surrogate for detection), % jack pine and black spruce cover, a spatial gradient (X+Y), and the non-proportional allocation of sample points among ECSS.

³Lower 85% percentile confidence limit on mean predicted probability of observing \geq 1 pellet.

⁴Upper 85% percentile confidence limit on mean predicted probability of observing \geq 1 pellet.

ECSS ¹	No. possible sample pts	Prop. sample	No. survey pts	Sample fraction	No. routes	No. pts pellets detected	No. pts fresh pellets detected	No. pts roost piles detected	Mean pellet grp count ²	SD ³	Mean predict pellet-grp count ⁴	85% LCL⁵	85% UCL ⁶
NSU	865	0.46	364	0.42	43	108	27	98	5.2	6.20	0.64	0.41	1.04
NMOP	407	0.22	115	0.28	11	24	5	19	5.9	6.42	0.90	0.30	3.36
NMDLP	590	0.32	88	0.15	11	3	0	1	3.7	4.62	0.07	0.03	0.17
All	1862	1.00	567	0.3	65	135	32	118	5.3	6.18	0.70	0.32	1.12

Table 3. Sample statistics and count indices for a spruce grouse pellet survey at points (pts) in northern Minnesota during spring 2016.

¹ECSS = Ecological Classification System Section (NSU = Northern Superior Uplands; NMOP = Northern Minnesota & Ontario Peatlands; NMDLP = Northern Minnesota Drift & Lake Plains).

²Mean pellet-group count (excluding zero counts).

³Standard deviation of the mean pellet-group count.

⁴Mean predicted pellet-group count after adjusting for snow coverage (surrogate for detection), % jack pine and black spruce cover, a spatial gradient (X+Y), and the non-proportional allocation of sample points among ECSS.

⁵Lower 85% percentile confidence limit on mean predicted pellet-group count after adjusting for snow coverage.

⁶Upper 85% percentile confidence limit on mean predicted pellet-group count after adjusting for snow coverage.

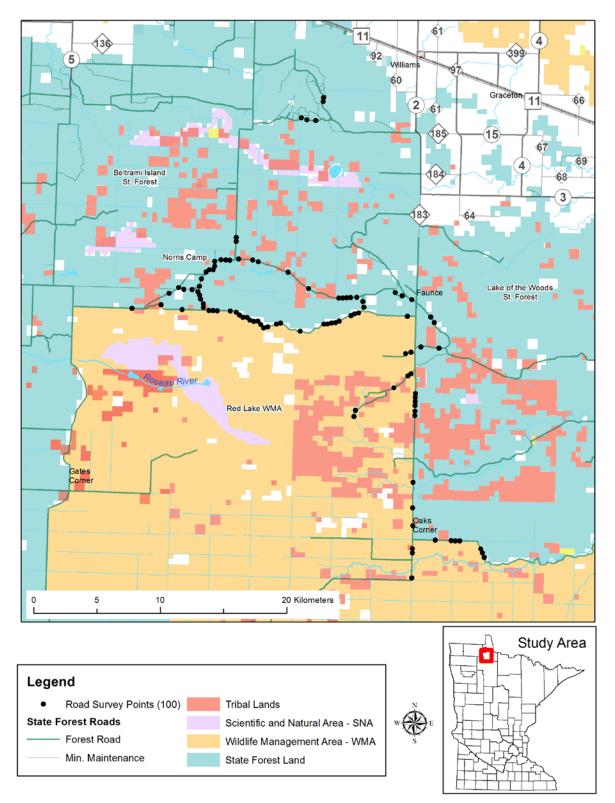


Figure 1. Study area at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2015. The study area was reduced to focus on areas where spruce grouse were detected in 2014. Off-road points were 300 m from road points and alternated sides except when access was prohibited.

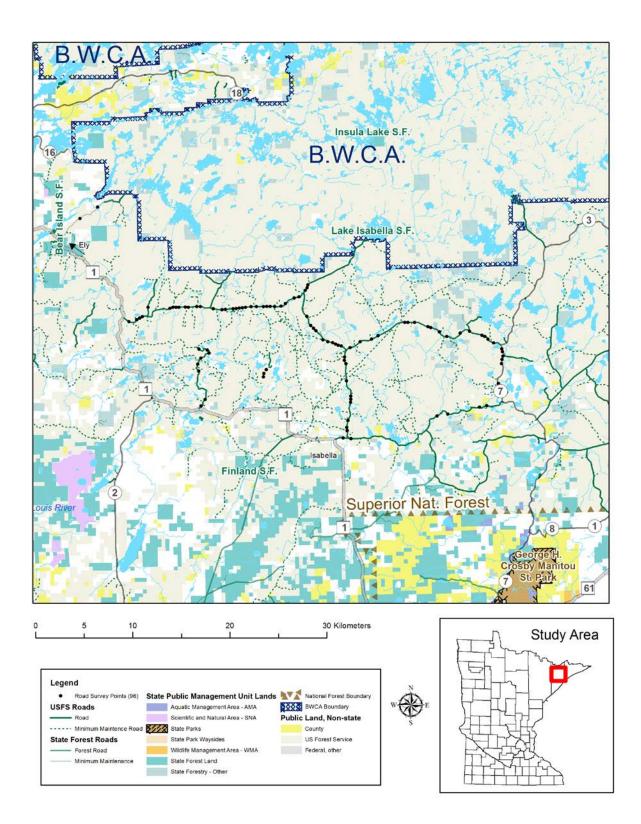


Figure 2. Study area near Isabella (NE) in 2015. Points indicate survey locations along roads. Off-road points were within 300 m of road points and alternated sides except when access was prohibited.

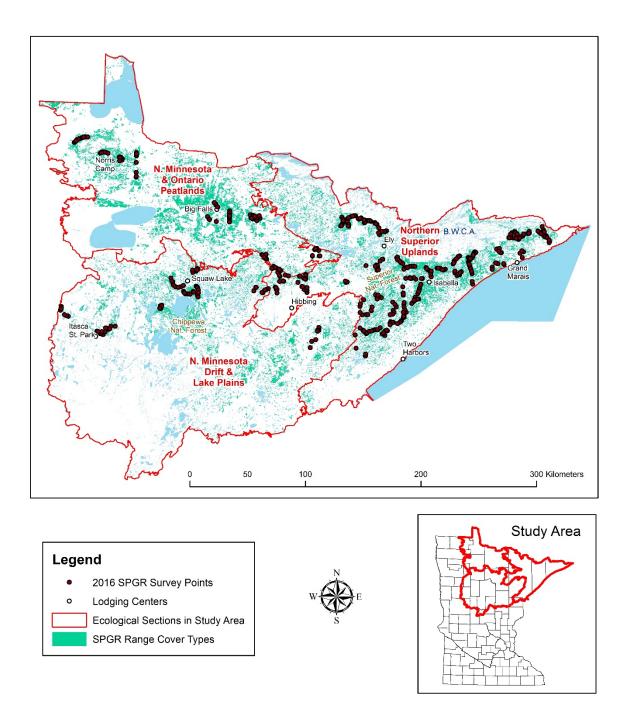


Figure 3. Spruce grouse study area in 2016. Survey points are depicted within the 3 Ecological Classification System sections.

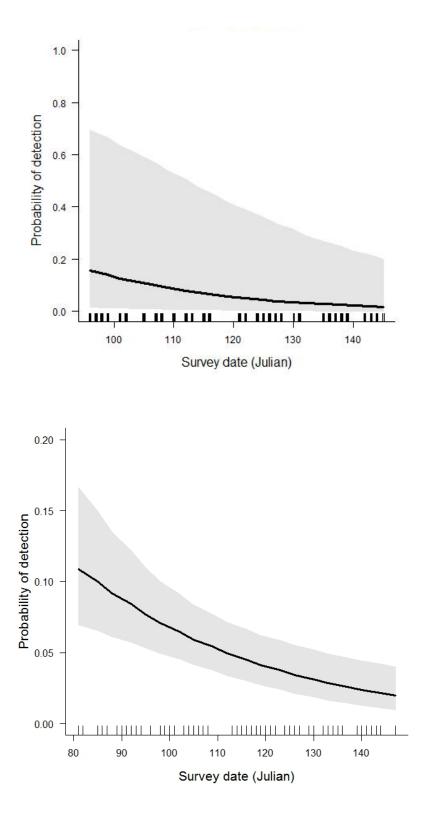


Figure 4. Mean conditional probability of detection (solid line; conditional on a bird being present and available for detection) in each listening interval as a function of survey date at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2014 (top) and both study areas in 2015 (bottom). Gray polygon denotes 95% confidence interval. The "rug" on the x-axis denotes the sample distribution.

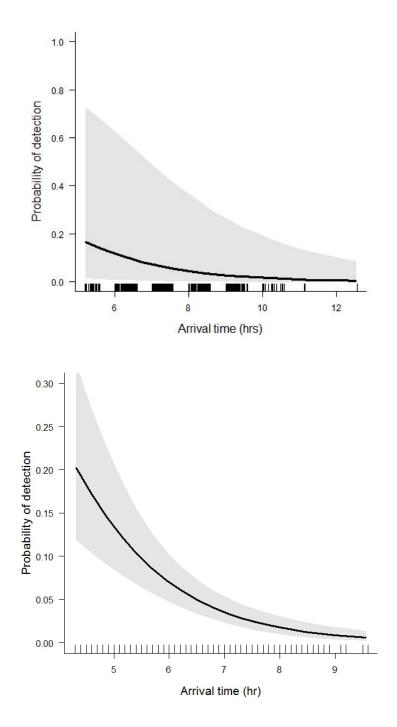


Figure 5. Relationship between spruce grouse call detections and cantus call survey arrival time (i.e., 6 = 0600 hours) at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2014 (top) and in both study areas in 2015 (bottom). Gray polygon denotes 95% confidence interval.

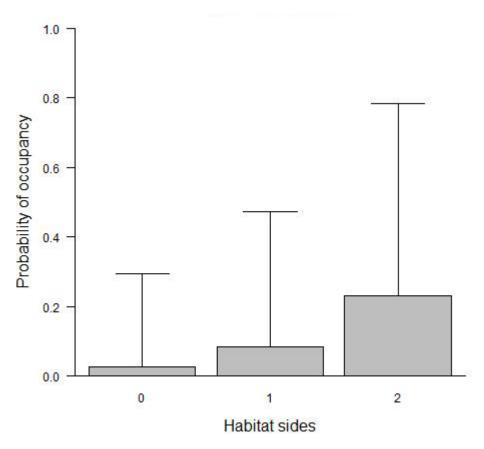


Figure 6. Relationship between the probability of spruce grouse occupancy and the presence of habitat on 0, 1, or 2 sides of the road during cantus call surveys at Red Lake Wildlife Management Area and Beltrami Island State Forest in 2014.

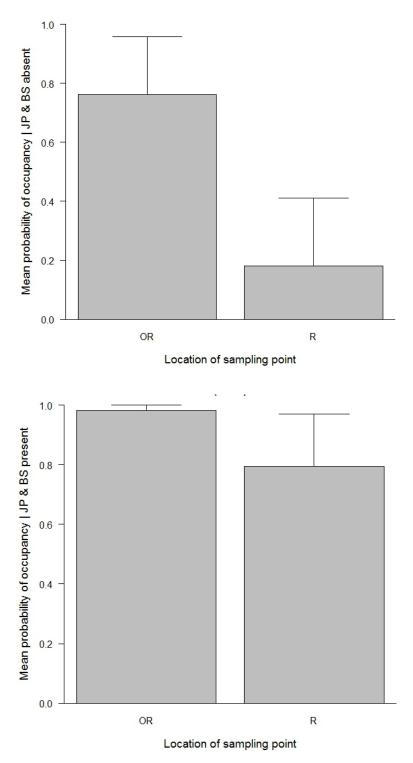


Figure 7. The mean probability of occupancy of spruce grouse at survey points located on roads (R) and off roads (OR) during cantus call surveys at points where jack pine or black spruce were not (top) and were (bottom) present in the Isabella (NE) study area in 2015.

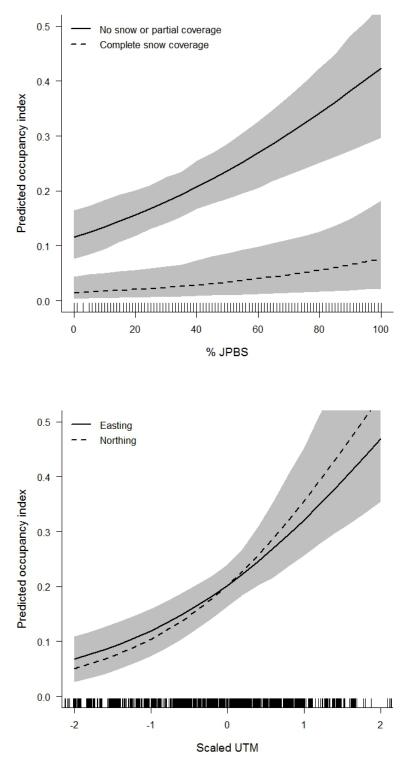


Figure 8. Mean probability of detecting spruce grouse pellets as a function of (A) percent cover of jack pine and black spruce habitats (%JPBS) and snow cover (surrogate for detection probability), and (B) the spatial location of survey points (with other covariates fixed at mean or base values). Figure is based on a generalized linear mixed-effects model fit to pellet-survey data in northern Minnesota during spring 2016.

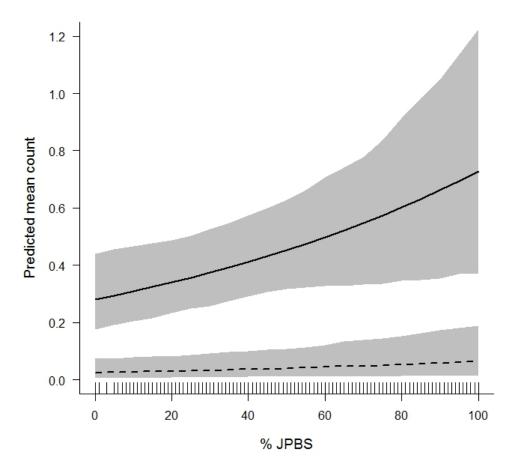


Figure 9. Mean count of spruce grouse pellet groups as a function of percent cover of jack pine and black spruce (%JPBS) habitats and snow cover (surrogate for detection probability). No snow or partial snow coverage is indicated by the solid line and the dashed line represents complete snow coverage. Figure is based on a generalized linear mixed-effects model fit to pellet-survey data in Minnesota during spring 2016.

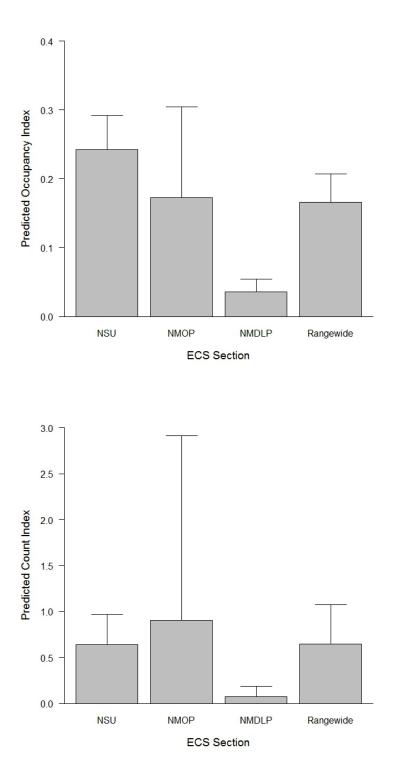
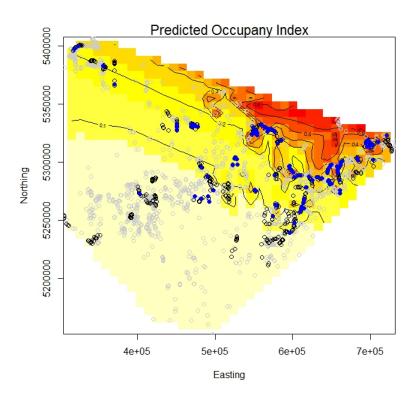


Figure 10. Potential monitoring metrics for spruce grouse in northern Minnesota during spring 2016. Figures are based on the arithmetic mean of model predictions applied to all potential roadside sampling points while holding the categorical predictor snow cover (surrogate for detection probability) to "None or partial." Ecological Classification System (ECS) sections included Northern Superior Uplands (NSU), Northern Minnesota & Ontario Peatlands (NMOP), and Northern Minnesota Drift & Lake Plains (NMDLP).



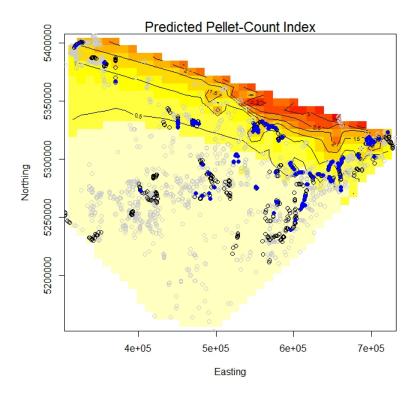


Figure 11. Smoothed prediction surface for spruce grouse monitoring metrics (red = highest; light yellow = lowest predicted index values) in northern Minnesota during spring 2016 based on a bivariate interpolation of model predictions. Contour lines with the highest predicted index values are also depicted.



ASSESSING THE VALUE OF PERMANENTLY MANAGED WILDLIFE OPENINGS FOR AMERICAN WOODCOCK

Lindsey Shartell

SUMMARY OF FINDINGS

This pilot study evaluates American woodcock (*Scolopax minor*) use of permanently managed forest wildlife openings. Field data collection began in April 2016 and will continue until September 2016. Spring singing ground surveys were completed at 98 sites in the Grand Rapids, Cloquet, and Red Lake WMA work areas. Woodcock were observed peenting or displaying within the opening at 56 sites (57%). Summer roosting ground surveys are currently being conducted and include monitoring woodcock use before and after summer mowing treatments. As part of these surveys, vegetation composition and structure is sampled to aid wildlife managers in creating optimal signing and roosting habitat for woodcock.

BACKGROUND

The American woodcock is a popular migratory game bird and Species of Greatest Conservation Need in Minnesota (MN DNR 2006). In 2014, Minnesota had an estimated 10,900 hunters take 18,600 woodcock, ranking Minnesota third highest in both woodcock hunter and harvest numbers (Copper and Rau 2014). Annual woodcock surveys have indicated a long-term (1968-2014) decline in singing male numbers across the full breeding range (Cooper and Rau 2014). These declines have been attributed to the loss of open and young (early successional) forest and shrub habitat due to succession, lack of disturbance, and development (Dessecker and McAuley 2001).

Woodcock require a variety of habitat components including dense young forests or shrublands and open singing and roosting grounds (Wildlife Management Institute 2009). In order to maintain and increase woodcock populations, forest management practices that create and maintain the necessary open components of their habitat are needed. Most forests associated with woodcock habitat do not naturally maintain permanent openings, but rather contain openings that are spatially and temporally dynamic. Historically disturbance by fire, wind, Native American activities, flooding, and beavers created openings and early successional habitat for American woodcock (DeGraaf and Yamasaki 2003). Many of the disturbances that created and maintained open areas are now prevented and replaced with the permanent management of openings using mowing and/or herbicide application. Current land management practices, however, can emulate the natural creation of dynamic openings through forest harvest, log landings, logging roads and trails, and other disturbances, eliminating the need for and cost of maintaining permanent openings. In addition, managing habitat based on the natural disturbance regime creates a more resilient ecosystem and better balances the many needs and goals of the system. For example, temporary openings may later provide dense young forest or shrub habitat that woodcock also require.

The secretive nature and cryptic coloration of the woodcock makes it difficult to estimate population size and management effects. There have been past studies assessing the use of openings by woodcock, but these are more often focused on wintering grounds (for example Glasgow 1958, Stribling and Doerr 1985, Berdeen and Krementz 1998) than on the spring and Page 93

summer range (though see Sheldon 1961). There is some documentation of the use of aspen clearcuts in Wisconsin and young pine plantations (1-5 year old) in Arkansas by woodcock in spring and summer (Hale and Gregg 1978, Long and Locher 2013), but additional research comparing the use and characteristics of temporary openings like these to permanent openings would improve our understanding and provide context for management in Minnesota.

The MN DNR maintains a number of permanent wildlife openings to provide singing and roosting grounds for woodcock, as well as habitat for a variety of other game and non-game species such as deer and bear. Wildlife managers have expressed differing opinions, however, on the need for permanently managed wildlife openings. Managers have also asked for information on best management practices for maintaining forest wildlife openings for woodcock, in particular, mowing frequency, timing of mowing, and vegetation structure necessary to maximize use by woodcock. The information gained in this study will allow for the development of better management practices for land managers and landowners interested in providing wildlife openings for woodcock and other wildlife.

OBJECTIVE

Assess woodcock use of managed forest openings and relate use to treatment/disturbance regime, persistence, size, vegetation composition and structure, and surrounding landscape (e.g. forest composition, landform, soil).

METHODS

Singing ground surveys for American woodcock were conducted from late April through May in forest openings within the Grand Rapids, Cloquet, and Red Lake WMA work areas. Surveys followed SGS protocol where possible (Cooper and Rau 2014). Surveys generally took place 15 to 60 minutes after sunset, when temperature was above 40 F, and there was no heavy precipitation or strong wind. Openings in close proximity were grouped to allow surveying multiple openings per evening. At each opening observers recorded their GPS location (UTM coordinates), time of sunset, sky condition, temperature, wind speed, precipitation, and any noise disturbance present at the time of the survey. Observers listened for and recorded the number of different woodcock heard peenting or observed displaying (heard and/or seen) within the opening during a listening period of at least 5 minutes. Observers also recorded other observations of woodcock (not within the opening) along with time and approximate location (direction and distance) of the woodcock.

Roosting ground surveys began in June and will continue until fall migration. These include before and after surveys at mowing sites and controls treated in late June. Roosting surveys were conducted using crepuscular flight surveys (Glasgow 1958, Berdeen and Krementz 1998) and spotlighting. Observers recorded their GPS location (UTM coordinates), time of sunset, sky condition, temperature, wind speed, precipitation, and any noise disturbance present at the time of the survey. The observers recorded the number of woodcock flying into the opening or heard peenting (when not seen) from 20 minutes before sunset to 40 minutes after sunset. After the survey window, observers systematically walked openings using spotlights and recorded the number of woodcock flushed or spotted, noting the flush direction and distance of woodcock to avoid double counting.

Vegetation within forest openings was sampled along two transects using a line intersect/intercept method (Canfield 1941). The first transect (Transect A) was placed across the widest part of the opening from edge to edge (as determined in GIS and in the field) and the second transect (Transect B) was placed perpendicular to the first crossing the opening from edge to edge to edge. The transect start and end points were marked using a flag and flagging to aid resampling, UTM coordinates were taken at each (using point averaging to increase accuracy). A measuring tape was stretched tight from the starting point to the end point and secured in place by rebar. The direction of the transect (azimuth) from the start point facing the end point was recorded, and a photo of the site from the start point facing the end point of the transect Page 94

was taken. Observers also described the habitat across the entire opening (e.g., number of trees, distribution of trees, percent shrub cover) and the surrounding habitat by type (e.g. upland forest, lowland forest, upland shrub), tree or shrub species, and coarse age class (young, middle, old). Vegetation was sampled along the right edge of the measuring tape (from the start point looking towards the end point). For each change in cover, the start distance to the nearest tenth of a meter (e.g., 1.1 m, 5.8 m), the cover type code, abundance, and height class was recorded (Table 1). For shrubs and trees taller than 1.5 m, the actual height to the nearest meter was recorded.

To assess the use of openings in this study by other wildlife (e.g. deer, bear) the presence of scat encountered within 0.5 m of the transect was recorded along with the distance and suspected species.

PRELIMINARY RESULTS

Singing ground surveys were conducted at 98 sites. American woodcock were observed peenting or displaying within the opening at 56 sites (57%). Woodcock were heard peenting from surrounding vegetation outside of the opening at 50 sites, 22 of which had no woodcock within the opening. Field work and data entry are still underway so no further results are available at this time.

ACKNOWLEDGEMENTS

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Stribling HL, Doerr PD. 1985. Nocturnal use of fields by American woodcock. Journal of Wildlife Management 49:485-491.

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Table 1. Codes used to describe vegetation composition and structure along transects within forest openings.

Cover type codes	Abundance codes	Height codes
G = Graminoid H = Herbaceous W = Woody (<0.5 m tall) S = Shrub (0.5 - 2 m tall) T = Tree (>2 m tall) B = Bare Ground C = Coarse Woody Debris	R = Rare, < 25% S = Sparse, 25-50% M = Moderate, 50-75% D = Dense, > 75%	0 = < 3 cm 1 = 3-10 cm 2 = 10-30 cm 3 = 30-50 cm 4 = 0.5 - 1.5 m



USING MOVEMENT BEHAVIOR OF ADULT FEMALE MOOSE TO ESTIMATE SURVIVAL AND CAUSE-SPECIFIC MORTALITY OF CALVES IN NORTHEASTERN MINNESOTA

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SUMMARY OF FINDINGS

Continuing research on cause-specific mortality and annual survival of moose (Alces americanus) calves in northeastern Minnesota is critical to understanding the long-term trajectory of the population. Thirty-five global positioning system (GPS)-collared adult females were computer-monitored beginning in late April for calving movements, or a long distance movement followed by intense localization. We observed 28 of 35 (80.0%) make a calving movement, and along with additional visual observations of calves, determined 31 of the 35 (88.6%) cows were pregnant. Mean birth-date was 12 May 2016 (median = 11 May 2016, 24 Apr-10 June [range]). Following confirmation of calf presence (e.g., calf pellets, tracks, afterbirth), cows were monitored for a rapid, long distance movement ("flee") followed by a return to the origin of the flee, indicating a possible predator attack. We observed evidence of 14 mortalities with a mean age at death of 13.8 days (± 3.2 [SE], 2.7–33.7). Specific causes of mortality included: 10 wolf-kills, 2 bear-kills, 1 unknown predator-kill, and 1 death following a possible vehicle collision. The mean distance cows fled from the mortality site was 1,634 m (± 444, 126–5,805, n = 12). Eight of 12 cows returned to the mortality site a mean 2.6 ± 0.5 times. Mean search time by field staff when a mortality occurred was 67.6 min (± 9.9, 25–134, n = 14), covering 2.7 hectares (± 0.5, 0.9–7.4, n = 13). Calf survival to 30 days of age was $61.5\% \pm 10\%$. Understanding movement behaviors of cows can yield important insight into mechanisms driving the decline of the population in northeastern Minnesota and aid in future management decisions.

INTRODUCTION

The near disappearance of moose (*Alces americanus*) in northwestern Minnesota since the mid-1980s and a dramatic decline of northeastern Minnesota's population since 2006 (Murray et al. 2006; Lenarz et al. 2009, 2010; DelGiudice 2016) prompted aggressive studies of survival and cause-specific mortality of adults and calves in 2013 in northeastern Minnesota (Figure 1) using cutting-edge global positioning system (GPS)-collar technology (Carstensen et al. 2015, Severud et al. 2015*a*). Earlier work (2002–2008) in northeastern Minnesota focused on moose survival and employed very high frequency (VHF) telemetry. Current studies switched to GPS collars to facilitate more expeditious investigations of adult and calf mortalities (Butler et al. 2013, Severud et al. 2015*a*).

Adult survival and reproduction are the primary drivers of ungulate population performance (Gaillard et al. 2000, Raithel et al. 2007). Several studies have reported that low and highly variable juvenile survival contribute to population declines (Hatter and Janz 1994, Cooley et al. 2008, Forrester and Wittmer 2013). Ungulate calves are particularly vulnerable to predation within the first few months of life (Franzmann et al. 1980, Keech et al. 2011, Patterson et al. 2013). In the most recent study of cause-specific mortality of moose calves in northeastern Minnesota, 50%

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of the collared individuals died within 50 days of birth (Severud et al. 2015*b*). The majority of calves were killed by wolves (*Canis lupus*), and a smaller proportion by black bears (*Ursus americanus*). Other studies have reported similar findings (Ballard et al. 1981, Osborne et al. 1991, Keech et al. 2011, Patterson et al. 2013). Wolves may prey on calves throughout the entire year, whereas bears have their greatest impact closer to parturition when the calves are less mobile (DelGiudice et al. 2009, Basille et al. 2015).

A primary objective of 1 of the studies initiated by the Minnesota Department of Natural Resources (MNDNR) in 2013 has been to assess annual variation of cause-specific mortality of calves. Unique challenges to the study's protocol for capturing and handling neonates in 2013 and 2014 (e.g., capture-induced abandonment, DelGiudice et al. 2015) and adults in 2015 (e.g., capture-related mortality, Carstensen et al. 2015) resulted in a Governor's executive order that captures be discontinued. Consequently, since 2015, research has continued without the benefit of neonates fitted with GPS collars (Severud et al. 2015*a, b*). In 2015, we monitored 60 cows with functioning GPS collars for calving activity (i.e., calving movement), and subsequently for a "predation movement" relative to a potential calf mortality. A predation movement was described as a cow making a sudden long-distance movement ("flee"), followed by a return to the origin of the flee, and often multiple times (Figure 2; T. R. Obermoller et al., University of Minnesota, unpublished data).

Dams with young calves have reduced movements (Testa et al. 2000), which allowed us to differentiate between cows with and without calves. The calves' limited mobility at an early age makes them particularly vulnerable to wolf and bear predation. Once dams lose their calf or calves, their movements may increase by ~12% within 48 hours (Testa et al. 2000). DeMars et al. (2013) applied a movement threshold (using a 3-day average) to caribou (*Rangifer tarandus*) dams. When her movements exceeded 186.5 m/hour, her calf was assumed to be dead. "Normal movements" of females then resumed, because they were no longer limited by the mobility of a calf. These authors successfully detected calf survival up to 4 weeks of age using this threshold. We attempted to improve our understanding of the temporal and spatial aspects of maternal movement patterns relative to calf mortalities using 2013 and 2014 movement data of GPS-collared moose dams relative to known mortalities of their GPS-collared calves. We then applied that understanding to support detection and investigation of mortality events of calves born in spring 2016.

OBJECTIVES

- 1. Determine the pregnancy rate of GPS-collared adult females by intense computermonitoring of movements associated with calving activity and field confirmation
- 2. Increase our understanding of dam movements relative to cause-specific mortality of calves
- 3. Identify, locate, and assign cause of mortality to moose calves with field confirmation
- 4. Determine seasonal survival rates of moose calves

METHODS

Adult moose (128 females, 51 males) were captured and fitted with GPS collars (Vectronic Aerospace GmbH, Berlin, Germany) in winters 2013–2015 as part of a companion study examining survival and cause-specific mortality in northeastern Minnesota (Butler et al. 2013; Carstensen et al. 2014, 2015). Due to natural mortalities, malfunctioning GPS collars, and battery expiration, 35 adult females with functioning GPS collars were available for intense computer-monitoring during and beyond the 2016 calving season. Twenty-three cow collars were programmed to take locations every 4 hours and transmit these locations to our base station after 6 successful fixes. The remaining 12 cow collars were locked in "mortality mode," programmed to take locations every hour and transmit these locations after 11 successful fixes. In early May, all cows were monitored for a calving movement, which is a long distance movement followed by an intense localization (McGraw et al. 2014, Severud et al. 2015*b*).

highlighting calving movements were generated twice daily based on a 4-hour fix-rate for each cow (Severud et al. 2015*a*; J. D. Forester, University of Minnesota, unpublished data). We verified calving by examining the calving site for calf presence (e.g., tracks, pellets, hair) or evidence of birth (e.g., scrape in the earth, afterbirth) after the cow left the area to avoid disturbing her or her calf. In a few cases, we confirmed the presence of a calf by a public-reported visual or by searching a subsequent localization. Following verification of a calf by 1 of these methods, we monitored each cow's locations daily for a previously described predation movement.

In 2013 and 2014, we captured and fitted 74 neonates with GPS collars (with mortality accelerometers) to monitor them for survival and cause-specific mortality events (Severud et al. 2015*a*, *b*). We analyzed movement patterns of GPS-collared dams of GPS-collared calves that died of known causes in 2013 and 2014 to determine characteristics indicative of specific causes of mortality (e.g., wolf or bear predation, abandonment). Specifically, we used temporal and spatial analyses of mortalities of 2013 and 2014 to aid in identifying calf mortality events in 2015 and 2016. In 2015, a 50% success rate of detecting mortalities associated with a predation movement was documented until moose neonates were approximately 3 weeks of age, after which, identifying mortality sites became increasingly difficult, because of increased movement rates of the cow-calf pair (Severud et al. 2015*b*).

When we observed a predation movement we deployed a team to the site for an investigation. At the site, we searched in the immediate area for any sign of calf mortality. If no evidence was immediately found, a search was conducted within a more expanded radius to more efficiently and thoroughly cover the surrounding area. We conducted the search as a 3- to 4person team; 1 person carried a handheld GPS and hiked in each cardinal direction, and the other team members spread out to the right of the first person in 10-m intervals. We hiked in this manner for ~200 m, returned to the origin, and repeated this process in the remaining cardinal directions (Figure 3). Due to various constraints, (e.g., calf age, habitat type, topographical limitations, and lack of cow or calf sign) the search area was adjusted as needed. We searched for carcass remains, sign of a specific predator, and site evidence (e.g. broken vegetation, blood sprays) to lead us to a cause of death. Evidence indicative of a bear-kill included cached body parts, peeled or inverted hide, selective feeding on viscera or sensory organs, and claw marks across the body, whereas a wolf-kill would be indicated by long bones chewed on the ends, presence of the rumen and its contents, scattered remains over a large area, and puncture wounds on the head, neck, or hindquarters when present (Ballard et al. 1981, Severud et al. 2015a, b). To the best of our knowledge, all aforementioned information was used to assign specific causes of mortality.

Following mortality or unusual movements, we performed survival checks to try to determine whether the cow had a remaining twin or calf, respectively, with her. We executed survival checks by investigating localized areas previously occupied by the cow. Opportunistically, we placed camera traps in areas the cow frequently occupied to attempt to capture evidence of a calf. This fall we will conduct helicopter surveys to check all GPS-collared cows and determine 6-month calf survival or confirm our findings from this summer. We will check all cows again in late spring to determine near-annual survival (i.e., recruitment).

RESULTS

Thirty-one of 35 (88.6%) cows monitored this spring were determined to be pregnant via calving movement and site confirmation or by visual observation of a calf or calves. We observed 28 of the 35 (80.0%) cows, or 90.3% (28 of 31) of the pregnant cows, make a calving movement. Mean duration of the calving movement was 24.5 hours (\pm 2.5 [SE], 3.2–63.8 [range], n = 28), and mean total path length over this period was 5.3 km (\pm 0.8, 0.1–18.3, n = 28). Mean displacement from the start of the calving movement to the birth location was 2.3 km (\pm 0.6, 0.1–15.2, n = 28). Of the 3 remaining cows that did not make a calving movement, one came back "on air" during the calving season and a calf was confirmed via camera trap, another did not make a calving movement, but was seen with a calf by the public, and the remaining cow was killed by

wolves with a calf *in utero* (M. Carstensen, Minnesota Department of Natural Resources, unpublished data).

We confirmed evidence of a calf for 27 of 31 of our cows via calf pellets, tracks, afterbirth, or visualization of the calf (e.g., camera trap or seen by public). In the 4 remaining cases we were only able to confirm the presence of a calving bed, but subsequent evidence of reduced movements by the cow further increased our confidence a calf was still present. The mean birth-date was 12 May 2016 (median = 11 May, 24 Apr–10 Jun), with 83.3% of the localizations occurring during 4–20 May 2016.

As of 13 July 2016, we have documented 14 calf mortalities from 27 mortality investigations, giving a 51.9% overall success rate. We found mortalities at 12 of 21 (57.1%) investigations where a cow made a predation movement. Following a mortality we checked and confirmed evidence (e.g., tracks, pellets, hair) of a twin for 5 cows. The remaining cows were checked and had no confirmed evidence or had increased movement rates indicating a calf was not present. Based on the preponderance of evidence at each mortality site, we recorded 10 (71.4%) wolf-kills, 2 (14.3%) bear-kills, 1 (7.1%) unknown predator-kill, and 1 (7.1%) death following a possible vehicle collision (Figure 4). Accounting for both calf mortalities and confirmation of calf presence (e.g., calf pellets, tracks, afterbirth, or visualization of calf), 30-day calf survival was 61.5% \pm 10 (Figure 5). Calves died at a mean 13.8 days (\pm 3.2, 2.7–33.7, n =13) of age. We also had 3 cases where a cow made a predation movement, but no evidence of a calf mortality was found. The cows' behaviors (increased movements) following the predation movement was suggestive a calf or calves had been lost; we believe the mortalities were not found within the searched area or the mortality occurred beyond this area. Additionally, 2 separate road-kill incidents of 3 calves were reported from uncollared cows: a female calf and a pair of male twins, the first road-kill cases reported in our 4-year (2013-2016) study.

The mean distance cows fled following a mortality was 1,633.5 m (± 444, 126–5,805, n = 12). Cows that made return-trips to the mortality site, returned a mean 2.6 times (± 0.5, 1–5, n = 8). The cow's return-trips were a mean 106 m (± 25.9, 33.8–230, n = 7) from the mortality site. The mean search time when a mortality occurred was 67.6 min (± 9.9, 25–134, n = 14). In contrast, mean search time when no mortality was detected was 94.8 min (± 9.7, 39–142, n = 12). When a mortality was located, the mean area covered was 2.7 hectares (± 0.5, 0.9–7.4, n = 13), whereas the searched area was 5.5 hectares (± 0.5, 3.8–8.4, n = 10) when no mortality was located.

DISCUSSION

Identifying parturition via the calving movement was again found to be a reliable tool for estimating pregnancy rates. We had only 1 case where a cow did not make the calving movement and was subsequently seen with a calf. With this tool, recapturing cows to fit vaginal implant transmitters (VITs) each year is unnecessary, and it is advantageous by reducing costs and stress to the animals. Total path length and displacement of calving movements in 2016 were similar to movements from 2012 to 2015 (McGraw et al. 2014, Severud et al. 2015a,*b*). We found 80% of our cows (90% of our pregnant cows) made a calving movement; similarly, 82% of cows were observed making a calving movement in 2015. Our mean birth-date was 12 May, very similar to what was reported in 2013 and 2015 in northeastern Minnesota (Severud et al. 2015*a*,*b*). The mean birth-date of 19 May 2014 was much later and may have resulted from a severe and prolonged winter (Severud et al. 2015*a*). Normal birth-dates, as in 2013 and 2015–2016, may indicate generally good health conditions of adult females during the calving period and during the previous rut.

In 2016, we had a 21% increase in success rate from 2015 locating mortalities using the predation movement (Severud et al. 2015*b*). We also located 3 mortalities where the calf was > 30 days of age at mortality; none were located past 21 days of age in 2015. We believe the addition of conducting formal searches (patterns rather than casual searches) for mortality evidence increased our ability to detect mortalities compared to in 2015. Our percentage of predator-kills was similar to those of the first 3 years of this study (2013–2015), increasing our

confidence that predators, especially wolves, are the leading cause of calf mortality in northeastern Minnesota. Calf survival to 30 days was 60.9 and 61.5% in 2015 and 2016, respectively. High variation in juvenile survival can play a significant role in population declines (Gaillard et al. 2000, Raithel et al. 2007); these survival rates appear consistent with the population's recent apparent stability.

Identifying calf mortalities via predation movements of collared dams allows researchers to locate mortalities without the need to put collars on moose neonates. Return-visits were the most reliable tool for identifying mortality; in 2015, all mortalities with calf remains included return-visits by the cow (Severud et al. 2015*b*). With this behavior, we had success identifying mortalities with 1- and 4-hour fix-intervals, although shorter fix-intervals are advantageous. Monitoring cows with 1-hour fix-rates was easier, because there was more information available on each cow's movements. Increasing the amount of information available would increase the likelihood of both identifying and finding mortalities in the field.

Without GPS collars on neonates, we were unable to obtain more accurate estimates of calf production (forced to assume twinning rates), morphological measurements, or fine-scale habitat data, which could reveal important information relative to the declining population. Also, determining cause-specific mortality requires an extensive amount of effort in initially identifying possible mortalities (intense computer-monitoring), and subsequently, for confirming them by conducting extensive searches in the field. Neonate capture and GPS-collaring overcome all of these limitations.

ACKNOWLEDGEMENTS

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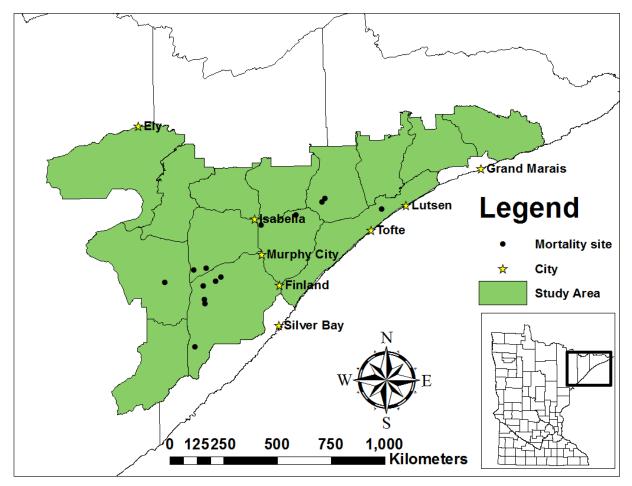


Figure 1. Moose calf study area (6,068 km²) for survival and cause-specific mortality study in northeastern Minnesota, 2016. Mortality sites (n = 14) of moose calves during May–June 2016.

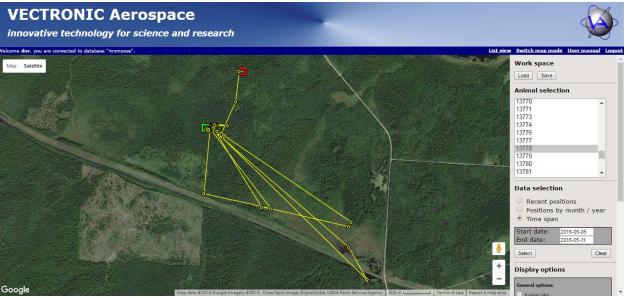


Figure 2. Vectronic Aerospace website (https://www.vectronic-wildlife.com) displaying the path of Cow 13778 in northeastern Minnesota during 5–11 May 2016. The green and red squares represent the beginning and end of the interval, respectively. The cow's movements show flees and return-visits to the green square; a mortality occurred on 5 May 2016. This cow made 3 return-visits before leaving the area. We found 3 wolf scats at the mortality site; within these scats were calf hair, teeth, vertebrae and other bone fragments. The estimated age at mortality was 2.7 days.

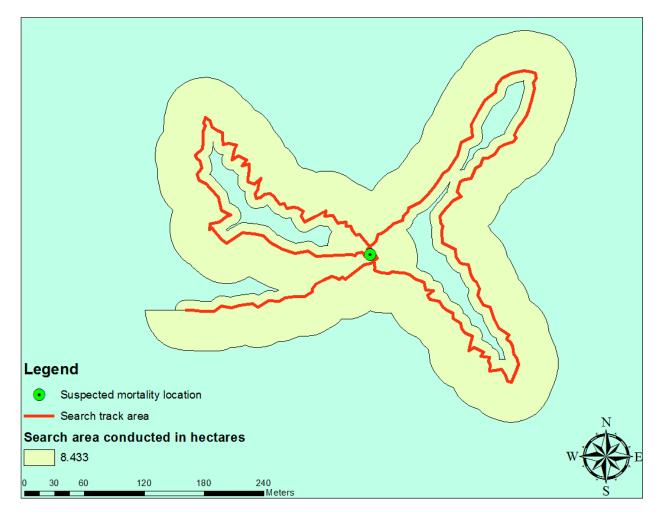


Figure 3. Moose calf mortality search area using ArcMap to buffer the search track. The center of the search area was the suspected mortality location in northeastern Minnesota, 21 May 2016. Cow 13821 made a flee and return-visits to the site. We searched an area of 8.4 hectares, but no mortality evidence was found.

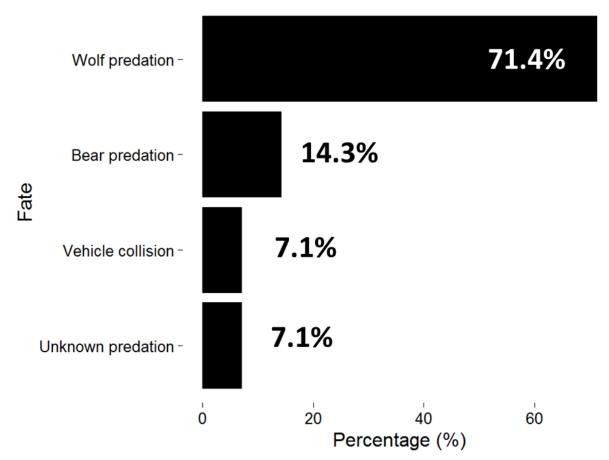


Figure 4. Cause-specific mortality of moose calves (n = 14) in northeastern Minnesota, May–July 2016.

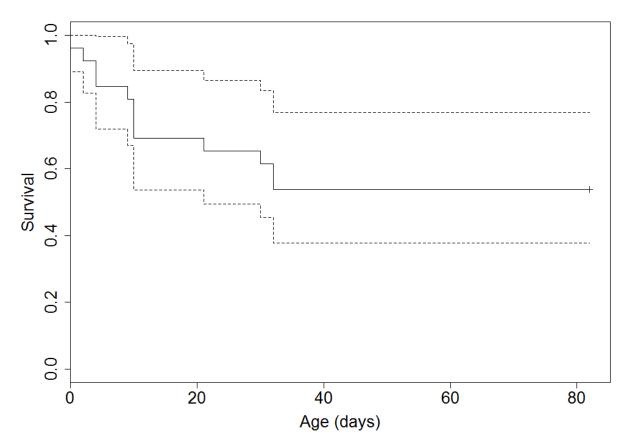


Figure 5. Kaplan-Meier 30-day survival (\pm 95% confidence intervals) of moose calves in northeastern Minnesota, May–July 2016.



SURVIVAL, CAUSE-SPECIFIC MORTALITY, AND SPACE USE OF MOOSE CALVES IN A DECLINING POPULATION

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SUMMARY OF FINDINGS

Adult survival and recruitment are important drivers of large herbivore population dynamics. The moose (Alces americanus) population in northeastern Minnesota has been exhibiting a downward trend from 2006 to 2016. Our research was initiated because neonatal and seasonal survival rates and specific causes of mortality (e.g., predation, undernutrition, disease) of calves were largely unknown. We remotely monitored global positioning system (GPS)-collared adult female moose during the calving season to locate and GPS-collar neonates in 2013 and 2014 (n = 49 and 25, respectively). Due to the Governor of Minnesota's Executive Order 15-10 (28 Apr 2015), we were unable to continue handling or collaring neonates in 2015, but instead used behavioral cues of existing GPS-collared cows to identify calving behavior and calf mortality due to predation. We additionally conducted helicopter surveys to assess apparent seasonal survival rates. Survival of calves dropped precipitously to 58% by 30 days of age in 2013 and 2014, and then to 34% by 9 months of age. Median age of death of calves that died before 1 year of age was 17.5 days. Hazard started low at birth and spiked at ~20 days old. Similar patterns were seen in 2015, with a 30-day survival rate of 63% and 10-month survival rate of 40.5%. Over-winter survival was generally high in all 3 years. Predation was the leading cause of mortality in 2013 and 2014, with 84% of mortalities due to wolves (Canis lupus) or black bears (Ursus americanus). Predation was an important cause of mortality in 2015 as well, but the relative certainty in assigning cause was low. Calves were generally preved upon once the dam and calves departed their calving sites. Wolf predation was the leading cause of calf mortality in our study. Identifying specific causes of calf mortality and understanding their relations to various landscape characteristics and other extrinsic factors should yield insight into mechanisms contributing to the declining moose population in northeastern Minnesota and serve as a basis for an ecologically sound management response.

INTRODUCTION

Ungulate population declines have been attributed to poor juvenile survival (Pinard et al. 2012, Forrester and Wittmer 2013). Large herbivore population growth is most sensitive to variation in adult survival, but differences in temporal variation of juvenile survival may be important in accounting for between-year variation in population growth rate (Gaillard et al. 1998, 2000; Lenarz et al. 2010). When viable populations of predators are present, predation can be a primary cause of mortality of temperate ungulate neonates (Linnell et al. 1995, Carstensen et al. 2009, Severud et al. 2015*a*). Less is known about other specific ultimate and proximate sources of moose (*Alces americanus*) calf mortality or contributing factors. It also is unclear when predation is compensatory or additive to other sources of mortality (Franzmann et al. 1980, Linnell et al. 1995), although a recent study documented additive effects of predation on moose calves in Alaska (Keech et al. 2011). The degree of predation's impact on population-wide calf survival rates

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depends on the extant predator guild and relative densities of predator and prey (Eriksen et al. 2011, Patterson et al. 2013). The moose population in northeastern Minnesota has declined 55% from 2005 to 2016 (DelGiudice 2016). Survival and cause-specific mortality of calves was largely unknown in this area, but recruitment had been estimated at 0.40 mostly before the population began its decline (Lenarz et al. 2010).

Pregnant cow moose tend to move long distances prior to localizing ("calving movement") to give birth (Testa et al. 2000, McGraw et al. 2014, Severud et al. 2015*a*). Dams localize with their calves for up to 14 days at calving sites (Severud et al. 2015*a*). Dams also have been observed making repeated flees and return-trips to focal areas where calves have been preyed upon (e.g., a "predation movement," Severud et al. 2015*b*). Expandable global positioning system (GPS) collars have until now not been fitted to moose neonates, and have only recently been used on other ungulate neonates (white-tailed deer [*Odocoileus virginianus*], Long et al. 2010; fallow deer [*Dama dama*], Kjellander et al. 2012). Observable fine-scale movement patterns and habitat use of moose calves, made possible by GPS collars, also facilitated rapid investigation of mortality events to assign proximate causes and gather evidence for contributing factors. Having dams and their calf or calves fitted with GPS collars also allowed us to study the importance of proximity of dam and offspring to juvenile survival.

OBJECTIVES

- 1. Estimate neonatal (30-day), seasonal, and annual survival of moose calves
- 2. Quantify cause-specific mortality of moose calves
- 3. Identify potentially important covariates that influence survival or cause-specific mortality

METHODS

Our study area is the same as that of the Environmental and Natural Resources Trust Fund (ENRTF)-supported study in the Arrowhead region of northeastern Minnesota focused on survival and cause-specific mortality of adult moose (Carstensen et al. 2015). White-tailed deer populations occurred at pre-fawning densities of ≤4 deer/km² (Grund 2014). Major predators of moose in the area included gray wolves (*Canis lupus;* 3 wolves/100 km², Erb and Sampson 2013) and black bears (*Ursus americanus;* 23 bears/100 km², Garshelis and Noyce 2011). Moose had not been harvested in the state since 2012 (DelGiudice 2014).

As part of the adult moose mortality study, 84, 25, and 20 female moose were captured and fitted with Iridium GPS collars (Vectronic Aerospace GmbH, Berlin, Germany) during January 2013, February 2014, and February 2015, respectively (Butler et al. 2013; Carstensen et al. 2014, 2015). Blood was collected and analyzed for serum progesterone; \geq 2.0 ng/mL was indicative of pregnancy. We monitored cow movements during pre-parturition and calving, with particular attention afforded to pregnant cows, looking for calving movements (Bowyer et al. 1999, McGraw et al. 2014, Severud et al. 2015*a*).

In 2013 and 2014, calves were located and fitted with an expandable Globalstar GPS Calf Collar (Vectronic Aerospace, Berlin, Germany). Details of calf captures, handling protocols, and mortality investigations can be found in Severud et al. (2014, 2015*a*, *b*). In response to captureinduced abandonment of calves and capture-related mortality of adults (DelGiudice et al. 2014, 2015; Carstensen et al. 2015), the Governor of Minnesota issued Executive Order 15-10 (28 Apr 2015), barring state agencies from conducting or permitting any collaring of moose in the state. We then monitored existing collared adult females for a calving movement, and tracked dam behavior for indications of a predation movement.

We estimated birth-dates of all calves (2013–2015) based on dams' calving movements. We assumed calves were born 12 hours after the cow localized. In 2013 and 2014, time of death was estimated using the mortality mode of collars, and calf and dam locations relative to the mortality site. In response to a high rate of collar slippage in 2014, we conducted an apparent survival check flight in March 2015. In 2015, dam behavior was used to indicate calf mortality (time of the location from which the dam initially fled was also the estimated time of death). We

conducted flights via helicopter to assess seasonal apparent survival rates in 2015 during late November and early December (~190 days old) and late March 2016 (~320 days old).

We calculated Kaplan-Meier survival, hazard, and Cox proportional hazard using the R packages *survival, KMsurv*, and *muhaz*. Since calf births were tightly synchronized (Severud et al. 2015*b*), we calculated survival by calf age, with day 0 meaning birth. For smoothed empirical hazard curves, we used a global bandwidth and the product-limit method. We calculated cause-specific mortality rates with a cumulative incidence function using the R package *wild1*. Dam and calf location data were screened for locations that were thought to be erroneous fixes. We then calculated proximity between cows and calves. Summer field tests demonstrated mean linear error (\pm SE, range) of locations for adult collars of 3.7 m (\pm 0.3, 0–17) under open canopy and 7.0 m (\pm 0.3, 1–36) under dense canopy (\geq 80% closure), and for calf collars of 24.9 m (\pm 2.7, 1–274) under open canopy and 33.7 m (\pm 3.1, 1–236) under dense canopy.

RESULTS

We collared 49 calves from 31 dams in 2013 and 25 calves from 19 dams in 2014 (58% and 32% twinning rates, respectively). Overall, the sex ratio of collared calves was 36 females: 38 males. Seven dams abandoned 9 calves in 2013 and 6 dams abandoned 9 calves in 2014 (DelGiudice et al. 2014, 2015). These calves, as well as 2 additional calves that died during or shortly after capture from trampling by the dam and not nursing due to unknown causes (DelGiudice and Severud, in press), were not included in further survival analyses, leaving 54 calves. Of these 54 calves, 4 slipped their collars in 2013 and 10 in 2014, resulting in 40 calves to study survival and natural cause-specific mortality. In 2015, we observed calving movements or localization of 50 cows and tracked those dams for predation movements. Assuming a 30% twinning rate (M. Schrage, Fond du Lac Natural Resource Management Division, unpublished data), this yielded ~65 uncollared calves under observation during 2015. Median calving dates for 2013, 2014, and 2015 were 14, 19, and 10 May, respectively.

Blood profiles of calves sampled in 2013 are reported elsewhere (DelGiudice and Severud, in press). For pooled collared calves, mean total body mass at capture was 15.8 kg (\pm 0.3, 12–20.5, *n* = 38) and mean hind foot length (HFL) was 45.9 cm (\pm 0.3, 42–49, *n* = 42). Body mass and HFL were weakly correlated ($r^2 = 0.31$, *P* <0.001, Figure 1). There were no differences in mass or HFL by sex or between twins versus singletons. Mean rectal temperature was 101.6 °F (\pm 0.12, 99.9–103.4, *n* = 43). Mean dam age of all collared calves was 6.4 years old (\pm 0.5, 1–14, *n* = 43). Mean dam age of calves that died was 6.7 years old (\pm 0.7, 1–12, *n* = 23).

For pooled 2013 and 2014 collared calves, 30-day survival was 0.584 (95% Confidence Interval [CI] = 0.461–0.740, Figure 2) and declined to 0.341 (95% CI = 0.226–0.516) by 206 days of age (6–10 February 2014), when all remaining collars were removed (Figure 3). Incorporating slipped collar flight data, survival is further adjusted to 0.285 (95% CI = 0.178-0.457). In 2015, we observed calf mortalities during the first 30 days of life, as indicated by predation movements of dams. Based on suspected and confirmed calf mortalities, 30-day survival was 0.632 (95% CI = 0.518-0.770; Figure 2). For the uncollared 2015 cohort of calves, flights in early winter (30 Nov-3 Dec 2015) and late winter (28-29 Mar 2016) indicated an apparent survival rate of 0.442 and 0.405, respectively. In all 3 years, survival dropped dramatically from birth to age 50 days (Figures 2 and 3). Dam age, HFL, mass, sex, and twin status did not meet the assumptions of proportionality, so we could not run Cox proportional hazard models. The empirical hazard function was low initially, and then peaked at ~15 days old before declining, with a second spike in hazard around 90 days of age (Figure 4). Mean age of death of calves that died before 1 year of age was 36 days old (\pm 8, 3.5–206, n = 31), but the median age was 17.5 days, very close to the peak in hazard. Mortalities from predation (n = 26) occurred 32 days (± 6.5, 2–120) after leaving the calving site and occurred 1,553 m (± 289, 107-5,788) from the calving site.

We documented 31 natural mortalities of collared calves in 2013 and 2014. Specific causes of mortality included 20 wolf-kills, 5 bear-kills, 2 natural abandonments, and 1 each of the following: drowning, abandonment of unknown cause, unknown predation, and an infection resulting from wolf bites (Figure 5). The cause-specific mortality curves rose rapidly from birth to

50 days of age. Over the first 9 months of age, the cumulative probability of being preyed upon by wolves was 50.3% (90% CI = 37.1-82.2), with bear predation 11.8% (90% CI = 3.6-20.1) and other causes 9.6% (90% CI = 2.2-16.8). Predation accounted for 84% of all natural mortalities, with wolves having the greatest impact overall (77% of the predation events).

For uncollared calves born during 2015 we documented 11 natural mortalities, with 4 additional cases pending (no direct evidence of calf mortality, but predator scat [1 wolf, 5 bear] will be analyzed for presence of calf hair). We documented 8 wolf-kills, 1 bear-kill, and 2 unknown predator-kills (saliva evidence pending, calf remains located).

Most dams and their offspring (one outlier cow-calf pair excluded) were a mean of 101 m (\pm 1.5, 0–6,083) apart throughout the year. Much variation by individual and fate was apparent (Figure 6). The outlier was a twin that separated from its mother and twin in November. With this outlier included, the mean proximity of all dams and their offspring was 3,736 m

DISCUSSION

We documented high mortality rates of moose neonates in this declining population. However, the mortalities tended to occur once the dams and their calves departed from calving sites. Peak energetic demands for dams due to lactation occur 21–31 days postpartum (Schwartz and Renecker 2007), which coincides with the highest hazard calves experienced. This suggests that dams seeking out high quality or quantities of forage to meet this demand may be travelling in risky areas or that movement to new foraging patches is itself risky, potentially exposing dams with young calves to predation. Calving habitat may be an important determinant of neonatal survival (Bowyer et al. 1999). Future work will focus on habitat covariates (horizontal cover, forage availability, etc.) at pre-calving, calving, peak lactation, and mortality sites to quantify the potential safety of the calving sites and investigate if dams are making a tradeoff between safety and nutrition during the calf-rearing period (Bowyer et al. 1999). We will also compare movement characteristics of cow-calf groups that successfully avoided predation to those that did not.

Our near-recruitment rates for 2013–2014 and 2015, although estimated in different ways (via collaring of calves versus observing cow movements and subsequent survival flights), were similar. Both methods required collars on adult cows, yet without calf collars extensive field searches and helicopter flight time were required. Tracking GPS-collared cow movements was a highly reliable way to estimate calving rates and to a lesser degree calf mortality. Due to the Governor of Minnesota's Executive Order 15-10, we were unable to confirm presence of calves shortly after birth, nor handle or collar calves in 2015. Without observing neonates at calving sites, we could not estimate twinning rates. We also did not know when a calf had died, but used dam movements as an indication of calf mortality. This also delayed site investigations, frequently making assignment of mortality cause difficult. Only in cases where the calf was ≤23 days old and the dam fled and made 1-7 return trips were we successful in confirming calf mortality. In a subset of those cases we were able to assign cause of death. This technique may serve as a method to estimate early neonatal mortality, but has less power to detect mortality as calves age beyond 3 weeks (although see Obermoller et al., this volume). This method will not reliably detect calves that succumb to forms of mortality other than predation, because we have not documented cows fleeing from and returning to other mortality events (e.g., disease, drowning, abandonment, but see Obermoller et al., this volume).

Wolves accounted for the largest proportion of mortalities in all 3 years of the study. Wolf predation has been partially implicated in the decline of this population (Mech and Fieberg 2014) and has been shown to account for adult mortalities as well (Carstensen et al. 2015). However, adults have typically exhibited predisposing factors when preyed upon by wolves. The overall poor health of the northeastern Minnesota moose population (Carstensen et al. 2015, DelGiudice and Severud 2015) could potentially explain not only the high number of capture-induced abandonments we observed (DelGiudice et al. 2014, 2015), but also the high rates of predation on calves. Dams in other studies and study areas defended their calves less vigorously following harsh winters or if in poor nutritional condition (Keech et al. 2011, Patterson et al. 2013).

Dams and calves often were in close proximity throughout the first year of life. One outlier was a twin that did not follow its dam and twin across a large lake at about 175 days old. The lone twin returned to where the group was spending time and survived until mid-winter when she was captured to have her collar removed.

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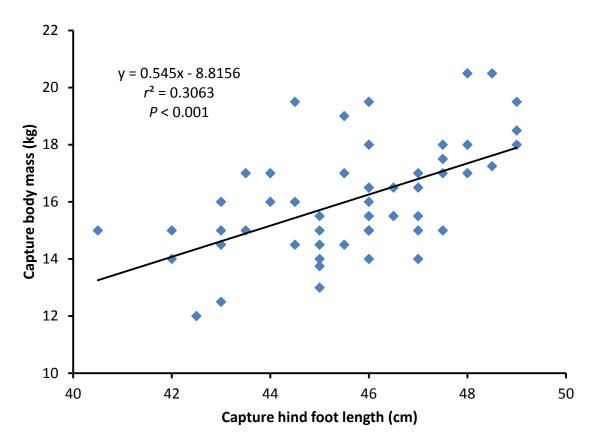


Figure 1. Relationship of hind foot length (cm) to body mass (kg) at capture of moose (*Alces americanus*) neonates (n = 54), northeastern Minnesota, 8–12 May 2013.

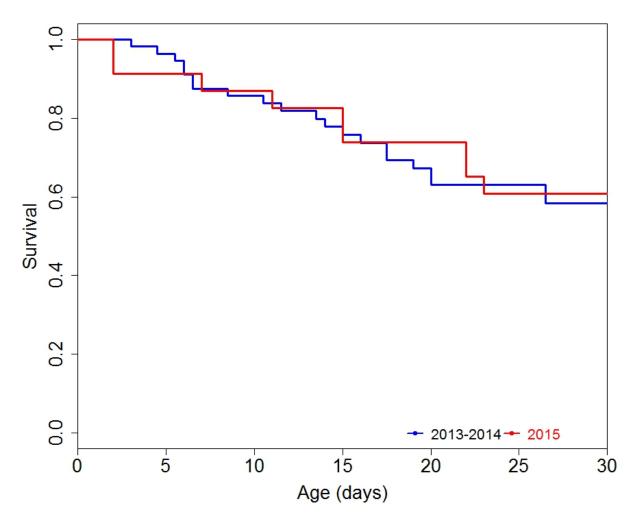


Figure 2. Kaplan-Meier 30-day survival for known moose (*Alces americanus*) calf mortalities, northeastern Minnesota, May–June 2013–2015. Mortality was confirmed by GPS collars (pooled 2013 and 2014, blue line, n = 54 calves monitored) or through investigations triggered by dam movement patterns and observation of calf remains (2015, red line, n = 65 calves monitored). Tick marks depict individuals censored due to slipped collars.

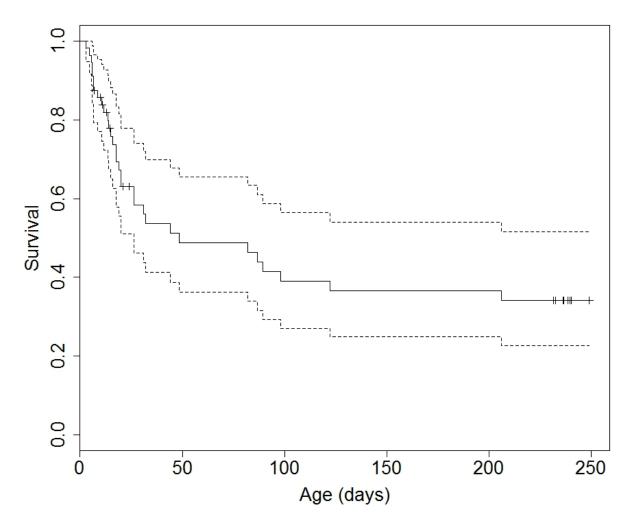


Figure 3. Kaplan-Meier 250-day survival for known moose (*Alces americanus*) calf mortalities (n = 54 calves monitored), northeastern Minnesota, May–February 2013–2015. Tick marks indicate individuals censored due to slipped or removed collars. Dashed lines represent 95% confidence intervals.

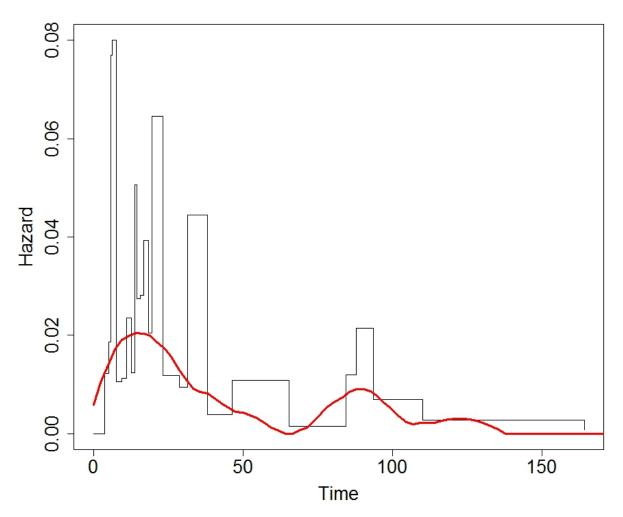


Figure 4. Empirical hazard function for known moose (*Alces americanus*) calf mortalities (n = 31 calves monitored), northeastern Minnesota, May–February 2013–2015. "Time" indicates calf age in days.

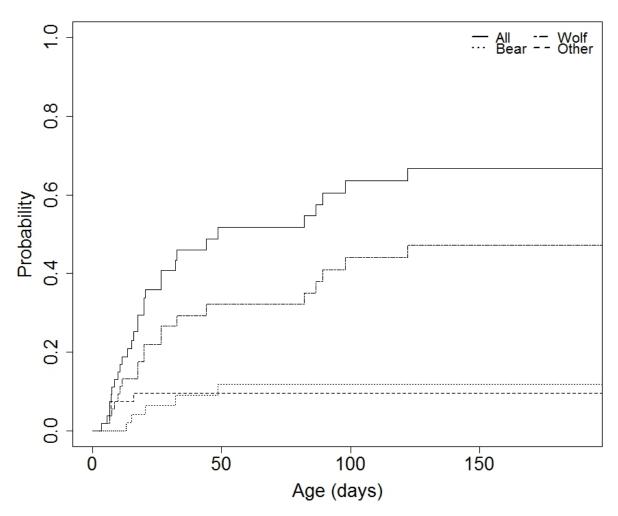


Figure 5. Cumulative incidence function for cause-specific mortality of moose (*Alces americanus*) calves in northeastern Minnesota (n = 40 calves monitored), May–February 2013–2015. Causes of mortality were wolf (*Canis lupus*) predation (20), black bear (*Ursus americanus*) predation (5), and other (natural abandonment [2], drowning [1], abandonment of unknown cause [1], unknown predator [1], and infection resulting from wolf attack [1]).

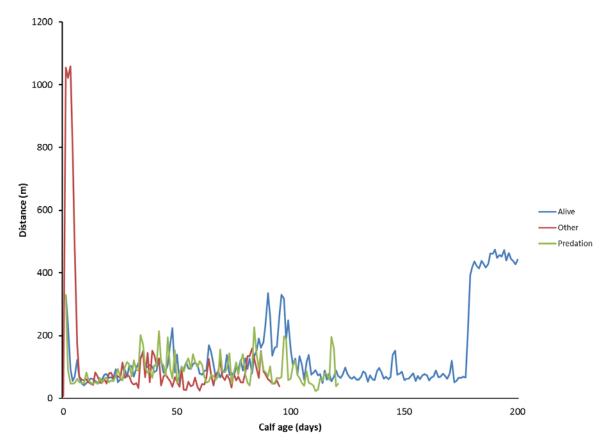


Figure 6. Mean daily proximity (distance) between moose (*Alces americanus*) dams and their calves, excluding an outlier calf that moved up to 28,595 m from its dam, by calf age (up to 200 days old) and fate type (Alive, Other [non-predation mortality], and Predation), northeastern Minnesota, May–February 2013–2015. Spikes in distance early in life are due to capture and handling.

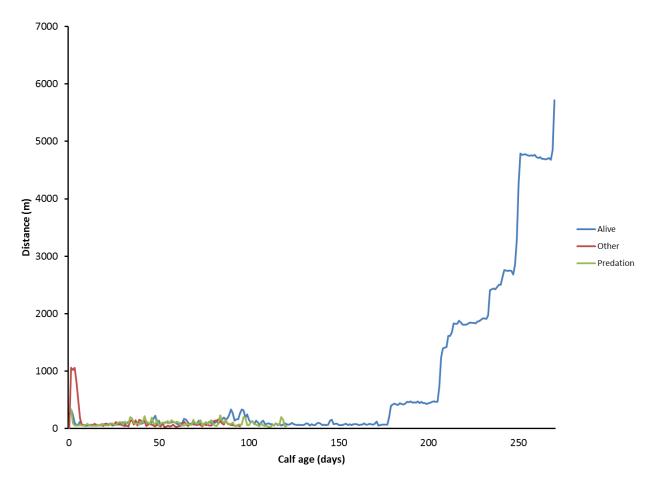


Figure 7. Mean daily proximity (distance) between moose (*Alces americanus*) dams and their calves, including a single calf that moved much further from its dam than any other collared calf (up to 28,595 m), by calf age (up to 270 days old) and fate type (Alive, Other [non-predation mortality], and Predation), northeastern Minnesota, May–February 2013–2015.



CLIMATE CHANGE, WINTER NUTRITIONAL RESTRICTION, AND THE DECLINE OF MOOSE IN NORTHEASTERN MINNESOTA, WINTERS 2013–2016

Glenn D. DelGiudice and William J. Severud¹

SUMMARY OF FINDINGS

The moose (Alces americanus) population in northeastern Minnesota has declined an estimated 55% from 2006 to 2016. As in northwestern Minnesota, a number of complex ecological relationships between undernutrition, pathogens, predation, and environmental factors (e.g., habitat, temperature) are likely exerting pressure on moose and contributing to this recent decline. Nutrition is centrally related to our understanding of all other aspects of wildlife ecology, including population performance. Winter nutritional restriction of moose and other northern ungulates may be physiologically assessed by serial collection and chemical analysis of fresh urine in snow (snow-urine); urea nitrogen:creatinine (UN:C) ratios have shown the greatest potential as a metric of winter nutritional status with values <3.0, 3.0-3.4, and ≥3.5 mg:mg being indicative of moderate (normal), moderately severe, and severe nutritional restriction, respectively. During 6 January-28 March 2013-2016, we collected annual totals of 123, 307, 165, and 189 moose snow-urine samples, and mean seasonal UN:C ratios were 3.7, 2.9, 2.9, and 3.5 mg:mg for the 4 winters, respectively. The mean population UN:C ratios for winters 2013 and 2016 were above the threshold indicative of severe nutritional restriction (i.e., a starvation diet) and accelerated body protein catabolism. During 2014 and 2015 the corresponding values were just below the moderately severe interval. Most indicative of the unique severity of nutritional restriction in 2013, nearly one-third of all samples collected yielded UN:C ratios >3.5 mg:mg.

Perhaps the ultimate value to management of assessments of nutritional status of freeranging animals comes when the findings can be related to the performance and dynamics of the population and other ecological factors challenging that performance. Presently, our populationlevel nutritional assessments are closely tracking population estimates ($r^2 = 0.90$) and calf production ($r^2 = 0.89$) of moose in northeastern Minnesota. Although nutritional restriction varied among the 4 winters, elevated UN:C values suggested a level of deprivation not supportive of population stability or growth, and variation in winter conditions, as indexed by the winter severity index (WSI), is not directly responsible. For the 4 winters, we also have documented that the level of severe nutritional restriction is inversely related (r = -0.88) to variation of *natural* winter (and winter-summer) survival of global positioning system (GPS)-collared adult moose, and both of these are related to the heat stress index calculated from January minimum temperatures \geq -5°C (HSI_{Min}, $r^2 = 0.74-0.76$). While such relationships do not substantiate cause-and-effect, presently they provide the best preliminary empirical evidence indicating that inadequate winter nutrition at the population level, and perhaps climate change, are intricately related to the declining trajectory of moose numbers in northeastern Minnesota.

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INTRODUCTION

Declines in regional populations of moose (*Alces americanus*) along the southern periphery of their global range have been common in recent decades. In northeastern Minnesota the estimated 2016 population (4,020 moose) was 55% less than in 2006 (8,840 moose,

DelGiudice 2016), exhibiting a trajectory similar to that documented previously for moose in northwestern Minnesota, where the population decreased from ~4,000 in the mid-1980s to <100 moose in 2007 (Murray et al. 2006). Mean annual mortality rates of collared adult moose have been similarly high (21%) in the northwest and northeast during the declines (Murray et al. 2006; Lenarz et al. 2009; R. A. Moen, unpublished data). In northwestern Minnesota, malnutrition and pathogens were identified as important factors influencing the population's decreasing trajectory (Murray et al. 2006). In northeastern Minnesota a recent (2013–2015) aggressive study of global positioning system (GPS)-collared, adult moose reported a mean annual mortality rate of 15%, with health-related factors (e.g., parasites, disease) accounting for two-thirds of the deaths, wolf (*Canis lupus*) predation for one-third, and complex interactions between the 2 categories well-documented (Carstensen et al. 2015). Climate change (i.e., warming temperatures) has been implicated in both population declines (Murray et al. 2006; Lenarz et al. 2009, 2010).

These temperature-survival relationships are complex, and indicate that climate change can directly and indirectly impact ungulate populations (Bastille-Rousseau et al. 2015, Davis et al. 2016, Street et al. 2016). Moose are particularly well-adapted to cold climates, but temperatures that exceed "heat stress" thresholds of 14 to 24°C during summer and –5°C during winter may increase metabolic rates, induce energy deficits, and hasten deterioration of body condition (Renecker and Hudson 1986, 1990; Broders et al. 2012; McCann et al. 2013). These thresholds may be influenced by exposure to solar radiation and wind (Renecker and Hudson 1990, McCann et al. 2013). Nutritional and health status (e.g., disease, parasites), behavioral responses (e.g., altering movement, foraging, and bedding patterns), and quality of available habitat have the potential to affect the animal's ability to mitigate negative impacts from heat stress (Van Beest et al. 2012, Street et al. 2016).

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

Winter nutritional restriction of moose and other northern ungulates can be physiologically assessed at the population level by serial collection and chemical analysis of fresh urine voided in snow (snow-urine; DelGiudice et al. 1988, 1997, 2001; Moen and DelGiudice 1997, Ditchkoff and Servello 2002). Urea nitrogen (interpreted as a ratio to creatinine, UN:C), the end-product of protein metabolism, is one of many chemistries investigated for its value as a physiological metric of the severity of nutritional restriction (DelGiudice et al. 1991*a*,*b*, 1994). In healthy moose urinary UN:C values decrease (N conservation) in response to diminishing intake of crude protein and digestible energy, but as dietary restriction and negative energy balance become more severe and fat reserves are depleted, ratios increase to notably elevated values in response to accelerated net catabolism of endogenous protein. Snow-urine UN:C ratios exhibited differential effects of a winter tick (*Dermacentor albipictus*) epizootic and habitat differences on the severity of nutritional restriction of moose on Isle Royale, and were strongly related to dynamics of the population, including a pronounced decline and recovery to historically high numbers (DelGiudice et al. 1997).

OBJECTIVES

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

We hypothesized that increasing winter ambient temperatures, exceeding the HSI threshold, are contributing to the severity of nutritional restriction and energy deficit of moose, decreases in survival by various proximate factors, and diminishing performance of the population. Findings will set the stage for additional work assessing nutritional relationships of moose to variations in habitat and other factors.

STUDY AREA

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

Wolves (*Canis lupus*) and American black bears (*Ursus americanus*) are predators of moose (Fritts and Mech 1981, Severud et al. 2015) with recent densities estimated at 3.4 wolves and 23 bears/100 km² (Erb and Sampson 2013, Garshelis and Noyce 2015). White-tailed deer (*Odocoileus virginianus*) are managed at pre-fawning densities of <4 deer/km², and are the primary prey of wolves in most of northern Minnesota (Nelson and Mech 1986, DelGiudice et al. 2002). The MNDNR assesses winter severity (1 Nov–31 May) by a winter severity index (WSI), calculated by accumulating 1 point for each day with a temperature $\leq 0^{\circ}$ F (–17.8°C, temperature-day) and 1 point for each day with snow depth ≥ 15 inches (38.1 cm, snow-day), for a potential total of 2 points per day. Maximum WSI values varied across moose range, 35–160, 184–245, 54–152, and 31–142, for winters 2012–13 to 2015–16, respectively (Minnesota State Climatology Office 2016). Mean daily minimum and maximum temperatures varied markedly during November–April from 2012–13 to 2015–16 at Ely, Minnesota (Midwestern Regional Climate Center 2016; Figure 2). The heat stress index (HSI_{Min} and HSI_{Max}) for moose during January and the "cold season" (Nov–Mar) was calculated by daily accumulation of degrees Celsius >–5°C for the maximum and minimum ambient temperatures, respectively (Renecker and Hudson 1986).

METHODS

We collected fresh snow-urine specimens of moose during 6 January–28 March 2013– 2016. We conducted snow-urine sampling according to a random design. Our field team drove (by truck or snowmobile) a route of approximately 201 km (125 miles) to distribute the sampling throughout the study area (Figure 1). Field technicians were not restricted to this route, but could deviate, particularly on foot, as dictated by the presence of fresh moose sign (e.g., tracks, urine specimens, pellets). Each field team used handheld GPS units loaded with several land coverages (R. G. Wright, Minnesota Information Technology @ Minnesota Department of Natural Resources, Section of Wildlife) and a Superior National Forest map (U. S. Forest Service) to navigate in the field.

To be able to associate urine chemistry data of collected snow-urines and nutritional assessments with specific temporal intervals, sampling generally was conducted within 7 days of a fresh snowfall, most often within 2–4 days. Upon observing fresh moose sign, technichans

tracked the individual(s) on foot as necessary until they came to a fresh snow-urine specimen. The objective for the collections was to sample primarily adult (>1 year old) moose (indicated by track and bed size). This was not particularly challenging, because by this time of year calves comprised only 13–17% of the population (DelGiudice 2016). We focused primarily on the adult age class to facilitate optimum comparability of data.

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

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

Winter (Jan–Mar) was divided into 6, 2-week sampling intervals (1–14 Jan, 15–31 Jan, 1– 15 Feb, 16–28 Feb, 1–15 Mar, and 16–31 Mar). Sample sizes for the snow-urine collections varied by interval due to variability of weather (i.e., snow conditions), equipment availability, logistical challenges, and ease of finding samples. Most of the UN:C data are reported by the entire winter or by sampling interval as means (\pm SE). Additionally, based on past work, urinary UN:C values were assigned to 1 of 3 levels of nutritional restriction: moderate or "normal," <3.0 mg:mg; moderately severe, 3.0–3.4 mg:mg; and severe, \geq 3.5 mg:mg (DelGiudice et al. 1997, 2001, 2010). We report the percentage of samples with UN:C values falling within each of these categories. We examined relationships between proportions of snow-urine specimens with UN:C values indicative of severe nutritional restriction (\geq 3.5 mg:mg) and populations estimates, seasonal survival, and HSI by simple linear regression analyses in Excel (Version 14.0.7153.5000, Microsoft Corporation 2010).

RESULTS AND DISCUSSION

During January–March 2013–2016, annual totals of 123, 307, 165, and 189 sufficiently concentrated moose snow-urine samples, respectively, were collected during 5–6, 2-week sampling intervals using our designated routes. The greater number of samples collected during 2014 was largely due to the early and prolonged deep snow cover.

Overall, mean UN:C ratios were 3.7, 2.9, 2.9, and 3.5 mg:mg for winters 2013–2016, respectively (Figure 3). The mean population UN:C ratio for entire winters 2013 and 2016 were above the threshold indicative of severe nutritional restriction (i.e., a starvation diet) and accelerated body protein catabolism. The elevated mean UN:C of 2016 was influenced largely by several collected samples that exhibited very high UN:C ratios indicative of a moribund condition (\geq 22.0 mg:mg), given that the proportion of samples in the lowest UN:C category was greatest that year (Figure 4). During 2014 and 2015 the population means were just below the defined moderately severe interval. Additionally indicative of the unique severity of nutritional restriction in 2013, nearly one-third of all samples collected yielded UN:C ratios \geq 3.5 mg:mg (Figure 4). The corresponding percentages of winters 2014–2016 were notably less than in 2013.

Mean urinary UN:C ratios by 2-week interval of winter 2013 indicated that nutritional restriction was normal or moderate during late-January, but became severe throughout February and early-March, and was still assessed as moderately severe in late-March (Figure 5). As severe nutritional restriction of certain individuals progresses with winter, those animals may be undersampled as some eventually die, and those still alive urinate less, which is a physiological mechanism to conserve water and electrolytes. Percentage of samples with urinary UN:C ratios indicative of severe nutritional restriction peaked (73.3%) in early-February and remained relatively high through late-March (36%) during 2013 (Figure 6). Such elevated values have been associated with long-term fasting in controlled nutrition studies of captive white-tailed deer and

starvation of free-ranging elk (*Cervus elaphus*), bison (*Bison bison*), and moose (DelGiudice et al. 1991*a*, 1994, 1997, 2001). The percentage of snow-urine specimens in 2013 with UN:C ratios indicative of moderately severe to severe nutritional restriction throughout the winter was 45.5% (Figure 4).

During 2014, mean urinary UN:C ratios in all 2-week intervals except early February remained just below the moderately severe category (Figure 5), and the percentage of samples with ratios indicative of severe nutritional restriction gradually decreased as this winter progressed (Figure 6), either due to an easing of conditions restricting access to forage or because severely stressed individuals were being under-sampled, which may be most plausible as previously explained. Adverse effects of the late, but prolonged conditions of winter 2013, including warm temperatures, may have contributed to the high spring-summer calf loss and absence of the need for dams to lactate (Severud et al. 2015). This also may have allowed the surviving animals to rebound nutritionally more quickly and to fare better during winter 2014. This would not be unlike the documented effects on the nutritional status and survival of northern Minnesota deer during the consecutive severe winters of 1996 and 1997 (DelGiudice et al. 2006; G. D. DelGiudice, unpublished data). Overall in winter 2014, UN:C values of 64% of the collected snow-urine samples classified nutritional restriction as moderate (normal), whereas 36% reflected moderately severe to severe restriction, which was less than in 2013 (Figure 4). Similar to winter 2014, severe nutritional restriction of moose was not as prevalent in 2015 as in 2013, but it was up slightly compared to 2014 (Figure 4). However, a higher percentage of moose appeared to be experiencing moderate or normal restriction and a smaller percentage moderately severe than in 2013 and 2014 (Figure 4). Rapidly diminishing snow cover prevented collection of snow-urine samples or assessments during the last 2 weeks of March 2015, certainly a positive factor relative to moose nutrition at that time. During winter 2016, maximum WSI values ranged from low to moderately severe, but this was clearly the warmest winter (Figure 2). Unexpectedly, 2016 had the greatest percentage of samples with urinary UN:C ratios indicative of moderate nutritional restriction (70.4%) and the smallest percentage with severe nutritional restriction (Figure 4).

According to maximum WSI values, winter 2014 was the most severe of the 4 in northeastern Minnesota moose range, followed by 2015, 2016, and 2013. Although the WSI numbers have value for annual comparisons of winter conditions, this WSI formula has far greater relevance to the size and energetics of white-tailed deer than for the much larger moose, which are not hindered as much by deep snow (DelGiudice et al. 2002, 2006; Schwartz and Renecker 2007). Furthermore, while the accumulation of snow-days and temperature-days has proven significant relative to the survival of white-tailed deer (DelGiudice et al. 2002), actual snow depth, its temporal occurrence, and duration may be of equal or greater importance for moose and deer (Telfer and Kelsall 1984, DelGiudice 1998, DelGiudice et al. 2002, Schwartz and Renecker 2007). During 2013 conditions became severe during mid- to late-winter; consequently, a high number of snow-days did not accumulate, but the season was prolonged. The severe nutritional restriction of moose in 2013 was most similar to that which occurred in moose during several winters (1988–1990) on Isle Royale associated with serious winter tick infestations and steep population decline (DelGiudice et al. 1997). Abundant evidence from the field in the MNDNR's ongoing studies similarly indicated that the winter tick infestation of moose in northeastern Minnesota was notably more serious during winter 2013 than in 2014 and 2015 as the population continued to decline (Carstensen et al. 2014; M. Carstensen, MNDNR, personal communication).

Perhaps the ultimate value to management of assessments of nutritional status of freeranging animals comes when the findings can be related to the performance and dynamics of the population and other ecological factors challenging that performance (DelGiudice et al. 1997, Cook et al. 2004). Presently, our population-level nutritional assessments are closely tracking (r^2 = 0.90) population estimates of moose from the annual aerial survey (Figure 7). What is most clear is that although restriction varies among the 4 winters, elevated UN:C values suggest a level of nutritional deprivation not supportive of positive population performance or growth. Unlike for white-tailed deer in northern Minnesota, warming winter temperatures rather than snow depth (DelGiudice et al. 2002, 2006) during winters 2013 to 2015 appeared to be having the most pronounced influence on the nutritional status of moose. As the January and winter HSI_{Max} values Page 128 increased, the incidence of severe nutritional restriction of moose increased ($r^2 \ge 0.93$, DelGiudice and Severud, unpublished data), which may have led to many of these animals becoming more vulnerable to various health-related causes of mortality and predation (Carstensen et al. 2015). Similar relationships were noted between winter nutritional restriction, winter tick epizootics, and decreasing moose on Isle Royale (DelGiudice et al. 1997). But, something new occurred during winter 2016, the warmest of winters since 2013 in northeastern Minnesota. The strong relationships between HSI_{Max} of January and winters 2013–2015 and severe nutritional restriction collapsed with winter 2016; severe nutritional restriction of moose was still noteworthy, but was the lowest of the 4 winters. Interestingly, there was still a reasonably strong relationship (r^2 = 0.763) between the January HSI_{Min} and the incidence of severe nutritional restriction for the 4 winters (Figure 8). Furthermore, variation in the occurrence of severe nutritional restriction at the population level remained inversely related (r = -0.88) to variation of natural survival rates of winter and winter-to-summer of GPS-collared adult moose (Figure 9), and January HSI_{Min} values were directly related to winter survival of these moose (Figure 10). Importantly, because these latter relationships are consistent with our association of severe nutritional restriction with the population estimates, it suggests that the current study cohort of GPS-collared moose is indeed representative of the free-ranging population in northeastern Minnesota. While these aforementioned relationships do not substantiate cause-and-effect, a preponderance of the empirical evidence is suggesting that winter nutritional restriction is a mechanistic thread from environmental variation (i.e., warming temperatures) to the performance and decline of the moose population in this region of Minnesota. Clearly, there is still much to understand about these relationships. New to this understanding are the effects of variation in severe nutritional restriction and the loss of breeding females on annual calf production (Figure 11).

In addition to the multi-year occurrence of severe nutritional restriction of moose, preliminary analyses reveal a vast spatial distribution throughout moose range of collected snowurine samples with UN:C ratios indicative of severe nutritional deprivation (see example in Figure 12). The wide temporal and spatial distributions of severe nutritional restriction suggest that habitat deficiencies at the landscape scale may constitute a primary contributing factor. We continue to apply significant efforts into investigating the habitat-nutrition relationships, but habitat deficiencies related to forage availability and quality, vegetative species composition, or less-than-optimum arrangements of forage openings and forest stands affording seasonal thermal cover remain unclear. Data from future winter nutritional assessments are required to provide additional support for our conclusions or to refute them. But the current data set, in combination with data from other ongoing habitat and nutritional studies, should provide a basis for formulating management recommendations that may be implemented and evaluated in the near future.

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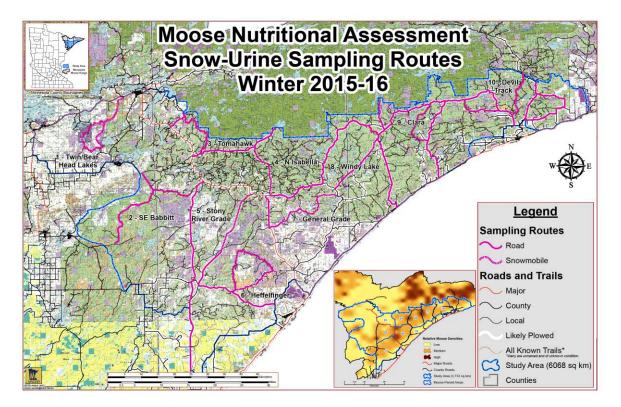
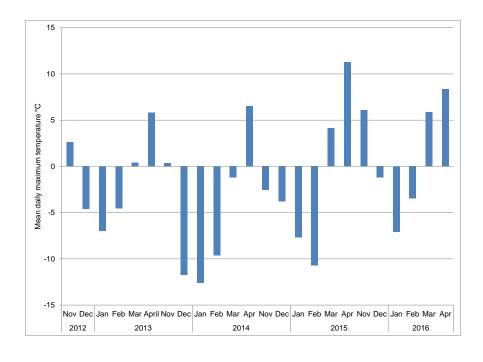


Figure 1. Map depicting the moose study area in northeastern Minnesota and the routes (i.e., roads and snowmobile trails in purple) used to distribute the sampling of fresh moose urine in snow (snow-urine) for nutritional assessments throughout the area, January–March 2013–2016.



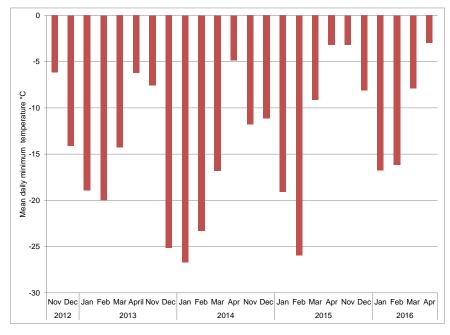


Figure 2. Mean daily maximum (top) and minimum (bottom) ambient temperatures, Ely, Minnesota, November–April 2012–2016 (Midwestern Regional Climate Center 2016).

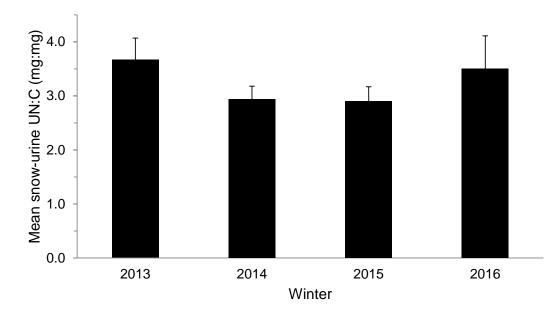


Figure 3. Overall mean (+ SE) urea nitrogen:creatinine (UN:C) ratios of samples of fresh urine voided in snow (snow-urine) by moose and serially collected for assessments of nutritional restriction throughout northeastern Minnesota, January–March 2013–2016.

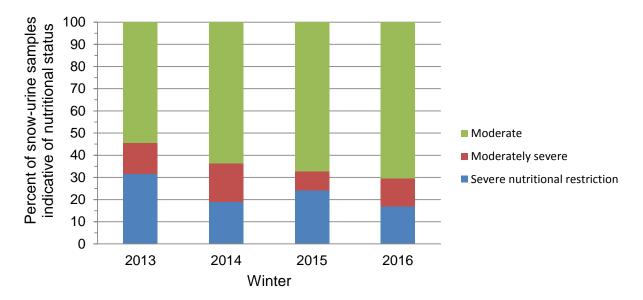


Figure 4. Overall percent of serially collected moose urine samples voided in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios indicative of moderate/normal (UN:C <3.0 mg:mg), moderately severe (UN:C = 3.0-3.4 mg:mg), and severe nutritional restriction (UN:C ≥ 3.5 mg:mg) throughout northeastern Minnesota, January–March 2013–2016.

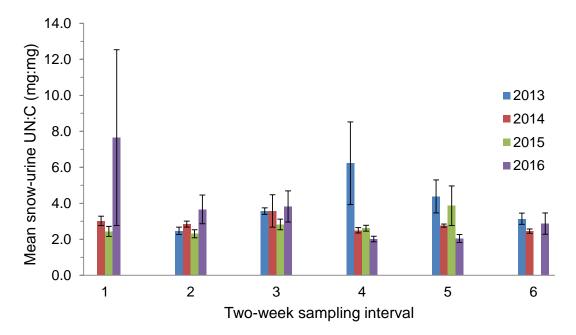


Figure 5. Mean (\pm SE) urea nitrogen:creatinine (UN:C) ratios of samples of fresh urine voided in snow (snow-urine) by moose and collected during 2-week sampling intervals for assessments of nutritional restriction throughout northeastern Minnesota, January–March 2013–2016.

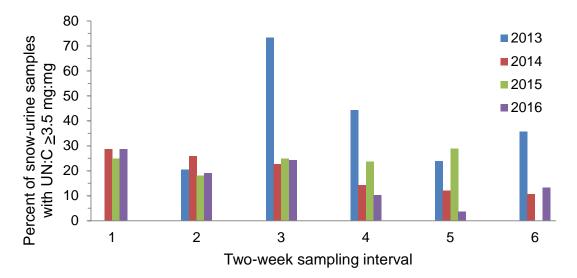


Figure 6. Percent of fresh urine samples voided in snow (snow-urine) by moose and collected during 2-week intervals with urea nitrogen:creatinine (UN:C) ratios indicative of severe nutritional restriction (UN:C \geq 3.5 mg:mg) throughout northeastern Minnesota, January–March 2013–2016.

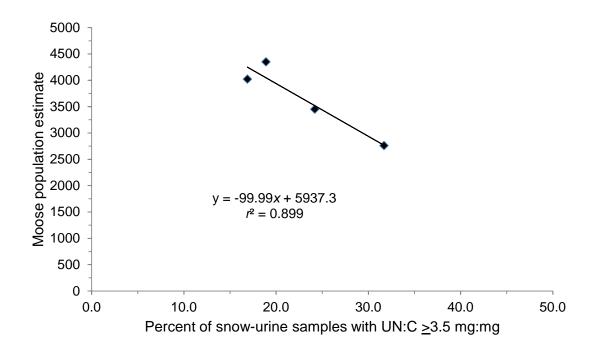


Figure 7. Relationship of the incidence of severe winter nutritional restriction of moose, indicated by the percentage of collected samples of urine in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios ≥3.5 mg:mg, to annual population estimates of moose in northeastern Minnesota (estimates from DelGiudice 2015), January–March 2013–2016.

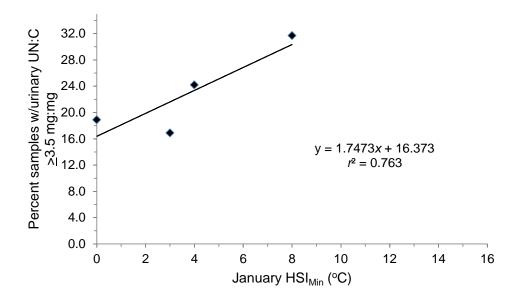


Figure 8. Relationships of January heat stress index values calculated from daily minimum temperatures (HSI_{Min}) to the incidence of severe nutritional restriction of moose, indicated by the percentage of collected samples of urine in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios \geq 3.5 mg:mg, northeastern Minnesota, 2013–2016.

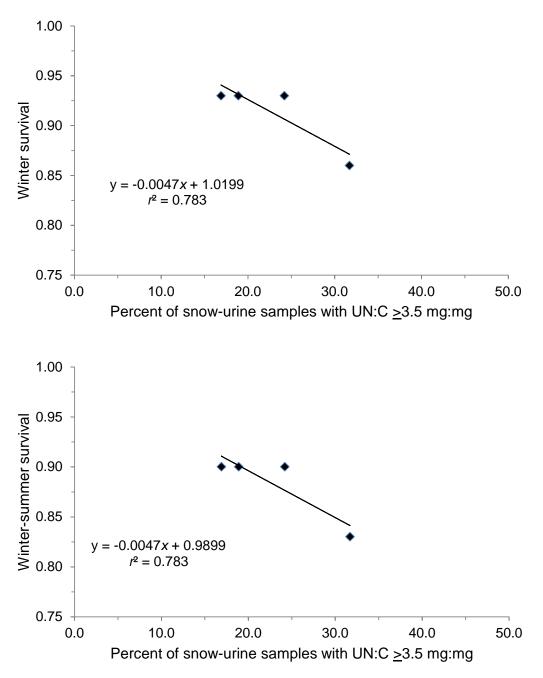


Figure 9. Relationships of the incidence of severe winter nutritional restriction of moose, indicated by the percentage of collected samples of urine in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios ≥3.5 mg:mg, to winter (top, 1 Nov–31 May) and winter-to-summer (bottom, 1 Nov–31 Aug) survival, northeastern Minnesota, 2013–2016.

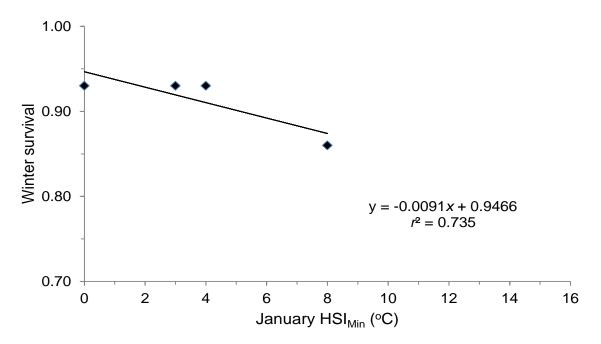


Figure 10. Relationships of January heat stress index values calculated from daily minimum temperatures (HSI_{Min}) to winter (1 Nov–31 May) survival of moose, northeastern Minnesota, 2013–2016.

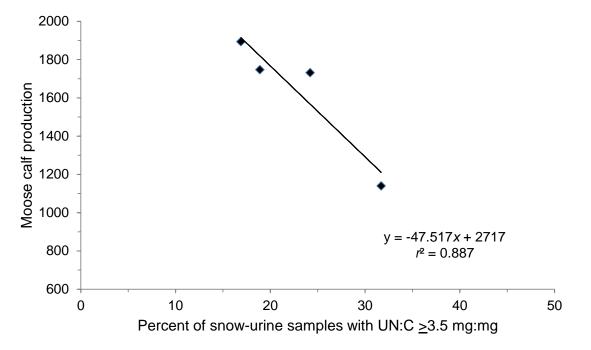


Figure 11. Relationships of the incidence of severe winter nutritional restriction of moose, indicated by the percentage of collected samples of urine in snow (snow-urine) with urea nitrogen:creatinine (UN:C) ratios \geq 3.5 mg:mg, to annual calf production, northeastern Minnesota, 2013–2016.

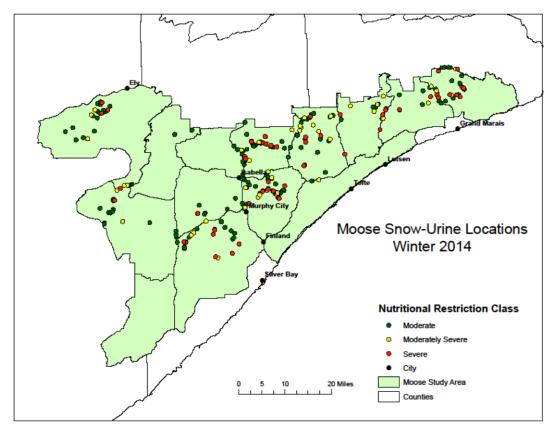


Figure 12. Spatial distribution of fresh urine samples of moose, serially collected for chemical analysis to assess the severity of winter nutritional restriction. Urinary urea nitrogen:creatinine (UN:C) ratios of <3.0, 3.0–3.4, and \geq 3.5 mg:mg are indicative of moderate/normal (green), moderately severe (yellow), and severe (red) nutritional restriction, northeastern Minnesota, 9 January–26 March 2014.



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

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SUMMARY OF FINDINGS

Camera-based surveys are increasingly being used to monitor wildlife species across large areas and a diverse range of habitats. We initiated a study in a forested area of northern Minnesota to assess various design and analysis guestions related to use of remotelytriggered cameras for simultaneously monitoring the occurrence of multiple species of carnivores. In spring 2016, we deployed 100 cameras in an area equivalent to 20 townships, with 5 cameras placed in each 9.65 x 9.65 km township. To test different lures and strategies for camera placement, we conducted a 2 x 2 factorial experiment following a randomized complete block design: four cameras were placed at randomly selected locations within forested areas, and were assigned one of 2 lures (salmon oil or a liquid version of the fattyacid scent used in tablet-form on the Minnesota Department of Natural Resources (MNDNR) scent-station survey) and one of two different placement strategies (on the closest suitable tree within 5 m from the randomly selected point, or at a user-chosen location within 90 m of the randomly selected point). We deployed an additional camera, without a lure, on a secondary road or trail within a forested area of each township. All cameras were active for a minimum of 6 weeks, and we recorded >678,000 photos. The most frequently detected carnivores were black bears (Ursus americanus); other carnivores detected included gray wolves (Canis lupus), coyotes (C. latrans), red (Vulpes vulpes) and gray (Urocyon cinereoargenteus) foxes, martens (Martes americana), fishers (Pekania pennanti), bobcats (Lynx rufus), raccoons (Procyon lotor), and striped skunks (Mephitis mephitis). We also frequently detected white-tailed deer (Odocoileus virginianus) and red squirrels (Tamiasciurus hudsonicus) and occasionally detected other herbivores (e.g., snowshoe hares (Lepus americanus), porcupines (Erethizon dorsatum), and moose (Alces alces)). More detailed analysis of the data is pending.

INTRODUCTION

Monitoring programs designed to track the distribution and actual or relative abundance of carnivores can be important for determining population status and for quantifying the effects of harvest, habitat change, and environmental variability on populations. The Minnesota Department of Natural Resources (MNDNR) currently relies on two track-based surveys (scent station and snow-track surveys) to monitor trends in a suite of 14 carnivores/furbearers. The data from these surveys have provided rough estimates of trend for many species, although interpretation must always be gualified with acknowledgment of two key, but untested, assumptions, namely that detection rates do not exhibit significant temporal or spatial trends and that road-based surveys adequately represent population-wide trends. Logistical challenges with conducting these surveys have also increased in the last decade due to loss of survey collaborators from other natural resource agencies, increased traffic or paving/plowing of roads, and less reliable snow in early winter. In the past decade, several key carnivore species had declined (e.g., fishers, martens, bears) and management intensity had increased on wolves. Given the importance of monitoring these species, statistical uncertainties with existing surveys, and increasing logistical challenges, we felt it

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was an opportune time to consider alternative ways to monitor carnivore populations. Camera surveys are an attractive option because they provide a means to estimate detection rates with little if any additional field effort, are less dependent on specific environmental conditions, and are more amenable to use of 'citizen scientists' with little formal training (photos can be verified by trained staff). Thus, remote cameras are increasingly being used or considered for large-scale multi-species occupancy surveys (e.g., O'Brien et al. 2010, Pettorelli et al. 2010, Ahumada et al. 2011, Kays et al. 2011, Fisher and Burton 2012).

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

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

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

Implementing a camera-based occupancy survey requires consideration of a variety of design and analysis options. While we do not delve into the details of each here, we highlight the following considerations: 1) camera selection and settings (Swann et al. 2004, Kays and Slauson 2008, Damm et al. 2010, Swann et al. 2011, Meek et al. 2012, Rovero et al. 2013, Weingarth et al. 2013, Wellington et al. 2013); 2) camera positioning; 3) whether to use baits/lures, and if so, which ones (Kays and Slauson 2008, Schlexer 2008, Du Preez et al. 2014); 4) time of year, which can affect species' behaviour and 'availability' as well as likelihood of meeting methodological assumptions (e.g., Kendall and White 2009, Rota et al. 2009); 5) number of cameras; 6) camera spacing and consideration of spatial correlation among sites (e.g., Sargeant et al. 2005, Hines et al. 2010, Magoun et al. 2010, Aing et al. 2011, Guillera-Arroita et al. 2011, Dorazio and Rodriguez 2012, Johnson et al. 2013); 7) whether or how best to discretize (e.g., hours, days, weeks) the temporally-continuous data from cameras into multiple survey occasions (e.g., Guillera-Arroita et al. 2011, Bischof et al. 2014); 8) site selection (e.g., random, systematic, convenience) and whether to allow flexibility in micro-site selection; and 9) approach to data analysis (e.g., single-species versus hierarchical community models; Dorazio and Royle 2005, Dorazio et al. 2006, Kery and Royle 2008, Zipkin et al. 2009, 2010, 2012, Giovanini et al. 2013, Pacifici et al. 2014).

Optimizing survey design becomes more complicated when multiple species with varying abundance and detection rates are involved. Biological characteristics of the species, such as home range size, movement patterns, and habitat preferences show large variation among carnivores (Boitani & Powell 2012). Consequently, a sampling design optimal for one species can violate important model assumptions for another. In the case of MNDNR surveys, where the suite of target species ranges from small to medium-sized mammals, such as skunks and martens, to large, roaming species like wolves and bears, design and analysis options that best account for or address this variability will be preferred. Recent attention has Page 141

been given to design of camera-based occupancy surveys targeting a community of carnivores (Hamel et al. 2013, Shannon et al. 2014), but their conclusions may not extend beyond the specifics of the biological system and analysis approaches considered therein.

OBJECTIVES

The broad objectives of this project were to:

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

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

STUDY AREA

In spring 2016, we implemented the first camera survey in one study area located in Itasca County, north-eastern Minnesota (Figure 1). This 1872 km² (48 x 39 km) area is mainly covered by forests and lakes and includes a high percentage of public land, including a portion of the Chippewa National Forest (SW portion of the study area), George Washington State Forest (NE portion), Scenic State Park (NC portion) and other state and county lands interspersed throughout. The fall camera session will be carried out in the same area and camera locations to allow comparison of estimated detection and occupancy rates between seasons.

METHODS

There are dozens of potential camera models that could have been selected for our study. Here we simply note that through consideration of available information and personal experience, we chose to utilize passive infrared (PIR) cameras with intermediate to fast trigger (<0.7 s) and recovery (<1.7 s) speeds, multi-picture capability (minimum 3) per trigger event, "no-glow" (black LED) infrared flash, and of moderate cost (maximum \$200 per camera). Through a competitive bid process, the camera model we deployed was the Bushnell Trophy Cam HD Aggressor No-Glow.

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

Our previous experience had been that few additional species are detected after 3–4 weeks of camera deployment. Although cameras can be left out indefinitely with only minimal additional financial cost related to personnel to review photos, long surveys increase risk of violating closure assumptions through mortality, immigration, or emigration. Hence, we chose to deploy cameras for 6 weeks during the first year, specifically May 1 to June 15 and September 1 to October 15.

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

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

Macro-site selection: In the first year, our focus was on evaluating the spatial sampling design in forested habitats. To identify suitable locations for camera deployment, we used Light Detection and Ranging (LiDAR) data (e.g., see Merrick et al. 2013) collected by the State of Minnesota in 2011

(http://www.mngeo.state.mn.us/chouse/elevation/lidar.html) to identify pixels (~ 20 X 20 m) with mean tree height >3 m (10 ft) and canopy cover >50% (Figure 2; details of this process will be incorporated in future reports). We then divided the study area into 20 contiguous blocks the size of townships (9.65 x 9.65 km). To ensure a minimum distance of 1.6 km (1 mi) between cameras both within and across blocks, we constrained the randomly selected points to lie within four equally-spaced sub-quadrats within each block (Figure 2). We then intersected the suitable locations (pixels) identified via LIDAR with the sub-quadrats and used the *Generate Random Points* tool in ArcGIS to select one random point falling within each of the four sub-quadrats in each block (Figure 2).

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

After selecting all locations and before deploying the cameras, each site was visualized on 2015 aerial photos to help ensure all requirements for deployment were likely met, including an additional requirement that each site was a minimum of 30 m (100 ft) from any non-forested edge.

Micro-site selection and covariates: Another important decision, after selecting the camera macro-sites, was how much flexibility should be allowed in determining the exact placement of the camera. While the use of lures effectively expands the area of camera 'coverage' well beyond the actual camera, within a given forest patch one can still potentially locate a microsite where the probability of carnivore use or detection will be higher. However, allowing flexibility in micro-site selection could introduce a source of heterogeneity in detection probabilities that may be difficult to quantify objectively. Using experienced biologists, we decided to test whether expert-based choices in fact increase detection rates. We accomplished this by dividing lured cameras into two camera placement strategies: 1)

not enhanced, meaning the camera was placed on a tree within a 5-m (15-ft) radius from the randomly selected point; or 2) *enhanced*, meaning the operator actively looked for an optimal deployment location within a 90-m (300-ft) radius of the randomly selected point.

At all camera stations, we recorded several vegetation characteristics (tree species diameter and dominance, shrub cover, canopy cover) and presence of game trails, natural 'bottlenecks', and other features within approximately 15 m of the final deployment location that could increase probability of detecting a carnivore. We also took a digital photo of angular (45°) canopy cover in 4 directions around the base of the camera tree, parallel and perpendicular to the camera-lure axis. While walking to each camera site (usually < 3 km), we also recorded presence of indirect carnivore sign (tracks, scats, dens). For trail cameras, we recorded trail width, ease of access (e.g., walk, ATV, vehicle), an index of frequency of use by humans, and vegetative coverage and height on the trail surface. Other variables (e.g., distance to main roads or water, landscape configuration metrics) will be measured using GIS. Although trail cameras were not designated an enhanced versus not enhanced treatment, we allowed flexibility in final deployment location of these cameras due to the need to position the camera on a tree at the desired angle and within sufficient distance of the trail to ensure trigger activation by animals; from the original coordinate, users were allowed a distance of 45 m (150 ft) in either direction down the trail to place the camera.

Experimental design: To test different lures and placement strategies, we conducted a 2 x 2 factorial experiment following a randomized complete block design. Along with the trail camera, 4 lured cameras were placed within each block at sites selected using the processes described above in the macro- and micro-site selection sections. Cameras at each randomly chosen site were randomly assigned 1 of 2 lure types (salmon oil or fatty acid scent oil) and 1 of 2 camera placement strategies (not enhanced or enhanced, Figure 3).

Camera deployment and settings: We deployed 100 passive infrared Bushnell Trophy Cam HD Aggressor No-Glow cameras, 80 at lured sites and 20 at un-lured trail sites. The general settings for all the cameras were based on pre-deployment testing. All cameras were attached to sturdy trees with bungee straps and placed about 75 cm (30 in) above the ground. The detection area in front of the cameras was cleared of vegetation (ferns, branches, leaves) that could obstruct the viewing area or cause false triggers, especially on windy days. At lured sites, we poured the lure on a tree located 4.5 to 9 m (15 to 30 ft) from the camera tree, with a preferred distance of 6 to 7.5 m (20 to 25 ft). We aimed trail cameras at a 45° angle to the main axis of the trail to ensure more opportunity to capture images of faster moving animals. We aimed all cameras north (ranging from northeast to northwest) when possible to reduce false triggers and blurred photos from direct sunlight.

All the cameras were programmed to record 3 mega-pixel images (color during daylight and black/white during night), with 3 'rapid-fire' pictures per trigger event and a 2-second delay between subsequent triggers. Additionally, a set of 3 rapid-fire time-lapse pictures were taken twice a day (noon and midnight) to check the functioning of the cameras and to record regular measures of daily temperature at each site. Date, time, temperature and camera Id were printed on all the images and recorded in the image metadata.

Photo processing and analysis: Identification of species is determined using experienced personnel, where after we are storing and managing photos using the open access software Camera Base (Atrium). We will use these data to compare detection rates for the two lures and the three camera placement strategies. In addition, we will calculate cumulative species richness curves to address questions related to survey duration and timing. Lastly, we will model occurrence and detection probabilities as functions of landscape features (e.g. bottlenecks, game trails) and forest characteristics (e.g. forest type, shrub cover) to provide information on species distribution and detectability. Further details of analysis methods will be presented in future reports.

RESULTS AND DISCUSSION

We recently completed our first sampling session (spring 2016) during which cameras recorded >678,000 pictures. Out of the 100 cameras deployed, only one was missing (site was logged) while two cameras malfunctioned. Bears altered camera

positioning on approximately 20 cameras, though only 10 were moved to an extent that the lure tree was no longer visible.

At this time, no formal analyses have been completed. Preliminary results suggest bears were the most commonly detected carnivore. Other detected species included gray wolves, coyotes, red and gray foxes, martens, fishers, bobcats, raccoons, and striped skunks (Figure 4). We also frequently detected white-tailed deer and red squirrels and occasionally detected other herbivores, such as snowshoe hares, porcupines, and moose.

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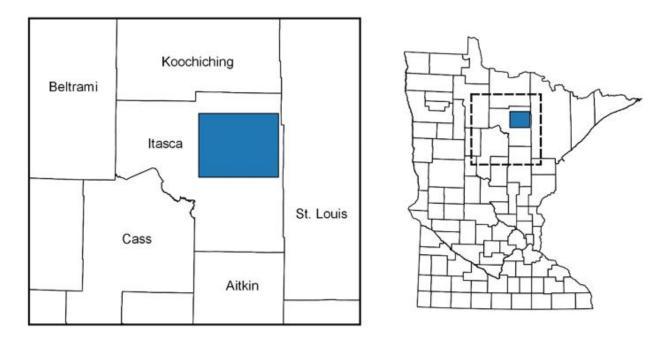
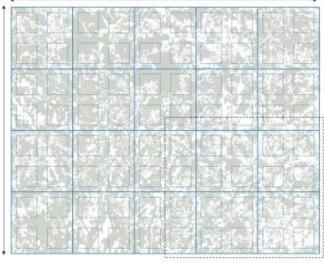
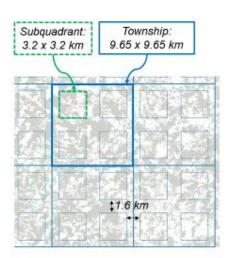


Figure 1. Location of the carnivore camera survey in the north-eastern portion of Itasca County, Minnesota.

~48 km



-39 km



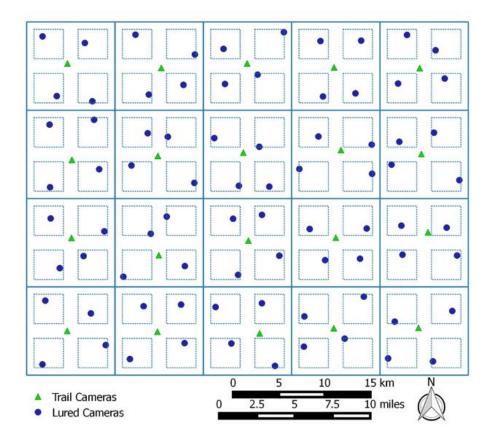


Figure 2. *Top*: Graphic of the study area showing forested habitat meeting our macro-site selection criteria (*top*: gray areas). In each township (solid blue lines; 9.65 x 9.65 km) we defined four 3.2 x 3.2 km sub-quadrats (green dotted lines). The spacing between adjacent sub-quadrats ensured a minimum distance of 1.6 km (1 mi) between cameras subject to different treatments. *Bottom:* One location for a lured camera was then randomly selected from the suitable area within each sub-quadrat. A fifth un-lured camera was placed outside the quadrats and on a trail nearest the center of the township.

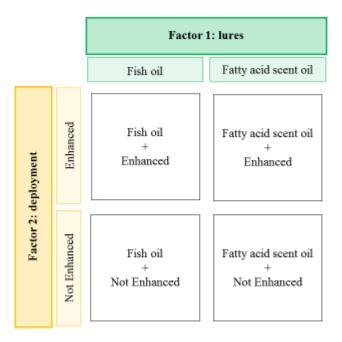


Figure 3. Factorial sampling design. In each township 4 cameras were randomly assigned to one of 4 different treatments given by the intersection between two factors: lure type and camera deployment strategy. The lure factor had 2 levels: *fatty acid scent oil* and *fish oil*; the second factor, camera deployment strategy, also had 2 levels: *not enhanced* (i.e., camera placed on nearest tree to the randomly selected UTM location) and *enhanced* (i.e., camera placed at a presumably optimal location within 90 m of the randomly selected point to increase carnivore detection).

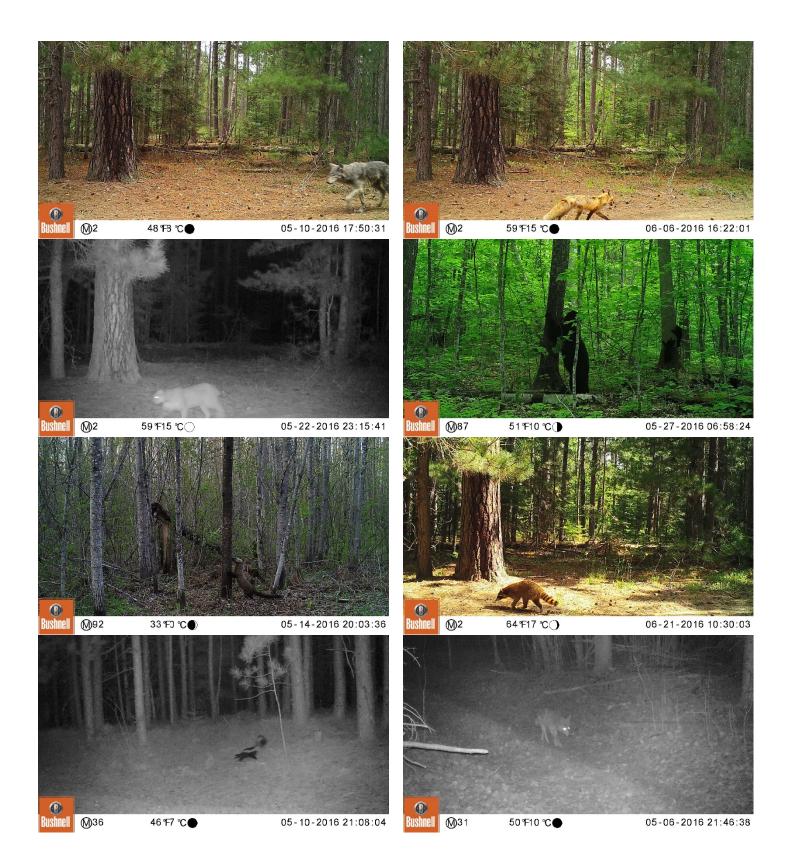


Figure 4. Example of images collected during the spring 2016 survey. From top-left to bottomright: gray wolf, red fox, bobcat, bear with two cubs, fisher, raccoon, striped skunk, and coyote.



REPRODUCTIVE ECOLOGY OF FISHERS AND AMERICAN MARTENS IN MINNESOTA

John Erb, Pam Coy, and Barry Sampson

SUMMARY OF FINDINGS

As part of a larger project on Martes ecology in Minnesota, we began monitoring reproductive success of radiocollared fishers (*Pekania pennanti*) and martens (*Martes americana*) during spring 2009. Including the pilot year of the study, 242 martens [115 females (F), 127 males (M)] and 114 fishers (65 F, 49 M) have been radiocollared. To date, age and reproductive status have been confirmed on 45 adult (≥2 years old) female martens. Pooling years, pregnancy rate has been 56% for 2-year-old martens, and 79% for martens 3 years or older. Average size of 27 marten litters is 2.96 (range = 1-4), with minimal difference between litter size of 2-year-old versus ≥3-year-old females. Based on initial data, it appears marten kits are typically born in late-April through early-May. We have located a total of 57 marten natal or maternal dens, of which 61% have been in tree [primarily aspen (Populus tremuloides) and cedar (Thuja occidentalis)] cavities, 33% in underground burrows, and 5% in hollow logs on the ground. We have also confirmed both age and reproductive status for 59 female fishers. Pooling years, fisher pregnancy rate has been 65% for 2-year-old fishers, and 94% for fishers 3 years or older. Average size of 45 fisher litters is 2.5 (range = 1-4). Data suggests that litter size for 2-year-old fishers is lower than for older females (2.15 versus 2.7). Based on data collected to date, it appears fisher kits are typically born in early- to mid-March in the southern and central part of the Minnesota fisher range and in late-March to mid-April near the northern boundary. We have confirmed 82 fisher natal or maternal dens, all but 3 being in elevated tree cavities. Cavities have been located in both live trees (70%) and snags (30%) with an overall average DBH of 20.0 in. Tree cavities used by female fishers have been located primarily in aspen (63%; Populus tremuloides, Populus grandidentata) and oak (11%, Quercus spp.) trees. Most female fishers appear to move kits from their natal den to at least 2 different maternal dens prior to June 1. Field work for this project ended during summer 2016, but full analysis of the data is not yet complete.

INTRODUCTION

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

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

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

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

In spite of these concerns, average litter size estimates from reproductive organs do not appear to be substantially biased. Strickland and Douglas (1987), summarizing data from 136 captive marten litters, computed average litter size of 2.9 for martens. This is within the range of average litter sizes reported from ovary or uterine analysis (2.5–3.5; Strickland et al. 1982, Strickland and Douglas 1987, Flynn and Schumacher 1995, 2009, Aune and Schladweiler 1997, MN DNR unpublished data). For fishers, the same appears to be true, with an average litter size of 2.8 from 60 captive fisher litters (reviewed in Strickland and Douglas 1987) and 19 wild litters (York 1996), which compares favorably to estimates based on reproductive organs (2.7–3.9 (CL), 2.7–3.2 (BC), and 2.5–2.9 (PC); review in Powell 1993).

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

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

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

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

OBJECTIVES

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

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

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

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

STUDY AREA

Marten research is focused on 1 study area located in northeastern Minnesota (Figure 1, Area 1), although 2 male marten were captured and radiocollared in Area 2 (Figure 1). Area 1 (approximately 700 km²) is nearly 90% public ownership, including portions of the Superior National Forest and state and county lands. Fishers are also present in this area at low to moderate density.

Fisher research has taken place in 3 areas (Figure 1; Areas 1, 2, and 3). The work in Area 3 is a collaborative effort between Camp Ripley Military Reservation, Central Lakes Community College, and the Minnesota Department of Natural Resources. Although we include animals captured in that area in our basic summaries, we do not discuss other aspects of that project in this report. Area 2 (1075 km²), our primary fisher study area, is approximately 67% public ownership, including portions of the Chippewa National Forest and state and county lands. Extremely few martens occupy Area 2.

METHODS

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

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

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

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

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

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

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

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

distribution of canopy layers. Lower-resolution LIDAR data (1 ppm) will also be analyzed in all study areas, along with higher-resolution (8 ppm) data for a portion of the marten study area.

To better understand any observed fluctuations in reproductive parameters, we are also collecting data on factors that may influence reproductive success, including winter severity and prey fluctuations. In each study area, a temperature monitor was placed on the north-facing side of a tree in each of 6 cover types. Each sensor records temperature every 30 minutes from 1 December to 1 June. At approximately 10-day intervals from 1 December to 1 April, we also recorded snow depth and 2 measures of snow compaction at 3 locations along transects situated in each of 6 cover types. Two snow compaction tools were constructed using PVC pipe, one each with an end-cap similar in diameter to a typical marten and fisher track in the snow. Each pipe length was then adjusted to ensure the pipe-specific load (g/cm²) was similar to marten and fisher foot-loading measures (females) reported by Krohn et al. (2004). Depth of snow compaction was recorded by dropping each load tool from 1 in. above snow level and measuring compaction depth.

Prev sampling transects have also been established in both study areas. Prev sampling is being conducted primarily to document between-area differences in prey abundance, annual within-area fluctuations in prey, and ultimately to assess whether fisher or marten habitat use, diet, survival, or reproductive success is correlated with prev dynamics. Prev-sampling transects (approximately 125 in each study area) consist of 10 sampling locations (2 parallel lines of 5 stations) spaced 20 m apart, with transects distributed in 6 cover types throughout each study area. Transects are generally oriented perpendicular to roads or trails, with the first plot 30 m off the trail. In spring, we count snowshoe hare pellets in a 1-m² plot at each sampling station (McCann et al. 2008). During fall, small mammal snap-trapping occurs for 2 consecutive days at the same sampling stations, similar to protocols used on an existing small mammal survey in Minnesota (Aarhus-Ward 2009). During both spring (hare pellet sampling) and fall (small mammal trapping), we also count the number of red squirrels (Tamiasciurus hudsonicus) observed or heard along each transect. Rather than using 10-min point counts (e.g., Mattson and Reinhart 1996. Bayne and Hobson 2000) with our small mammal and hare pellet stations as the sampling points, we record the number of unique squirrels detected per transect (summarized per unit time) while checking pellet plots and small mammal traps. Information on white-tailed deer and ruffed grouse (Bonasa umbellus) populations may be available from existing surveys or population models.

RESULTS AND DISCUSSION

Including the pilot year of the study, 242 martens (115F, 127M) and 114 fishers (65F, 49M) have been radiocollared. Because tooth aging has not yet been completed for all animals, some of which may be only 1 year of age (i.e., not capable of producing kits), we present results only for animals known to be \geq 2 years of age during spring den visits, or those of unknown age but for which we have confirmed parturition at the time of this writing (i.e., until age is known, we do not include animals that we have confirmed to be nulliparous). As of this writing, spring 2016 reproductive status assessment is largely complete.

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

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

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

Similar to martens, we treat female fishers that were alive during multiple parturition periods (years) as independent units. Excluding individuals known to be 1 year of age during the parturition period, we have confirmed both age and reproductive status for 59 female fishers (Table 1). Pooling years, pregnancy rate for female fishers has been 65% for 2-year-olds (n = 23), and 94% for fishers 3 years or older (n = 36, Table 1). We have been able to confidently assess litter size for 47 fisher litters. Overall average litter size is 2.5 (range = 1–4); litter size averaged 2.15 for 2-year-olds (n = 13) and 2.7 for ≥3-year-olds (n = 30, Table 1). Based on data collected to date, it appears fisher kits are typically born in early- to mid-March in the central and southern portion of their Minnesota range (Figure 1; Areas 2 and 3) and in late-March to mid-April further north (Figure 1, Area 1).

We have located 82 fisher natal or maternal dens to date (Table 3). For temporal reference, 66 (80%) of the fisher dens confirmed were documented to be used in March and April, with the few maternal dens located in May (n = 9) or after 1 June (n = 7) a result of time constraints and increasing difficulty in finding dens in summer. Of the 82 dens confirmed, all but 3 were in elevated tree cavities; the remaining 3 maternal dens were in large hollow logs either on or suspended above the ground (Table 3). Of the dens in tree cavities, 70% have been in live trees, whereas 30% have been in snags. Pooling live trees and snags (Table 3), most tree cavity dens used by fishers have been in aspen (n = 52) and oak (n = 9), with 1–5 dens located each in sugar maple (*Acer saccharum*), red maple, white cedar, white pine, red pine (*Pinus resinosa*), basswood (*Tilia Americana*), silver maple (*Acer saccharinum*), and American elm (*Ulmus Americana*). Average DBH for fisher den trees was 20.0 in. (range = 13.6–29.1, Table 3). Similar to martens, most female fishers appear to move their kits from their natal den to 1 or more maternal dens in the first 8 weeks following birth.

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Table 1. Parturition rate and litter size for radiocollared female fishers and martens in Minnesota from 2008 to 2016¹.

	Parturition Rate		Litter Size		
Species*Age	# females	% with litters	# litters	Average	Range
Martens					
2-year-olds	16	56	8	3.0	2 - 4
≥3-year-olds	29	79	18	2.94	1 - 4
All	48 ²	69	27 ³	2.96	1 - 4
Fishers					
2-year-olds	23	65	13	2.15	1 - 4
≥3-year-olds	36	94	30	2.7	1 - 4
All	61 ²	82	47 ³	2.5	1 - 4

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

Table 2. Natal and maternal den structures (n = 57) used by radiocollared female martens in Minnesota from 2008 to 2016.

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

Den Structure	# dens	% of total	Average DBH (in.)	DBH Range (in.)
Above-Ground, Tree Cavities	79	96.1	20.0	13.6 – 29.1
By tree status:				
Cavity, live tree	55	67.1	20.3	13.9 – 29.1
Cavity, snag	24	29.0	19.2	13.6 – 26.1
By tree species:				
Aspen cavities	52	63.4	19.8	13.6 – 29.1
Oak cavities	9	11.0	20.1	15.1 – 28.0
White pine cavities	5	6.1	23.1	19.0 – 25.6
Red Maple cavities	5	6.1	20.1	18.0 – 23.6
Sugar Maple cavities	2	2.6	20.6	19.1 – 22.1
Cedar cavities	2	2.4	17.1	13.9 – 20.3
Red Pine cavities	1	1.2	17.1	
Basswood cavities	1	1.2	16.5	
American Elm cavities	1	1.2	19.2	
Silver Maple	1	1.2	15.6	
Hollow Logs	3	3.9	15.7	13.0 – 18.3

Table 3. Natal and maternal den structures (n = 82) used by radiocollared female fishers in Minnesota from 2008 to 2016.

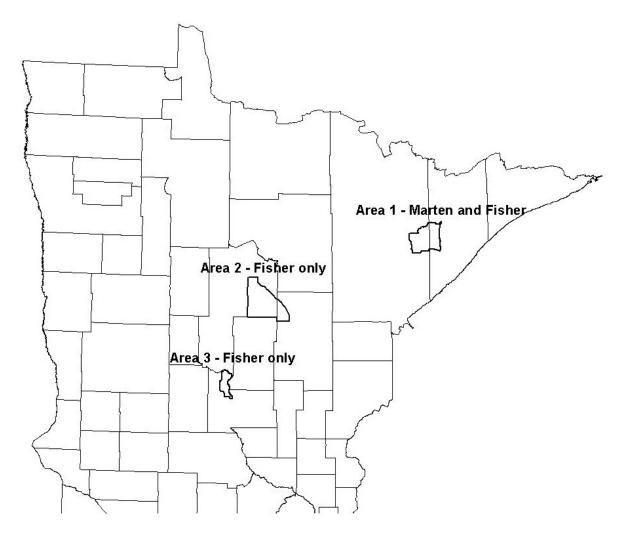


Figure 1. Fisher and American marten study areas in Minnesota, 2008–2016.



SURVIVAL AND CAUSES OF MORTALITY FOR FISHERS AND MARTENS IN MINNESOTA

John Erb, Pam Coy, and Barry Sampson

SUMMARY OF FINDINGS

As part of a larger project on *Martes* ecology in Minnesota, we began monitoring survival of radio-collared fishers (Pekania pennanti) and martens (Martes americana) during winter 2007-08. Radio-collaring efforts have now ended. Including the pilot year of the study, 242 martens [115 females (F), 127 males (M)] and 114 fishers (65F, 49M) were radio-collared. An additional 6 animals (3 martens, 3 fishers) were ear-tagged only. No martens are currently being monitored; radio-contact was lost on 76 (33F, 43M), 8 (4F, 4M) slipped their collars, 10 (9F, 1M) whose collars are inaccessible have either slipped their collars or died, and 148 deaths have been confirmed (of which 12 were censored due to death within 2 weeks of capture). Of the 136 noncensored marten deaths (60F, 76M), most have been from legal fur trapping (n = 53; 14F, 39M) and predation (n = 65; 39F, 26M). Approximately 92% of the marten predation deaths have been attributed to mammalian carnivores and 8% to raptors. Although natural mortality of martens >0.6 vears of age has occurred in most seasons, it is highest in spring and lowest in fall. No significant sex bias has been observed in overall mortality; female martens are 46% of the non-censored sample and 44% of the non-censored deaths. However, marten harvest mortality (including accidental trapping) has been notably male-biased (74% male) while natural mortality has been slightly female-biased (55% female).

Of the 114 fishers radio-collared, 4 are still being monitored (all females), radio contact was lost on 19 (10F, 9M), 19 slipped their collars (10F, 9M), collars were removed from 3 (1F, 2M), 11 (6F, 5M) have either slipped their collars or died (collars inaccessible), and 58 deaths (34F, 24M) have been confirmed (of which 1 was censored due to death within 2 weeks of capture). Of the 57 non-censored fisher deaths, most have been from predation (n = 26; 21F, 5M) and fur trapping (n = 18; 7F, 11M; 8 in-season, 10 accidentally out-of-season). Three fishers have been car-killed, 7 died from unknown but apparent natural mortality, and human-caused versus natural death could not be determined for 3 fishers. Similar to martens, natural mortality for fishers is highest in spring and lowest in fall. Of 21 female fishers predated, 19 were killed by other mammalian carnivores, 1 by a raptor, and 1 by an unknown predator. Conversely, 4 of the 5 male fisher predation mortalities were attributed to raptors (all bald eagles). Of particular note, 20 of the 21 female fishers killed by predators were adults, and 15 of them were killed while they still had dependent young, indirectly resulting in the death of all their offspring. The deaths of these 15 nursing females and their litters represent approximately 29% of the reproductive 'opportunities' for adult female fishers monitored during the kit-rearing season since the study began. Because the magnitude of this mortality would not likely have been sustainable for an extended period, we suspect that survival patterns have probably changed in the last 10 to 15 years. We continue to explore several hypotheses, but believe a partial explanation may be that cumulative changes in the environment have had both direct (e.g., reduction in denning habitat quality) and indirect (e.g., weather and habitat more favorable to competing bobcats) effects on survival of female fishers in the core of Minnesota's fisher range.

INTRODUCTION

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

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

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

Krohn et al. (1994) estimated 11% annual non-harvest mortality for adult fisher in Maine, while York (1996) estimated 19% and 7% annual non-harvest mortality (including 4% poaching mortality on males) for adult male and female fisher, respectively, in Massachusetts. Excluding the first 4-5 months of life, juvenile fisher non-harvest mortality rates have been estimated to be 28% in Maine (Krohn et al. 1994), and 0% (females) and 23% (males) in Massachusetts (York 1996). While mortality may be higher in the first months of life than the rest of the year, if we assume a similar non-harvest mortality rate during the first 4-5 months of life, we calculate that annual non-harvest mortality for juvenile fishers would be approximately 56% in Maine. Combining minimum summer survival estimates for kits with telemetry estimates of survival the rest of the vear, York (1996) estimated approximately 67% (males) and 22% (females) annual non-harvest mortality for juvenile fishers in Massachusetts. Kelly (1977, in Paragi et al. 1994) reportedly estimated 18% annual mortality of juveniles and 44% annual mortality for adult fisher in New Hampshire. More recently, Koen et al. (2007) estimated annual mortality rate (including harvest mortality) of fishers in Ontario to be 55-67% for males, and 29-37% for females. While non-harvest mortality of adult fishers is often presumed to be 'low', it has not always proven to be Page 167

the case. Furthermore, there is limited data on which to assess the amount of geographic or temporal variation in non-harvest mortality of fisher.

Natural mortality, particularly via predation, appears more common with martens. Marten survival data is available from Wisconsin (McCann et al. 2010), Maine (Hodgman et al. 1994, 1997), Ontario (Thompson 1994), Oregon (Bull and Heater 2001), British Columbia (Poole et al. 2004), Alaska (Flynn and Schumacher 1995, 2009), Quebec (Potvin and Breton 1997), and Newfoundland (Fredrickson 1990). Although we do not summarize details of these studies here, a couple conclusions are worthwhile. First, when comparing across studies, annual adult nonharvest mortality rates varied from 7-48%. Juvenile data was rarely separated, but a few studies pooled ages, and mortality rates also were within the above interval. While this variability may be attributable to both sampling and biological variability, the wide range suggests that it is risky to assume results from any area are applicable elsewhere. Secondly, at least 1 study (Maine; Hodgman et al. 1997) has documented significantly higher natural mortality for females compared to males, and other researchers have postulated this to be common given the typical male-biased harvest, 50:50 sex ratio at birth, and often balanced adult sex ratio (Strickland et al. 1982, Strickland and Douglas 1987). Due to male-biased harvest and our assumed sex-related equality in non-harvest mortality, our marten population model previously projected a very female-biased population, contradicting our preliminary capture results and suggesting that our model inputs were overestimating female survival, underestimating male survival, or incorrectly assuming a 50:50 birth sex ratio.

OBJECTIVES

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

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

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

STUDY AREA

Marten research is focused on 1 study area located in northeastern Minnesota (Figure 1; Area 1), though 2 martens have been captured and radio-collared in Area 2 (Figure 1). Area 1 (approximately 700 km²) is approximately 90% public ownership, including portions of the Superior National Forest and state and county lands. Fishers are also present in this area at low to moderate density.

Fisher research has taken place in 3 areas (Figure 1; Areas 1, 2, and 3). The work in Area 3 is a collaborative effort between Camp Ripley Military Reservation, Central Lakes Community College, and the Minnesota Department of Natural Resources. Although we do include animals captured in that area in our basic summaries, we do not discuss other aspects of that project in this report. Area 2 (1075 km²), our primary fisher study area, is approximately 67% public ownership, including portions of the Chippewa National Forest and state and county lands. Extremely few martens occupy Area 2.

METHODS

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

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

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

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

Radiolocations were obtained year-round from fixed-wing aircraft at approximately weekly intervals, with intensive ground telemetry primarily during certain focal periods (e.g., denning season). When a radiocollar emitted mortality signal, we usually investigated and recovered the animal or collar within 1–3 days. To determine cause of mortality, we used a combination of field investigation and animal necropsy. Starting in the second year of the project, we also collected forensic samples (hair by wound, wound swabs) from all animals exhibiting signs of being predated, particularly if a mammalian predator was suspected (Wengert et al. 2013). Forensic samples were submitted to either the University of California-Davis Veterinary Genetics Laboratory or Integral Ecology Research Center (Blue Lake, CA) for analysis. If non-predation natural causes were suspected after initial analysis (i.e., no visible trauma), carcasses in suitable condition were submitted to the University of Minnesota's Veterinary Pathology Lab for a full pathological exam.

RESULTS AND DISCUSSION

Including the pilot year of the study, 242 martens (115 F, 127 M) and 114 fishers (65 F, 49 M) have been radiocollared. An additional 6 animals (3 martens, 3 fishers) were ear-tagged

only. Tooth aging has not yet been completed for all animals; however, we note that because capture operations took place during winter, all animals were a minimum of 7 months of age at initial capture. We have yet to derive formal estimates of survival rate. Instead, we provide a simple overview of the fate of collared animals in this summary.

No martens are currently being monitored. Twelve martens died within 14 days postrelease and will ultimately be censored from survival analysis. Cause of death for these 12 martens was predation (n = 7), capture-related complications (n = 4), and 1 whose collar became lodged in a rocky crevice after release. Excluding these 12 animals, of the 230 collared martens monitored, radio contact has now been lost on 84 (37%; n = 76 missing, n = 8 slipped collars), the status of 10 (4%) is uncertain due to unrecoverable collars or collars found with no other evidence, and 136 (59%) have died (Table 1). Of the 136 non-censored deaths, most have been from legal fur trapping (n = 53; 14F, 39M) and predation (n = 65; 39F, 26M; Table 2). Nine animals died of other natural causes, including being crushed by a tree, perforation and blockage of the intestine from a piece of bone, starvation related to an intestinal polyp, and 6 from unknown but assumed natural causes (Table 2).

Of the 65 non-censored marten predation deaths, 61 could be attributed to either avian or mammalian predation. Evidence suggests 56 (92%) were killed by mammalian predators and 5 (8%) by raptors. Although predation deaths have occurred in nearly all months, predation and overall natural mortality is highest in the spring and lowest in the fall (Figure 2). Forensic (DNA) analysis of samples collected from predated marten (mammalian predation only) is still incomplete. To date, field evidence and DNA analysis suggests bobcats (*Lynx rufus*) as the most common mammalian predator, with red fox (*Vulpes vulpes*), fisher, and lynx (*Lynx canadensis*) also confirmed in at least one instance each.

Excluding martens censored within 14 days of capture, our sample of radiocollared marten contained 46% females. In comparison, female martens accounted for 44% of the total marten deaths, 26% of the total deaths due to harvest, and 55% of the predation deaths. Although there is no apparent sex-bias to overall mortality, marten harvest has been notably male-biased whereas natural mortality has been female-biased.

Of the 114 fishers radiocollared, 4 (3%) are still being monitored, radio contact has been lost with 41 (36%; n = 19 missing; n = 22 collars broke, slipped, or removed), the fate of 11 (10%) is uncertain due to unrecoverable collars or collars found with no other evidence, and 58 (51%) have died (Table 1). General cause of death (human versus natural) could be assigned to 54 of the 58 fisher deaths. Of these, 21 (38%) were attributable to humans (8 trapped during legal season, 10 accidental trapping, and 3 car-killed) whereas 33 (62%) were attributable to natural causes (26 predated, 7 unknown natural cause; Table 2). The seasonal pattern of natural mortality for fishers has been similar to that of martens, being greatest in spring and lowest in fall (Figure 3).

Of the 26 predated fishers, 21 were females (Table 2). Of the 21 females predated, only 1 was attributed to an avian predator [Great-horned owl (*Bubo virginianus*) suspected]. Conversely, 4 of the 5 male fisher predation deaths were attributed to raptors (all bald eagles; *Haliaeetus leucocephalus*), though scavenging cannot be rulted out in 1 case where only the radiocollar was retrieved directly underneath an active eagle nest. We are awaiting forensic DNA analysis on many fishers killed by mammalian predators. However, similar to martens, field evidence and forensic DNA analysis completed so far suggests bobcats as the most common predator, with canids (wolf or coyote) suspected in at least 2 fisher deaths.

Of particular note, 20 of the 21 female fishers killed by other predators were adults (≥2 years old), and 15 of those 20 were nursing females whose deaths resulted in complete litter loss. The deaths of these kit-rearing females and their litters represented 29% of the parous females and litters monitored during spring and early summer since the study began.

We suspect that 2 broad factors may explain the high mortality of kit-rearing female fishers during late-winter and spring: increased activity and increased vulnerability (independent of activity level). Given the potential for negative energy balance during parts of winter, compounded in early spring by the added energy demands of gestation and lactation, female fishers may need to increase activity in spring to meet energy demands. Combined with the need to locate suitable Page 170

(and multiple) natal or maternal dens, this activity, much of which may be in localized areas near den trees and hence more predictable and detectable to other carnivores, may increase predation risk. Preliminary data from temperature data loggers attached to radiocollars suggest that fishers spend increasing amounts of time (compared to winter) outside of den and rest sites during late-winter and spring. Secondly, independent of their activity level, fishers may be more vulnerable in spring because concealment cover is diminished (i.e., before 'green-up') and interspecific competition may be high due to potential prey for carnivores being at the low point in the annual cycle. Collectively, this may yield a period of high energetic demand that overlaps with a high risk, competitive environment for female fishers. Progressing into summer and fall, concealment cover is maximal, prey abundance (for all carnivores) is maximal, energetic demands of female fishers decrease as kits are weaned, and female movements may be less restricted (i.e., less predictable) with mobile kits.

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

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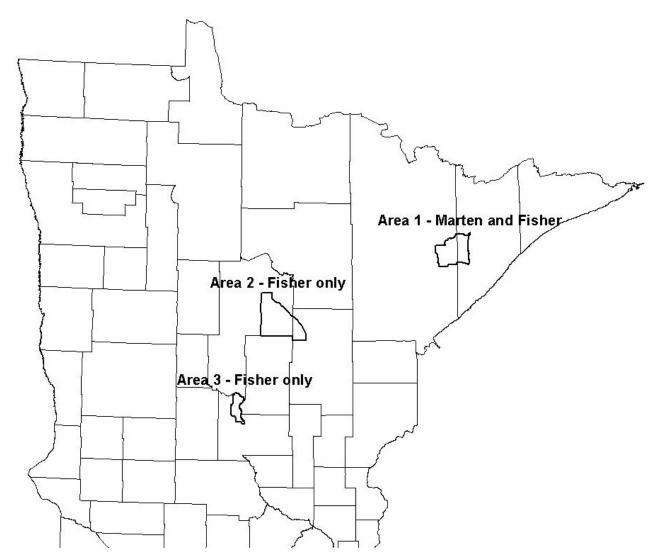


Figure 1. Fisher and marten study areas in Minnesota 2007–2016.

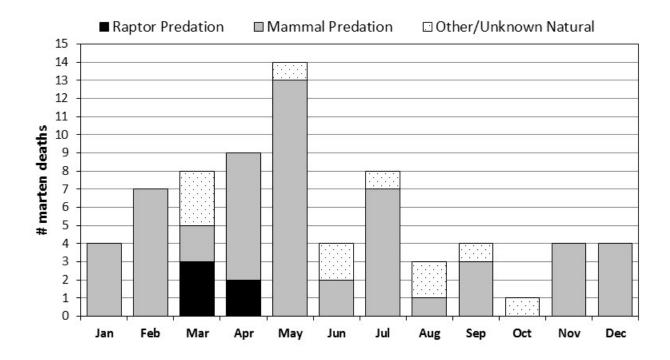


Figure 2. Seasonal timing of natural mortality for martens in northeast Minnesota, 2007–2016.

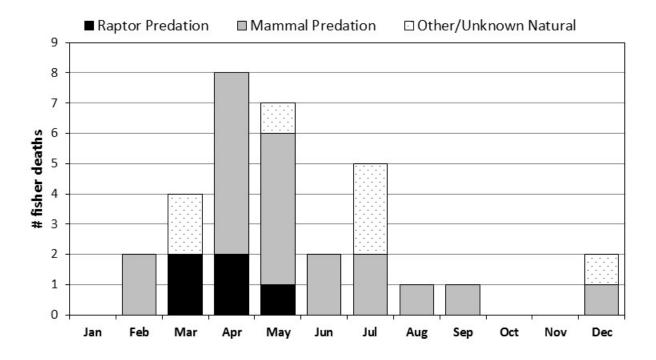


Figure 3. Seasonal timing of natural mortality for fishers in northeast Minnesota, 2007–2016.

Table 1. Number and status of fishers and martens radiocollared¹ in Minnesota from 2007 to 2016.

Sex*Species	# Active	# Deaths	# Missing	# Slipped Collars	Unknown ²	Total
Male Martens	0	76	43	4	1	124
Female Martens	0	60	33	4	9	106
Male Fishers	0	24	9	11	5	49
Female Fishers	4	34	10	11	6	65

¹ Excludes radiocollared animals that died within 2 weeks of capture and release.

² Unknown represents collars not yet retrieved from tree cavities or underground locations (presumed dead or slipped collars), or retrieved but with uncertainty whether the animal slipped the collar or had died.

Table 2. Cause of death for fishers and martens radiocollared1 in Minnesota from 2007 to 2016.

Sex*Species	Predation	Natural Accident	Disease/ Illness	Unknown Natural	Car- Killed	Trapped In Season	Trapped Out of Season	Collar Complication	Unknown ²	Total
Male Martens	26	2	1	4	0	40	2	1	0	72
Fem. Martens	39	0	0	2	1	14	2	2	0	60
Male Fishers	6	0	0	4	2	4	6	0	1	23
Fem. Fishers	21	0	0	3	1	4	3	0	2	34

¹ Excludes radiocollared animals that died within 2 weeks of capture and release.

² Unknown represents animals where evidence was insufficient to assign to natural versus human-related cause.



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

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

SUMMARY OF FINDINGS

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

We were interested in using LiDAR to supplement field data collected as part of a longterm project on fisher and marten ecology in Minnesota. Our objectives were to evaluate the potential of LiDAR technology to quantify both coarse- and fine-scale forest habitat metrics and to evaluate the effect of pulse density on prediction accuracy. We acquired high-density LiDAR data (8 pulses/m²) for a portion of our marten study area and selected 200 random locations within that portion to collect detailed vegetation measurements. Random sites were selected using a LiDAR-informed stratified random sampling design. We measured vegetation on 100 plots during summer 2015, and will measure all remaining plots during summer 2016. We defer reporting results of statistical analysis until all field data is collected and more comprehensive analysis is completed.

INTRODUCTION

To create and implement effective habitat management plans, wildlife managers depend on reliable knowledge of species-specific habitat requirements, accurate information on the current abundance and distribution of suitable habitat features, and an understanding of how management actions influence habitat suitability over a range of spatio-temporal scales. Forest wildlife species vary in their dependence on specific habitat characteristics. For some species, habitat requirements may be adequately described using coarse-resolution data such as forest cover type, stand age or successional stage, or proximity to permanent water or other specific landscape features. For these species, broad-scale forest inventory data and GIS layers derived from passive remote sensing technologies (e.g., satellite imagery, aerial photographs) are often

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

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

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

The potential for LiDAR to improve wildlife research and management has been recognized for some time. LiDAR data can be used to improve wildlife-habitat modeling in two different ways (Merrick et al. 2013, Vierling et al. 2008). First, it provides a tool that can be used with telemetry data or known species distributions to better understand resource selection. Forest attributes can be measured at fine spatial scales with LiDAR, allowing researchers to assess resource use at scales near those at which animals respond to structural attributes (Vierling et al. 2008). By providing spatially-continuous data, LiDAR data allows researchers to directly address how both landscape composition and configuration influence habitat selection. Furthermore, LiDAR can be used to investigate resource selection across a wide range of spatial scales including sites used for specific behaviors, individual home ranges, and entire wildlife Page 177

management units or other regional units. Second, LiDAR can be used to predict habitat suitability or species distributions based on prior knowledge of habitat requirements or life-history characteristics. The ability to translate habitat models into spatially-explicit maps is particularly useful for wildlife management, for example, by providing accurate predictions of the distribution and abundance of suitable habitat or by allowing managers to monitor changes in habitat suitability through time with repeated LiDAR acquisitions.

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

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

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

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

STUDY AREA

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

METHODS

There are two LiDAR datasets available that provide variable coverage of our study area (Table 1). Both datasets are discrete, multiple-return LiDAR data acquired from fixed wing aircraft during leaf-off conditions. The first dataset (hereafter, statewide data) was collected during spring 2011 part of the Minnesota elevation mapping project as (http://www.mngeo.state.mn.us/chouse/elevation/lidar.html) and provides complete coverage for Carleton, Cook, Lake, and St. Louis counties. The second dataset (hereafter, high-density data) was acquired in spring 2014 over a 25 square-mile portion of the marten study area. In general, specifications from both datasets (Table 1) match recommendations for forest inventory analysis (White et al. 2013). Those that do not (e.g., scan angle) are consistent with published studies that have successfully modeled forest structure using LiDAR (e.g., Treitz et al. 2012 used a scan angle of ±20°).

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

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

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

At each randomly-selected location, we measured structural variables within a 400-m² (11.3-m radius) circular plot. Plot size was selected to match recommendations for LiDAR-based forest inventory modeling (Laes et al. 2011, White et al. 2013) and corresponds to a 20-m pixel for landscape-level application of predictive models. Structural attributes were selected based on their importance to marten habitat from published literature (e.g., Andruskiw et al. 2008, Allen 1982, Raphael and Jones 1997, Slauson et al. 2007) and previous research in Minnesota (Joyce 2013; Table 2). Sampling protocols were largely based on USDA Forest Inventory and Analysis program protocols to maintain consistency with previous data collected at rest sites and reproductive dens used by radiocollared marten in Minnesota (Joyce 2013). All field measurements were taken in full leaf-on condition, although canopy cover and understory density also were sampled during leaf-off condition. During field sampling, locations of field plots were recorded using a Trimble GeoExplorer3 or similar mapping-grade GPS receiver. We took a minimum of 200 GPS points at a rate of 12 points per minute at each plot center. We used of a mobile beacon for real-time differential correction and applied differential post-processing to obtain sub-meter accuracy.

LiDAR can be used to directly measure a subset of the forest attributes being measured at field plots (e.g., canopy height, canopy cover/closure, canopy structure metrics; Merrick et al. 2013, White et al. 2013). For remaining attributes, we will create predictive models using multiple linear regression with LiDAR metrics as explanatory variables and attributes summarized from field plot data as response variables. We will use FUSION software (McGaughey 2013) to extract LiDAR point clouds corresponding to field plots and summarize statistical properties of individual point clouds based on return height, return intensity, or point density for use as explanatory variables in statistical modeling. Candidate models will be created using combinations of noncollinear predictor variables with the number of predictor variables included in candidate models based on effective sample size to avoid over-fitting data (Babyak 2004, Guidice et al. 2012). Candidate models will be compared using an information-theoretic approach to select the bestsupported model(s) from the candidate set (Burnham and Anderson 2002). Models will be further evaluated using R², root mean squared error (RMSE), and bias estimates.

To evaluate the effect of LiDAR pulse density on accuracy of predictive models we will subsample LiDAR data to obtain 7 different pulse densities (8, 6, 4, 2, 1, 0.5, and 0.25 pulses/m²) using FUSION software. Subsampling will be performed in a way that accurately simulates data acquired at specific pulse densities (i.e., we wish to thin the density of laser pulses rather than the number of returns per pulse). Predictive models will be created at each pulse density, and prediction accuracy will be plotted as a function of pulse density (Jakubowski et al. 2013). Prediction accuracy will be assessed using R², RMSE, and bias. From these plots we will determine the minimum pulse density necessary to create accurate predictive models (turning point, *sensu* Jakubowski et al. 2013) as well as the pulse density corresponding to the most accurate predictive model (best accuracy *sensu* Jakubowski et al. 2013). Results from this analysis will determine which forest attributes can be predicted throughout the entire marten study area using statewide LiDAR data (0.45 pulses/m²).

RESULTS AND DISCUSSION

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

Our 200 randomly-selected field plots included 115 plots in upland soil types and 85 plots in lowland soil types. During summer 2015, we measured 100 forest inventory plots. Data from these plots have been entered and checked for errors. The remaining 100 plots will be measured during summer 2016, with data entry and leaf-off vegetation measurements continuing into fall 2016. We have started preliminary statistical analysis, but we defer a more comprehensive statistical analysis until our full field dataset is compiled.

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Table 1. Specifications for statewide and high-density LiDAR datasets.

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

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

Table 2. Partial list of forest attributes that will be estimated using LiDAR data. Attributes were selected because of their biological significance to martens.

^aCitation for biological significance of attribute to martens.

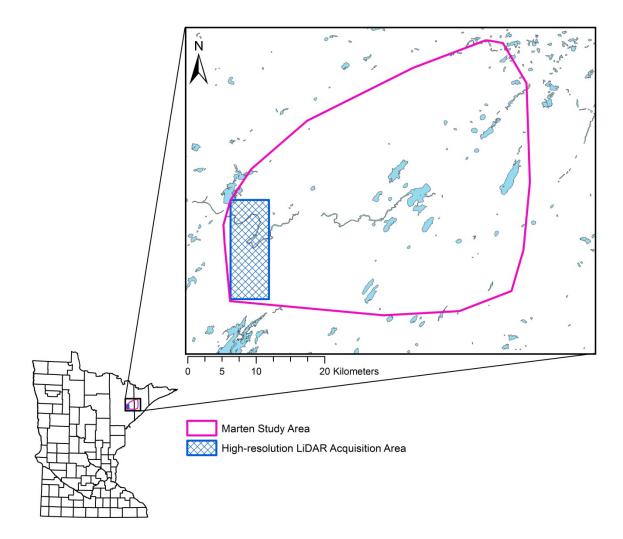


Figure 1. Map of primary marten study area in northeastern Minnesota with location where highdensity LiDAR data were acquired.

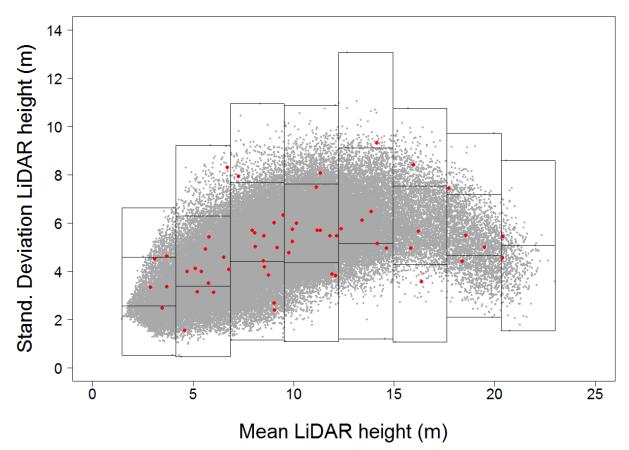


Figure 2. Sampling space for LiDAR-informed stratified random sampling design. Structural variability within the study area is represented by mean and standard deviation in LiDAR return height for each 20 m pixel in the study area (gray circles). Black squares represent strata from which a random sample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

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DETERMINING CAUSE- SPECIFIC MORTALITY OF ADULT MOOSE IN NORTHEAST MINNESOTA, February 2013 – July 2016

Michelle Carstensen, Erik C. Hildebrand, Dawn Plattner, Margaret Dexter, Christopher Jennelle, and Robert G. Wright (Minnesota IT Services)

SUMMARY OF FINDINGS

The primary goal of this study is to improve our understanding of the causes of nonhunting mortality in northeastern Minnesota's declining moose (Alces alces) population. Our goal is to respond to potential mortalities within 24 hours of death, prior to decomposition of tissues, and determine proximate cause of death and contributing factors. From 2013-2015, we captured and radio collared a total of 173 adult moose (123 females, 50 males). Mean age at capture was 6.1 (±0.3) years of age; range was 1 to 16 years. A total of 50 collared moose have died, excluding 12 capture-related mortalities that will be censored from subsequent survival analyses. Annual mortality was 19%, 12%, 15% in 2013, 2014, and 2015, respectively. The mortality rate from January–July 2016 was 7%. Overall proximate causes of death included: 18 wolf predation events (36%), 15 parasitic infections (30%), 11 bacterial infections (22%), 1 accident (2%), and 5 undetermined health issues (10%). At least 40% of the moose killed by wolves had other serious health issues that may have predisposed them to predation. Parelaphostrongylus tenuis was confirmed in 24% of all moose mortalities as either the direct cause (n=6, 12%) or a contributing factor (n=6, 12%) in their deaths. Whole carcasses were retrieved for 18 (36%) of mortalities, with field necropsies performed on the remaining 32 (64%) moose. Response times from initial mortality notification (e.g., text message or email) to a team in the field at the death site were ≤24 hours in 32 cases (64%), between 24 and 48 hours in 11 cases (22%), and >48 hours in 7 cases (14%). There are currently 74 moose remaining in the study with active collars, but 25 of these have collars that are experiencing significant transmission failures and we are not certain of their status; thus, 49 moose are actively transmitting data.

INTRODUCTION

Until recently, 2 geographically distinct moose (Alces alces) populations occurred in Minnesota (MN), one in the northwestern (NW) and the other in the northeastern (NE) part of the state. Since the mid-1980s the NW population has decreased from an estimated 4,000 to less than 100 moose, and since 2006 the NE population has declined 55% from an estimated 8,840 to 4.020 moose (DelGiudice 2016). However, there is some evidence that the moose population in the NE may be stabilizing over the last 4 years (2012-2016) at approximately 4,000 animals. Mean annual mortality rates of adults have been similarly high (21%) in both regions (Murray et al. 2006, Lenarz et al. 2009). Parasites, including liver flukes (Fascioloides magna) and brainworm (Parelaphostrongylus tenius) and other non-specific health-related issues have been documented in the majority of collared moose deaths through these past research efforts. Climate change has also been implicated as an underlying factor in both population declines. There were inverse relationships between warming ambient temperatures and decreasing survival of adult moose (Murray et al. 2006; Lenarz et al. 2010). Trends in temperature and precipitation patterns are likely to increase in intensity over the next century. If moose are unable to sufficiently thermoregulate above certain ambient temperature thresholds, we might expect to see increased body temperatures and energy expenditures required to stay

cool, which over time could have negative consequences for body condition, reproduction, and survival. Currently, no data exist to support the direct adverse effects of ambient temperature on the physiology, survival, or reproduction of free-ranging moose.

This study will determine cause-specific mortality by deploying satellite- linked GPS collars on moose in NE MN and by preparing an extensive network of responders highly trained in conducting field necropsies. Moose mortalities will be thoroughly investigated within 24 hours of death to identify the proximate cause of mortality and to examine the influence of potential contributing factors. Once causes of death and major influential factors are identified, appropriate management actions may be taken to address the population decline. Our main objectives are to 1) determine causes of non-hunting mortality (i.e., identify specific disease and parasite agents) and assess the role nutrition plays as a contributing factor; and 2) investigate how ambient temperatures relate to moose productivity, reproductive success, and survival in NE MN by applying an unprecedented field approach and comprehensive data collection methods.

Recently, a minimally invasive telemetry system for ruminants, called a mortality implant transmitter (MIT), has been developed to allow nearly continuous monitoring of body temperature with a battery lifetime of approximately 2 years. Using these MITs and GPS collars on adult moose in this study will allow us to correlate ambient temperature with their physiology, behavior (habitat use and activity), and fitness (survival and reproduction). This study will be the first to examine these relationships in a way that includes monitoring body temperature. The results of this study will be critical to an improved understanding of if, when, and how moose are able to successfully modulate their internal body temperature. Such an understanding should prove valuable in the formulation of future population and habitat management strategies and activities.

METHODS

Moose (*n*=173; 123 females, 50 males) were captured within the 3,732.8 km² study area located between 47°12'N and 47°95'N latitude and 90°33'W and 91°72'W in NE MN (Fig. 1) from 2013 to 2015, as described previously (Butler et al. 2013; Carstensen et al. 2014, 2015). All moose were fitted with GPS-Iridium satellite collars (Vectronic Aerospace GmbH; Berlin, Germany). Mortality implant transmitters (Vectronic Aerospace GmbH) were placed orally into a subset of the captured moose and provided immediate notification of mortality and recorded internal body temperature. External temperature loggers (Hobo TidbitV2; Onset Corporation, Bourne, MA) were attached to the GPS collars and were programmed to collect ambient temperature every 60 minutes. Additional ambient temperature loggers (black globes and white funnels) were placed in 7 open habitat sites throughout the study area. Data from the temperature loggers will be used along with data from 12 National Oceanic and Atmospheric Association and Remote Automatic Weather Stations in NE MN to determine the best ambient temperature predictor for moose with MITs in this study.

Moose mortality response teams have 8 primary team leaders that have undergone extensive necropsy training, and they are supported by about 20 secondary and tertiary team members (including MNDNR, tribal, academic, US Forest Service, and other personnel) available upon request. Every effort is made to respond to a moose mortality event with 24 hours of notification and to remove carcasses intact from the field and deliver them to the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL) for a complete necropsy by a board-certified pathologist. If a moose was found to be alive, but obviously ill, it was euthanized (via gunshot to the neck). If carcass extraction was not possible, a thorough and complete field necropsy was performed, guided by an established protocol. Samples were submitted to the UMN VDL for diagnostic evaluation (Carstensen et al. 2014, 2015).

Moose age was determined by cementum annuli at time of capture and we used oneway analysis of variance to compare age among years. A two-sample T-test was used to compare the mean age of moose killed by predators to those that died of health-related causes. Dead moose were categorized by age as young (\leq 3 years), prime (4–8 years), and old (\geq 9 years) and chi-square analyses was used to compared age cohorts by predator and healthrelated causes of death. Annual (Jan-Dec) survival rates were estimated using Kaplan-Meier to allow for staggered entry design.

RESULTS AND DISCUSSION

Annual survival and cause-specific mortality

From 2013–2015 a total of 173 adult moose (123 females, 50 males) were captured and radio collared. Mean age at capture was $6.1 (\pm 0.3, n=163)$ years for all moose; range was 1 to 16 years. Age of moose at capture was similar [*F-stat*=1.65, *p*=0.19] among years (6.0 years in 2013, n=101; 5.8 years in 2014, n=32; and 7.2 years in 2015, n=30). Annual (January–December) survival rate was 81%, 88%, and 85% in 2013 through 2015, respectively; 93% of moose have survived from January–July 2016 (Fig. 2). A total of 50 collared moose (37 females, 13 males) have died since this study began; which excludes 12 capture-related mortalities that are censored from subsequent survival analyses. Overall proximate causes of death included: 18 wolf predation events (36%), 15 parasitic infections (30%), 11 bacterial infections (22%), 1 accident (2%), and 5 undetermined health issues (10%; Fig. 3). Health-related causes were attributed to 64% of total deaths, with the remaining 36% being predator-related.

Seven (40%) of the wolf-killed moose had significant health conditions that likely predisposed them to predation, including encephalitis and meningitis in the brain, *P. tenuis* infections, winter tick infestations, and pneumonia in the lungs (Fig. 4a). Unfortunately, diagnostics were limited in 11 of the wolf-killed moose due to the degree of carcass consumption prior to the mortality team's arrival to the scene. It is possible that health issues may have compromised some of these moose as well.

Parasitic infections were the second leading cause of moose deaths (Fig. 4b). P. tenuis directly led to the death of 6 moose in this study; however, this parasite was also implicated in 5 wolf-caused deaths and 1 bacterial infection. Overall 24% of the moose in this study have been impacted by P. tenuis and this is likely an underestimate, as not all dead moose could be evaluated for this parasite. Winter tick (Dermacentor albipictus) infestations were primarily seen in spring 2013 (attributed to 3 moose deaths), as the severe and prolonged winters in 2012-13 and 2013-14 likely reduced tick survival. However, the past 2 winters have been extremely mild and it's likely that winter tick loads have recently increased on moose. In spring 2016, one moose in the study died from winter ticks; however, significant tick infestations were observed in other moose as well. It's likely that moose surviving into spring 2017 will experience a significant winter tick burden and may result in an increase in tick-related mortalities. Most moose in this study had livers that were damaged by liver flukes (F. magna), the severity of which varied from mild cases to severe infections that directly caused of the death of 3 moose. Similarly, the majority of moose in this study had hydatid cysts in the lungs or liver, caused by Echinococcosis granulosis, but only 2 moose had severe enough infections with this parasite to cause mortality. We also observed one moose with an extensive cysticercus (Taenia krabbei) infection throughout the body, including the heart, which resulted in death due to reduced cardiac function.

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

There are currently 74 moose remaining in the study with active collars, but 25 of these have collars that are experiencing significant transmission failures and we are not certain of their status; thus, 49 moose are actively transmitting data.

Timing of mortalities

Timing of these mortalities suggest that most deaths occur in spring (48%, March–May); however, moose died in all seasons (winter 18%, summer 20%, and fall 14%; Fig. 5). Health-related mortalities occurred during all months of the study; however, there were no wolf-related deaths in October through January (Fig. 6).

Mean age of moose (*n*=50) at death was 8.4 years (±0.5 year); range was 1 to 15 years old. Mean age of moose that died from health-related causes (*n*=31; excluding 1 moose that fell through the ice as accidental death) was 8.1 years (±0.6 year), similar [*T*-stat=2.0, *p*=0.6] to those (*n*=18) that died of wolf-related causes (8.6 ±1.0 years). Interestingly, both health and predator-related causes of death impacted nearly every age cohort in this study (Fig. 7), yet there was some evidence (X^2 stat= 4.5, *p*=0.10) to support that wolves were more selective for the young (≤3 years of age) or old (≥9 years of age) cohorts and more prime-aged moose were dying of health-related issues.

Mortality Response Times

Whole carcasses were retrieved for 18 (36%) of mortalities, with field necropsies performed on the remaining 32 (64%) moose. Response times from initial mortality notification (e.g., text message or email) to a team in the field at the death site were \leq 24 hours in 32 cases (64%), between 24 and 48 hours in 11 cases (22%), and >48 hours in 7 cases (14%). Delays in mortality responses >24 hours have been due to collar failures and wolves actively feeding on the moose carcass and preventing the collar from sending a mortality alert.

Mortality Implant Transmitters

We successfully deployed 61 MITs in moose during this study. To date, 28 moose with working MITs have died in the study and their body temperature data was collected; however, 6 of these moose were capture-related mortalities and their data will be censored from further analyses.

From December 2014 to October 2015, a MIT calibration project was conducted with the Moose Research Center within Alaska's Department of Game and Fish. Thus far, the study has shown the MIT to be a highly accurate measurement of internal body temperature in moose. On average, the MIT was only 0.03°C different than body temperature determined by vaginal implant transmitters. Further, preliminary analyses of MIT data recovered from a subset of moose (*n*=8) that have died in our study indicated prolonged elevated temperatures (>102°F) for 10-30% of readings during the summer months. Analyses to determine the role of ambient temperature relative to internal body temperature of moose and subsequent habitat selection and survival is pending.

ACKNOWLEDGEMENTS

This project is very demanding and would not be possible without the assistance of the following groups and individuals: the Environment and Natural Resources Trust Fund for funding the majority of this project, Dr. Arno Wuenshmann and Dr. Anibal Armien (UMN VDL) for their diagnostic investigations of the mortalities, Mike Schrage (Fond du Lac Natural Resources) and Andy Edwards (1854 Treaty Authority) for their assistance in the field and during captures, Richard Gerhold and Caroline Grunenwald (University of Tennessee) for assisting with the identification of microfilaria and P. tenuis, Ulrike Munderloh (University of MN, Department of Entomology) for testing samples for tick-borne illness, J. P. Dubey (USDA, ARS) for neospora and toxoplasma testing, our team of primary responders (Dave Pauly, Nancy Hansen, Dave Ingebrigtsen, Jessica Holmes, Bailey Petersen, and John Giudice; MNDNR), our team of secondary responders (Bob Fashingbauer, Bob Kirsch, Bryan Lueth, Carolin Humpal, Jim LaBarre, Leslie McInenly, Lindsey Shartell, Meadow Kouffeld-Hansen, Steve Piepgras, Tim Pharis, Tom Rusch, Ted Dick, Penny Backman, Marshall Deters, and Jeff Hines; MNDNR), Dan Ryan and Dave Grosshuesch (US Forest Service), Brandon Seitz (Grand Portage National Monument), EJ Issac and Seth Moore (Grand Portage Band), Lance Overland (Fond du Lac Resources), Nick Bogyo (1854 Treaty Authority), Bill Severud and Tyler Obermoller (UMN) for

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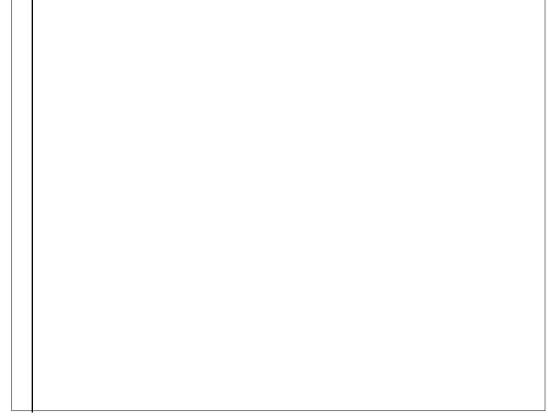


Figure 1. Study area in northeast Minnesota where 179 moose (included 6 recaptures) have been captured and radiocollared (2013–2015) to study cause-specific mortality.

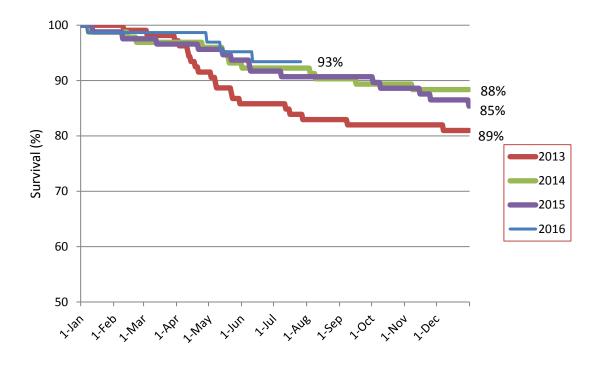
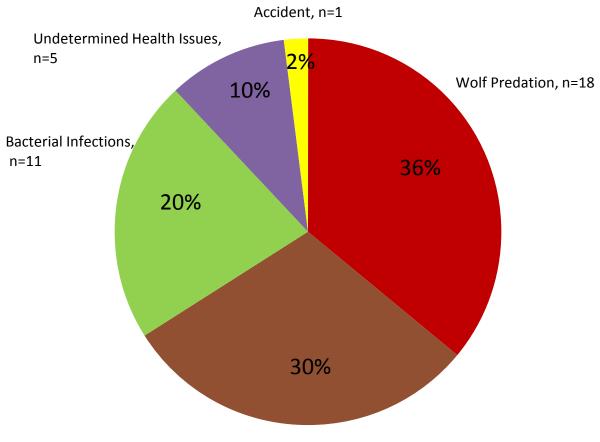
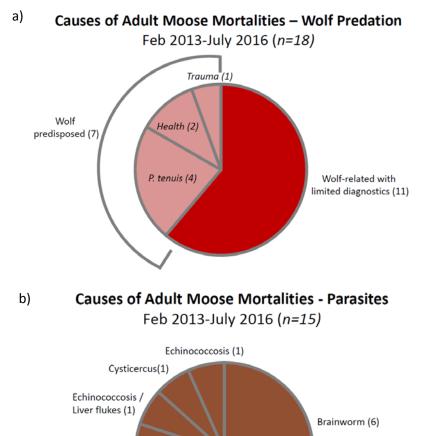


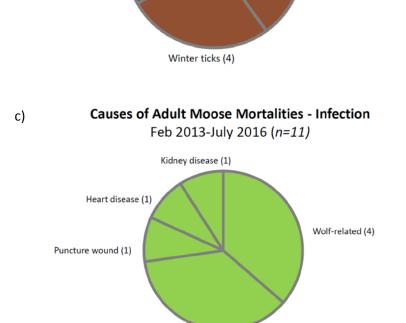
Figure 2. Annual survival of radio-collared, adult moose (*n*=173) captured from 2013-2015 in northeast Minnesota.



Parasites, n=15

Figure 3. Cause-specific mortality of radiocollared, adult moose (n=50) from February 2013 to July 2016, northeast Minnesota.





Liver flukes (2)

Figure 4. Breakdown of adult moose mortalities caused by wolf predation (a), parasites (b), and bacterial infections (c), Feb 2013-July 2016, northeast Minnesota

Other trauma (4)

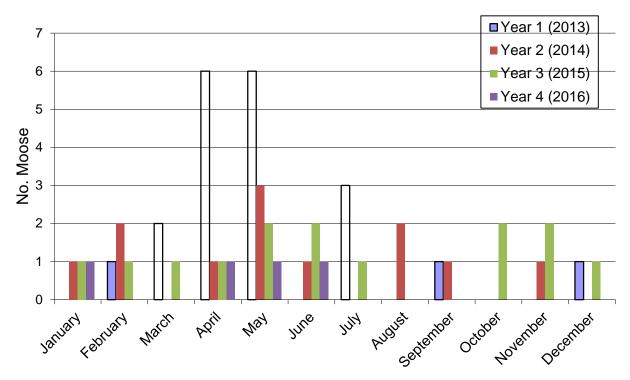


Figure 5. Timing of mortalities for radio-collared, adult moose (*n*=50) from January 2013 through July 2016, northeast Minnesota.

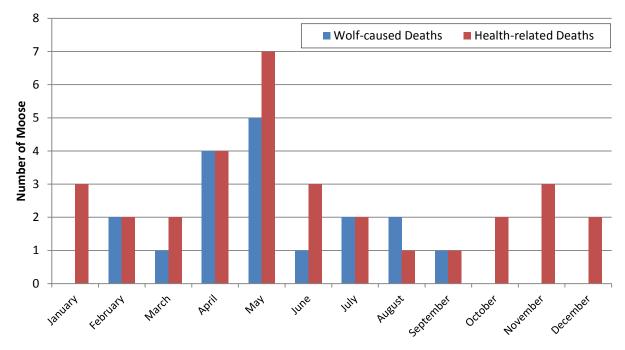


Figure 6. Timing of wolf-caused (n=18) and health-caused (n=32) moose mortalities, 2013-2016, in northeast Minnesota.

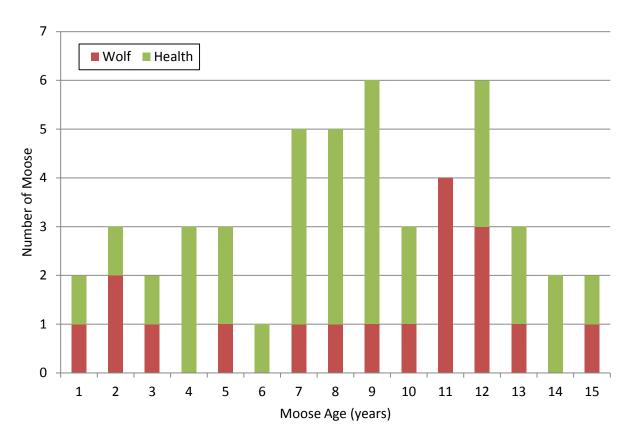


Figure 7. Age of radio-collared, adult moose (n=50) that died from health-related (green) or wolf-related (red) causes (2013-2016), in northeast Minnesota.



SURVEILLANCE FOR HIGHLY PATHOGENIC AVIAN INFLUENZA IN MINNESOTA'S WILD BIRDS IN 2015-16

Chris Jennelle, Michelle Carstensen, Erik Hildebrand, and Lou Cornicelli

SUMMARY OF FINDINGS

Since detection of highly pathogenic avian influenza (HPAI) strain H5N2 in a poultry facility in Pope County MN on February 27 2015, the Minnesota Department of Natural Resources (MNDNR) partnered with the United States Department of Agriculture's Wildlife Services (USDA-WS), the United States Geological Survey's National Wildlife Health Center (USGS), the United States Fish and Wildlife Service (USFWS), the University of Minnesota (UMN), and the University of Georgia - Southeast Cooperative Wildlife Disease Study (SCWDS) to conduct surveillance for the virus in Minnesota wild birds. The H5N2 HPAI virus strain is a combination of the highly pathogenic Eurasian H5 and low pathogenic North American H2 subtypes. From March through June 2015, 104 poultry facilities in MN were confirmed positive for HPAI, resulting in severe economic losses to producers and local economic disruption. In June and July 2015 the MNDNR collected 619 swab and blood samples of resident Canada geese (Branta canadensis) in central Minnesota. While only two geese were shedding a strain of low pathogenicity avian influenza (LPAI) virus, blood serum analysis suggested that one adult female was previously exposed to the Eurasian H5 HPAI strain. From August through September 2015, the MNDNR collected swab and blood samples from 369 live dabbling ducks; 21% were actively shedding a strain of LPAI, with 23% having serological evidence of prior exposure to avian influenza. Only one hatch-year mallard showed serological evidence of prior exposure to Eurasian H5 HPAI (but confirmation tests are pending). From September through November 2015, the MNDNR collected 907 tracheal and cloacal samples (combined) from hunter-harvested dabbling ducks across Minnesota: 20% were shedding LPAI. As part of USDA national surveillance efforts, the MNDNR collected additional oropharyngeal and cloacal samples (combined) from 545 dabbing ducks across summer, fall, and winter in specific watersheds; 21% were shedding LPAI. Since the start of the 2015 poultry outbreak through July 20 2016, MNDNR has collected 184 morbidity and mortality samples from wild birds (across most taxonomic orders): only 1 HPAI positive case was confirmed on April 29 2015 from a Cooper's hawk (Accipiter cooperil). In partnership with the USGS and UMN, MNDNR facilitated avian influenza testing of 200 common terns (Sterna hirundo) near Duluth, Minnesota via oropharyngeal and cloacal swabs; results are pending.

INTRODUCTION

Avian Influenza (AI) is a viral infection that occurs naturally in wild birds, especially waterfowl, gulls, and shorebirds. It is caused by type A influenza viruses that have 2 important surface antigens, hemagglutinin (H) and neuraminidase (N), that give rise to 144 possible virus subtypes. Influenza viruses vary widely in pathogenicity and ability to spread among birds. The emergence of an Asian strain of highly pathogenic avian influenza (HPAI) H5N1 virus in 1996, and subsequent spread of the virus in Asia, Africa, and Europe, killed thousands of wild birds and millions of domestic poultry. In 1997, HPAI H5N1 became zoonotic in Hong Kong and to-date has infected at least 850 humans around the world, resulting in 449 deaths (World Health Organization 2016). Since there is a risk of worldwide pandemic due to quickly evolving strains

of HPAI, there is an urgent need to understand transmission dynamics, host-species susceptibility, and role of the environment in AI dynamics.

From 2006 to 2010, the MNDNR tested over 12,000 wild birds in MN for HPAI and none were detected to be actively shedding virus (Hildebrand et al. 2010). The migratory movements of waterfowl and other shorebirds and subsequent mixing of birds from Asia and North America in the northern latitude breeding grounds likely facilitated the mixing of low pathogenicity avian influenza (LPAI) and HPAI strains (Pasick et al. 2015). Such mixing has resulted in recent discovery (2014) of three reassortant highly pathogenic strains including H5N1 (World Organization for Animal Health 2014), H5N2 (World Organization for Animal Health 2014, Pasick et al. 2015), and H5N8 (Ip et al. 2015) in British Columbia and the western United States.

On March 3 2015, a poultry facility in MN was confirmed infected with HPAI H5N2; the first time any HPAI strain has been detected in Minnesota poultry. The scope of the outbreak in MN poultry facilities was unprecedented and by June 2015, 23 counties contained 104 confirmed infected farms and 9.3 million birds were euthanized (20% of MN's annual poultry population) causing an economic impact of 650 million dollars (University of Minnesota Extension 2015). The MNDNR responded to this outbreak by conducting extensive surveillance for avian influenza in wild birds in 2015 (Jennelle et al. 2015, 2016). Only one wild bird, a Cooper's hawk (Accipiter cooperii) from Yellow Medicine County was confirmed HPAI H5N2 positive in Minnesota on April 29, 2015 (Jennelle et al. 2015, 2016). A black-capped chickadee (Poecile atricapilus) captured in July 2015 from Ramsey County tested positive for the presence of Eurasian H5 RNA (clade 2.3.4.4 of which the highly pathogenic strain is derived); however, virus could not be isolated from the sample. Since this species is not a reservoir host for avian influenza and based on its natural history is not expected to have direct exposure to HPAI virus, its true disease status at sampling is uncertain. Our efforts to detect HPAI H5N2 in wild birds in 2015 included live-bird and hunterharvested sampling of waterfowl and the continued monitoring of morbidity/mortality events. These efforts permit the estimation of temporal and spatial detection limits for HPAI on the Minnesota landscape, which leads to development of specific hypotheses that can help us understand HPAI risk in wild birds.

METHODS

We collected samples for AI testing from three sources: public- or agency-reported morbid or dead wild birds (i.e., morbidity and mortality events), live-captured and released ducks and Canada geese through banding programs, and hunter-harvested ducks. Dabbling ducks were primarily sampled, including mallard (*Anas platyrhynchos*), blue-winged teal (*A. discors*), American green-winged teal (*A. crecca*), American wigeon (*A. americana*), gadwall (*A. strepera*), American black duck (*A. rubripes*), northern pintail (*A. acuta*), northern shovelor (*A. clypeata*), wood duck (*Aix sponsa*), and ring-necked duck (*Aythya collaris*). Morbidity and mortality samples depended on opportunistic circumstances and public willingness to report or submit dead birds, and were collected statewide. Sampling live wild ducks and hunter-harvested ducks afforded more control over sampling design elements; both spatial and temporal dimensions were within our design control.

Morbidity and Mortality Sampling

Through outreach on the MNDNR and Minnesota Board of Animal Health websites and official press releases, we solicited the public and agency staff to report any wild birds exhibiting neurological symptoms consistent with avian influenza virus (AIV infection, dead raptors or wild turkeys, and groups of dead birds at a site regardless of species anywhere in the state. We did not investigate reports of dead adult ducks as HPAI infection in adults is generally asymptomatic, but we would consider sampling juvenile ducks and any duck experiencing neurologic signs. We did investigate reports of dead Canada geese (particularly juveniles) because recent evidence in Wyoming and Michigan documented HPAI H5N2-infected geese with clinical signs of illness. We emphasized the need to report dead birds as soon as possible to ensure collection of viable tissue samples; generally we only collected samples from birds that were deceased for <24 hours.

Depending on the resources available for staff (e.g., Brain-Heart-Infusion (BHI) media and swabs), we either collected whole carcasses (double-bagged and frozen) or swabs from the trachea and cloaca of dead birds. Both swab samples from a sick bird were placed in the same BHI media, and kept cool in a portable cooler with ice packs or a refrigerator. Whole carcasses were shipped overnight to the US Geological Survey National Wildlife Health Center (USGS) for necropsy and AIV testing using real time reverse transcription polymerase chain reaction (rRT-PCR) test, which tests for AIV RNA. Swab samples were submitted to the US Department of Agriculture National Wildlife Disease Laboratory (USDA) in Fort Collins, CO or the Minnesota Veterinary Diagnostic Lab in St. Paul, MN for AIV testing using rRT-PCR. If samples tested AIV positive initially at any lab, they were forwarded to the National Veterinary Services Laboratories in Ames, IA for confirmation and strain-typing. We had no fixed sample goal for this surveillance effort due to the opportunistic nature of public discovery and reporting of sick or dead birds. We used these data as an auxiliary source of information in our surveillance efforts.

Live Duck Sampling

We designed a late-summer project to examine differences in seroprevalence and RNA shedding of AIV between regions of Minnesota with and without infected poultry farms (Figure 1). We chose 6 areas of the state with wetlands that were in counties both with and without infected farm facilities. We used swim-in traps and night-lighting to capture, band, and sample live ducks (mostly mallards, blue-winged teal, and wood ducks). We collected between 2-3mL of blood from each bird and used a centrifuge to obtain approximately 1 mL of serum, which was frozen prior to shipping. Serum was shipped to the University of Georgia for antibody analysis by our collaborator Dr. David Stallknecht. We also collected swab samples from the oropharyngeal and cloacal cavities of each bird in order to test for AIV shedding. Methods for collection, storage, and testing were the same as previously described.

Hunter-harvest Sampling

For the fall 2015 duck hunting season, we solicited waterfowl hunters in counties with and without HPAI infected poultry facilities. Hunter participation was voluntary. We initially chose two counties without infected facilities (Morrison and Todd) and 4 counties with infected poultry facilities (Kandiyohi, Meeker, Pope, and Stearns) to collect data. Several additional counties were added to the study area in order to achieve adequate sample sizes (Figure 2). Our sample goal was to collect tracheal and cloacal swabs from 800 birds, split evenly between counties with and without infected facilities. Methods for collection, storage, and testing were the same as for morbidity and mortality sampling.

As part of the 2015 USDA National Surveillance Plan, MN participated by collecting 545 tracheal and cloacal swab samples from ducks for AIV testing between summer and winter 2015. The samples collected were broken down by watershed (Mississippi Headwaters, Red River, St. Croix, Upper Mississippi – Black Root, and Western Lake Superior) and season (summer, fall, and winter). The source of samples was from live waterfowl or hunter-harvested waterfowl. We collected swab samples from the oropharyngeal cavity or trachea (depending on live or dead birds) and cloacal cavities of each bird in order to test for viral shedding. Methods for collection, storage, and testing were the same as previously described.

RESULTS AND DISCUSSION

From March 1 2015 through July 20 2016, we collected 184 morbidity and mortality samples from wild birds (across most bird orders); only one H5N2 HPAI positive case was confirmed on April 29 2015 from a Cooper's hawk (*Accipiter cooperii*) that was found dead in Yellow Medicine County (Table 1). From August through September 2015, we collected swab and blood samples from 369 live dabbling ducks; 21% were shedding type-A influenza virus (non-HPAI). H5N2 HPAI virus was not detected in any swab samples. Although not directly part of MNDNR surveillance, our SCWDS collaborators sampled 358 live mallards and blue-winged teal from Marshall County, MN. No H5N2 HPAI virus was detected in their samples. From September Page 200

through November 2015, we collected samples from 907 hunter-harvested dabbling ducks across Minnesota; 20% were shedding a strain of LPAI virus; no H5N2 HPAI virus was detected. Additionally, we sampled all 545 ducks required for Minnesota as part of the USDA national surveillance plan between May 8 and December 4 2015. No H5N2 HPAI virus was detected in samples, but 17% of the sampled waterfowl were positive for a strain of LPAI.

Since the outbreak of HPAI began in Minnesota poultry in March 2015 through July 2016, the MNDNR and partners have collected and tested a total of 6,205 samples for HPAI, which included waterfowl feces, reported wild bird mortalities, hunter-harvested waterfowl, and live waterfowl (Figure 2). Only one Cooper's hawk was confirmed to be shedding H5N2 HPAI virus in Yellow Medicine County on 29 April 2015; it was approximately 12.5 miles from the nearest 2015 infected poultry facility. This predatory bird is typically found in woodlands and has a diet consisting mainly of small birds and mammals, and we suspect the infected hawk was exposed to HPAI through a food item.

Of 619 Canada geese blood samples collected to test for serological evidence of HPAI exposure in June and July 2015, one adult female indicated preliminary evidence of exposure to HPAI virus. This bird was sampled on June 23 2015 from Le Sueur County. Thirty-four percent of all goose blood samples indicated serological evidence of exposure to some LPAI virus strain; a result not unusual for geese. Of the 369 serum samples collected from dabbling ducks in southern Minnesota, one juvenile mallard indicated preliminary evidence of exposure to HPAI virus. This bird was sampled on August 18 2015 from Carver County. Twenty-three percent of serum samples from ducks indicated serological evidence of exposure to some LPAI virus strain; a result not unusual for ducks. Further confirmatory testing is required before a final conclusion can be made about possible HPAI exposure to the goose and mallard. If it is found that these birds were exposed to H5N2 HPAI virus, it is impossible to determine how, when, and where they were exposed.

Current Projects and Future Surveillance

In June 2016, MNDNR partnered with UMN and USGS to collect and test oropharyngeal and cloacal samples (combined) from 200 common terns in a breeding colony near Duluth, MN. These samples are currently being analyzed and results are pending. The MNDNR is also collaborating on a newly funded LCCMR project to investigate AI prevalence, exposure, and potential health effects on ring-billed and herring gulls across Minnesota.

As part of the USDA national surveillance plan for 2016 (USDA 2016a), Minnesota has been allocated a sample size of 1,040 dabbling ducks for avian influenza sampling and testing (Figure 3). Varying sample sizes are requested from six watersheds across Minnesota spanning summer, fall, and winter seasons in 2016 (USDA 2016b).

MNDNR sampling and testing of morbidity and mortality events is ongoing. We have adopted a risk-based approach to AIV surveillance in wild birds designed to respond to new detection events in a rapid and efficient manner. Three triggers will initiate intensive, and spatially and temporally designed AI surveillance efforts; if HPAI virus is detected in (1) wild, migratory birds in Minnesota through ongoing morbidity and mortality surveillance, (2) wild migratory birds in the Mississippi flyway, or (3) commercial or backyard poultry in Minnesota.

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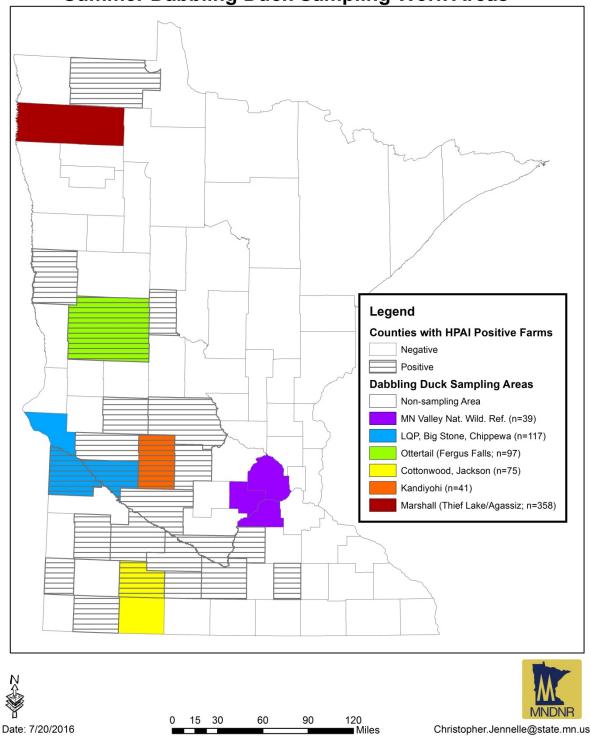
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Table 1. Cumulative species of wild bird morbidity & mortality samples (n = 184) submitted to the Minnesota Department of Natural Resources for avian influenza testing from March 2015 to July 19 2016. One Cooper's hawk tested positive for H5N2 highly pathogenic avian influenza and one American coot (*Fulica americana*) tested positive for low pathogenic avian influenza.

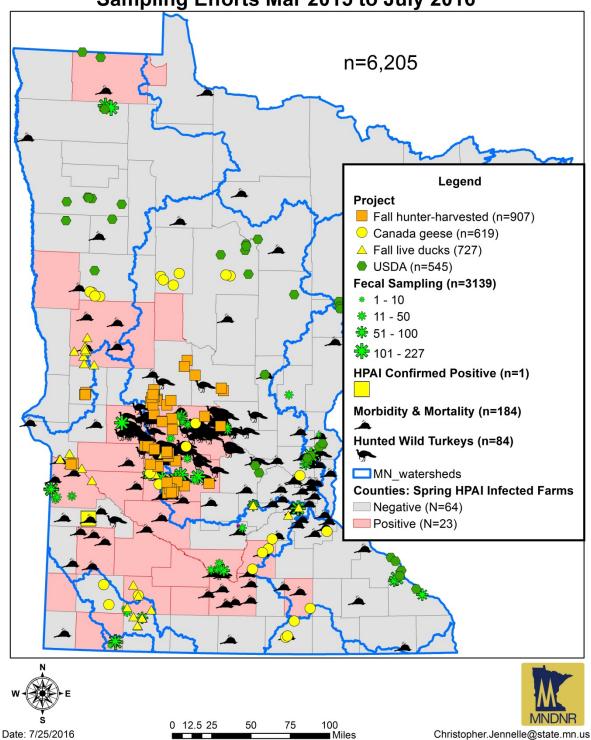
Agency	Species sampled	<u>n</u>
MNDNR	American coot	9
	American crow	1
	Bald eagle	6
	Belted kingfisher	4
	Blackbird	5
	Black-capped chickadee	1
	Broad-winged hawk	1
	Canada goose	14
	Cedar waxwing	2
	Common nighthawk	1
	Cooper's hawk	9
	Dark-eyed junco	1
	Downy woodpecker	1
	Finch	3
	Great horned owl	3
	Hawk	1
	Herring gull	1
	House finch	3
	House sparrow	15
	Mallard	15
	Mourning dove	1
	Northern shovelor	1
	Osprey	1
	Pelican	1
	Ring-necked duck	2
	Ring-necked pheasant	8
	Red-tailed hawk	4
	Ring-billed gull	3
	Rock dove	8
	Rose-breasted grosbeak	1
	Sandhill crane	2
	Sharp-shinned hawk	9
	Sora rail	1
	Starling	10
	Swainson's thrush	1
	Turkey vulture	1
	Trumpeter swan	5
	Unknown passerine	1
	Unknown sparrow	1
	Virginia rail	1
	Wild turkey	23
	Wood duck	3
Total		184

* Note that multiple birds may have been submitted for a given location and time



2015 MN Highly Pathogenic Avian Influnza Surveillance: Summer Dabbling Duck Sampling Work Areas

Figure 1. Study area layout for summer 2015 paired collection and testing of dabbing duck swab and blood samples in collaboration with SCWDS. A total of 727 paired samples were collected across six work areas throughout Minnesota.



MNDNR Highly Pathogenic Avian Influenza Surveillance: Sampling Efforts Mar 2015 to July 2016

Figure 2. The distribution of all MNDNR avian influenza surveillance efforts throughout Minnesota from March 2015 through July 2016.

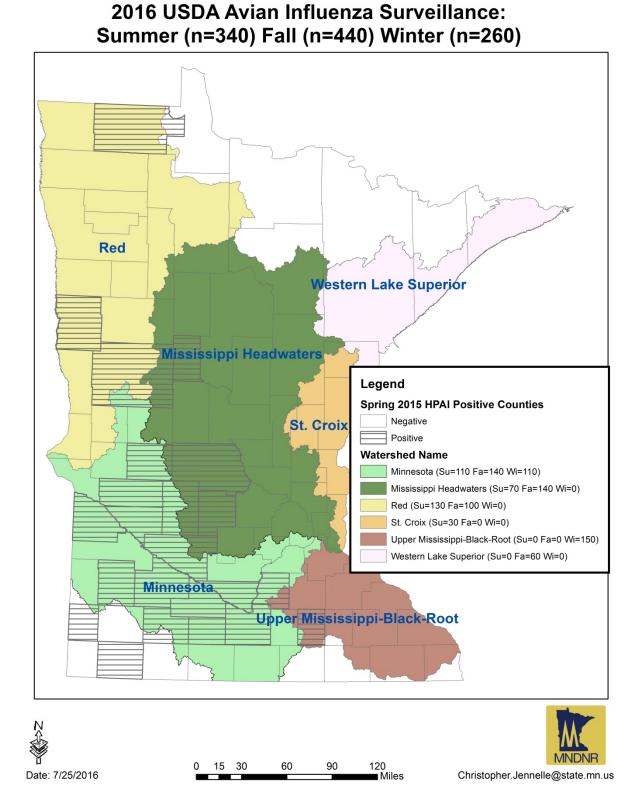


Figure 3. The USDA allocation of targeted MN watersheds for avian influenza sampling (n=1,040) for summer, fall, and winter 2016. The three sample sizes noted beside watersheds in the legend are the quotas requested by USDA for summer, fall, and winter sampling, respectively.



CALIBRATION OF A RUMEN BOLUS TO MEASURE INTERNAL BODY TEMPERATURE IN MOOSE

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SUMMARY OF FINDINGS

Mortality Implant Transmitters (MITs) were deployed in 61 wild moose (Alces alces) as an effort to understand physiological and behavioral responses of moose to increasing ambient temperature in Minnesota. To validate MIT derived body temperatures, we fit 10 captive female moose (>2 years old) at the Moose Research Center in Kenai, Alaska with MITs. All moose were also fit with vaginal implant transmitters (VITs) capable of measuring true body temperature. Both transmitters collected data in 5 min intervals for 12 months starting in December 2014. To assess how behavior affects rumen-measured temperatures we collected a total of 384 hours of behavioral observations during four, two-week windows distributed over that 12-month period and within each season. We observed a notable effect of drinking behaviors on MIT values, and developed an approach for censoring out these observations. Results suggest that after removing low temperatures due to drinking behaviors, MITs record internal body temperatures on average 0.03 °C (95% CI -0.57-0.55; mean MIT: 38.14 °C; mean VIT: 38.17 °C; n=760,439) lower than the VITs. Linear mixed effects models were developed to predict VIT temperature using corrected MIT temperatures, season, and moose ID as predictors. On average, the difference between predicted and observed VIT temperatures was 0.11 (95% CI -0.16-0.34) and 0.28 (95% CI -0.11-0.65) for winter and summer seasons respectively. This study demonstrates that, with minimal censoring and data correction, minimally invasive MITs can provide unprecedented data related to an animal's physiological response to its environment, with direct applications for understanding moose physiological and behavioral responses to increasing ambient temperatures in Minnesota.

INTRODUCTION

The rapid decline in moose numbers in Minnesota (MN) is unprecedented (DelGiudice 2016); moose have been extirpated from the northwest portion of the state and the northeast (NE) population has experienced a 55% decline in the past 10 years, from an estimated 8,840 in 2006 to approximately 4,020 moose in 2016 (Lenarz 2007, DelGiudice 2016). Adult non-hunting mortality rates have also been shown to be abnormally high in NE MN (21%; Lenarz et al. 2009; Murray et al. 2006) compared to populations in the core of their North American range (8-12%;

Mytton and Keith 1981, Larsen et al. 1989, Ballard et al. 1991, Stenhouse et al. 1995, Modafferi and Becker 1997). While the ultimate driver of this population decline remains unknown, recent research has demonstrated that health-related causes can be attributed to the majority off moose deaths (Murray et al. 2006; Carstensen et al. 2015).

Diseases, parasites, predators, habitat loss, and climate change are all factors that may have contributed, alone or in concert, to the population decline in MN (Murray et al. 2006; Lenarz et al. 2009, 2010; Mech and Fieberg 2014). Being a cold-adapted boreal species, moose are very intolerant of high temperatures (Renecker and Hudson 1986); because moose in MN are at the southern extreme of their range, they may consequently be particularly vulnerable to changes in climate (Renecker and Hudson 1986; Lenarz et al. 2010). Survival of radio-collared moose in NE MN has been shown to be negatively correlated with ambient air temperatures during winter (Lenarz et al. 2009). Interactions between moose and climate are not completely understood, but these findings suggest that increasing air temperatures may be contributing to the current population decline in NE MN.

Great Lakes temperatures are projected to increase in the near future (5 to 8 °C during spring/summer by 2025 to 2035, 5 to 8 °C during the fall/winter by 2100, Climate Change Assessment 2014 – Union of Climate Scientists), studying the effects of warming temperatures on moose is becoming increasingly important. Out of the limited body of research studying these effects; Renecker and Hudson 1986 found that temperatures larger than 5°C and 14-20°C in the winter and warm season (late spring to early fall) respectively are associated with increased metabolic, heart, and respiratory rates, reduced foot intake, and reduced body weight (Belovsky and Jordan 1978; Renecker and Hudson 1986, Renecker and Hudson 1990). McCann et al. 2013, found similar thresholds for late spring to early fall (17-24°C). However, the results of these studies were based on 2 and 4 moose respectively, and may not accurately represent thresholds for wild moose. Ambient temperatures above these thresholds, combined with the inability for moose to sufficiently thermoregulate, may lead to increased body temperatures, which feedbacks into increased energy expenditure to mitigate that heat increase. Such conclusions are speculative; however, and no data exist currently to directly support any negative effects of ambient temperature on the physiology, survival, and/or reproduction of wild moose living in natural settings, mostly due to technological limitations.

A minimally invasive telemetry system capable of measuring heart rate, body temperature, and locomotive activity for 2+ years (Signer et al. 2010) has recently been developed for ruminants, providing researchers with the opportunity to examine the effects of ambient temperature on moose behavior and physiology. Signer et al. 2010 used these mortality implant transmitters (MIT) to study alpine ibex (Capra ibex ibex). Rumen boluses have also been used in studies of dairy cattle (Loholter et al. 2013; Regev-Shosani et al. 2014) and red deer (Cervus elaphus; Turbill et al. 2011). However, the accuracy of MITs for measurement internal body temperature has yet to be tested. While core body temperatures remain very stable throughout the year, rumen temperatures have been shown to fluctuate (Degan and Young 1984; Nicol and Young 1990; Crater and Barboza 2007). Rumen temperatures have been observed to decline over different temporal scales due to water consumption in cattle (Dale et al. 1954; Cunningham et al. 1964), sheep (Brod et al. 1982; Dehority 2003), and more recently in muskoxen (Obvibus moschatus; Crater and Barboza 2007). Similar rumen temperature declines may be observed in winter feeding bouts in moose, particularly food covered by snow. Increases in rumen temperature have also been observed after feeding bouts due to microbial fermentation (Barnes

et al. 1983; Dehority 2003). These short-term changes in temperature need to be considered in the interpretation of body temperature taken with a rumen bolus, especially when trying to make correlations between body temperature and ambient temperatures.

OBJECTIVES

While recent work has been done to look at rumen temperature and possible factors affecting these readings, no work has been done to establish a relationship between rumen temperature and true core body temperature using MITs. As part of a larger project aimed at understanding cause-specific mortality of moose in northeastern Minnesota, we began efforts to validate MITs with the goal of helping guide future temperature-related moose research. Our specific objectives were to:

- 1. Quantify the relationship between rumen temperature measured by MITs and body temperature measured by VITs, and determine whether or not the MIT is an accurate device for recording body temperature of moose.
- 2. Determine if MIT-recorded temperatures are affected by moose behavior. Specifically, determine if abnormal (below or above normal) MIT temperatures are induced by moose behavior.
- 3. Establish a set of thresholds to screen out abnormal MIT readings and develop a MIT-VIT conversion model that can be applied to the MIT time series on wild moose in MN.

METHODS

Study area

The study was conducted at the Kenai Moose Research Center (MRC) on the Kenai Peninsula, Alaska. Captive moose were studied at the MRC located within Alaska GMU subunit 15A (Figure 1). The MRC is a 2400 acre facility built in the 1960's to study the relationships between moose and their environment (Hundertmark et al. 2000). All moose in this study were maintained in outdoor enclosures roughly 600-800 acres in size. Each enclosure encompasses a mix of five habitat types including open wetlands/grasslands, open crushed tree areas, aspen/birch (Populus tremuloides, Betula neoalaskana; ~25 year old), black spruce (Picea mariana; ~125 year old), and aspen/white spruce (Populus tremuloides, Picea glauca;~125 year old). For this particular study, the moose observed cohabitated two different enclosures. Moose were maintained at densities of 4-8 adult females per enclosure depending on the time of year and any conspecific aggression. Moose primarily feed on natural vegetation within the enclosures. Moose are only supplemented during times of low nutritional condition (late winter) and during research studies requiring genetic markers achieved through feeding. Similarly, moose have access to water from lakes and wetlands throughout the enclosures. Cattle troughs are available during the warm season (late spring to early fall) for enclosures with less wetlands.

Animal handling

Five female moose (>3 years old) were chemically immobilized with 0.45 mg (0.15 mL) of carfentanil and 25 mg (0.25 mL) of xylazine and five female moose (>2 and <3 years old) were chemically immobilized with 0.3 mg (0.10 mL) of carfentanil and 15 mg (0.15 mL) of xylazine during routine immobilizations at the MRC (December, April, June, September). During December 2014, eight female moose in enclosure 2 and two female moose in enclosure 3 were

fitted with a Vectronic GPS collar (GPS Plus Iridium; Vectronic Aerospace GmbH; Berlin Germany). GPS collars collected data on activity (i.e., counts of movements in the X and Y plane), position, and temperature. Collars recorded GPS locations at 30 minute intervals, and activity with a three-axis accelerometer at five minute intervals. Temperature sensors within the canister of the collar recorded temperatures at 5 minute intervals. To make evaluations of the ambient temperature quality taken by the GPS collars we attached both a Thermocron i-button (diameter: 17.35 mm; thickness: 6 mm; Mixim Integrated, San Jose, California) and a HOBO tidbit logger (Length: 30 mm, diameter: 41 mm; thickness: 17 mm; Onset Computer Corporation, Bourne, Massachusetts) to the outside of each collar canister. I-buttons and tidbit loggers recorded temperatures every five minutes. GPS collars were able to be removed and/or adjusted from captive moose at any time without the need for immobilization.

To measure rumen-record body temperature, mortality implant transmitters (MIT; length: 72 mm; diameter: 21 mm; weight: 120 g; Vectronic Aerospace GmbH; Berlin Germany) were deployed as a rumen bolus during the December 2014 immobilizations at the MRC. Deployment followed protocol outlined by Minicucci et al. (unpublished manuscript; figure 2); all adult moose were hand sedated using carfentanil and xylazine, outlined above, and then initially reversed using tolazaline. Following tolazaline application, after consistent swallowing was observed, a V-grip applicator (Genesis Industries, Elmwood, Wisconsin) was used to place the MIT at the base of tongue to allow for natural swallowing to deliver the MIT to the rumen. After correct placement, naltrexone was administered to reverse the carfentinil (Minicucci et al. unpublished manuscript). Each MIT was programmed to transmit rumen temperature, heart rate and activity to the Vectronic GPS collar at five minute intervals. MITs will remain in the moose for the duration of their life.

To establish a relationship between core body temperature and rumen temperature, vaginal implant transmitters (VIT) were deployed as a measure of core body temperature in the 10 captive female moose during each of the routine immobilizations starting in December 2014. Modified or T-shaped VITs were first used with farmed fallow deer (Dama dama) and then domestic sheep (Ovis aries) for administering drugs related to fertility (Asher and Smith 1987; Rathbone et al. 1997). In wild ungulates, VITs have been deployed successfully for parturition studies in North America with moose, black-tailed deer, elk, and mule deer (Carstensen et al. 2003; Johnson et al. 2006; Bishop et al. 2011; Patterson et al. 2013; Gilbert et al. 2014). It wasn't until more recently that VITs were modified to include temperatures loggers capable of recording core body temperature (Burfeind et al 2011; Burdick et al. 2012). The eight female moose in enclosure 2 were fit with modified VITs with archive temperature and activity sensors (VIT; diameter: 19 mm; length: 80 mm; wing span: 150 mm; Advanced Telemetry Systems (ATS); Isanti, Minnesota). The two female moose in enclosure 3 were fit with a modified VIT (VIT; diameter: 20 mm; length: 70 mm; wing span: 70 mm; Vectronic Aerospace GmbH; Berlin Germany). ATS VIT temperatures were recorded at 5 minute intervals and data was downloaded after removal. Each Vectronic VIT transmitted vaginal temperature and activity to the Vectronic GPS collar at 5 minute intervals. Each VIT was deployed for a minimum of 2 weeks after each immobilization, and were allowed to remain in for a longer time period if no negative effects are observed. VIT could be manually removed at any time without sedation.

Animal Observations

To determine the effects of behavior on both rumen and core body temperature, we directly observed eight of the collared captive moose at the MRC for six hour intervals during four

2-week time periods, totaling 384 observation hours. Each of the eight moose was observed twice during each two week period. To address seasonality, observation time periods were spread across the four seasons during 2015 (January, April, July, and October). Animal behaviors were recorded as time spent foraging (low, medium, and high), resting, ruminating, drinking/eating snow, walking, standing, running, shaking, grooming, and interacting (i.e., boxing). Behaviors were recorded using Recon data loggers (Trimble Navigation Limited Trimble, Sunnyvale, California) to the nearest second in a procedure similar to Moen (1996).

Statistical Methods

To determine correlations between MIT and specific behaviors, interval lengths for each behavior were matched temporally with MIT temperatures. Targeted behaviors were determined from previous knowledge of behaviors affecting rumen temperature (Degan and Young 1984; Nicol and Young 1990; Crater and Barboza 2007). Summary statistics were calculated for targeted behaviors (drinking, ruminating, running, etc.) including duration, max increase/decrease in MIT temperature due to specified behavior from the average temperature of the previous behavior, time from behavior start to max/min MIT temperature during this behavior time sequence, and time until MIT temperature normalization (within in one SD of the behavior start MIT temperature). Twenty minute time intervals containing only one target behavior beginning at target behavior start times were paired with random 20 minute time intervals from the same 6 hour observation period. Change in MIT temperature over the 20 minute intervals were compared using paired t-tests. To establish a set of thresholds for censoring out abnormal MIT temperatures due to behavior, an empirical cumulative distribution function (ECDF) was used to first determine the distribution of normal MIT temperatures (all MIT temperatures before the first targeted behavior start time during an observation window). The ECDF function was used to determine where behaviorally effected MIT temperatures (post targeted behavior start time during an observation window) fell within the distribution of normal temperatures.

Accuracy of MIT-recorded body temperatures were first visualized with simultaneous VITrecorded body temperatures using time series graphs using R statistical software (R Core Team 2016). The relationship between MIT and VIT data was quantified using linear mixed-effect models to account for individual variability amongst the 8 cows. To help with the interpretability of results and convergence of predictive models, MIT temperatures were centered on the mean. Models were created for each of the four seasons, and were done for 15 minute (MIT temperatures are logged every 15 minutes in wild MN moose), 90 minute, and daily temperature averages. Mixed models were allowed to have random slopes and intercepts and were defined as:

$$Y_i = X_i\beta + Z_ib + \varepsilon_i$$

Where VIT body temperatures (Y_i) were predicted using a fixed effect (β) for centered MIT temperature and random effects (b) for the interaction between centered MIT temperature and moose ID. Residual error was represented by ε_i . We evaluated how well MIT temperatures predicted core body temperatures using a k-fold cross-validation procedure (Hastie et al. 2009). Model cross-validation results were compared using root mean squared error (RMSE), defined as:

$$\text{RMSE} = \sqrt{\sum_{j} \frac{\frac{\sum_{i} (y_{ij} - \hat{y}_{ij})^2}{N_j}}{k}}$$

Where RMSE is the overall root mean squared error of all cross-validations, y_{ij} is the i-th MIT temperature of the j-th cross-validation, \hat{y}_{ij} is the i-th MIT predicted temperature of the j-th cross-validation, N_j is the number of observations of the j-th cross-validation, and k is the number of cross-validations. The goal was to evaluate how well each model predicts new moose MIT-derived body temperatures, an important need for using these models with MIT data from wild moose in Minnesota. Before prediction data could be evaluated, differences between VIT devices first needed to be determined. The difference (offset) between ATS and Vectronic VITs was calculated using linear mixed effect models. VIT body temperatures were predicted using a fixed effect for device type and a random effect for moose ID. The resulting difference in beta values was determined to be the offset between ATS and Vectronic VITS. To determine the mean difference and confidence intervals between the predicted and observed VIT temperatures for each season, we used a bootstrap. One thousand random samples were taken from the population (predicted VIT body temperatures); means and standard deviations were calculated across these 1000 samples and were used in determining the confidence intervals. Statistical significance was determined at α <0.05

RESULTS AND DISCUSSION

Mortality implant transmitter temperatures were found to be 0.04 °C lower than paired VIT temperatures for the eight moose implanted with ATS VITs (95% CI -0.63-0.55; mean MIT: 38.12 °C; mean VIT: 38.16 °C; n=760439; figure 6). Moose implanted with Vectronic VITs had MIT temperatures on average 0.27 °C higher than paired VIT temperatures for the two moose implanted with Vectronic VITs (95% CI -0.41-0.73; mean MIT: 38.11 °C; mean VIT: 37.84 °C; n=55495). Data from three moose implanted with ATS VITs had to be removed from analysis due to abnormally low VIT temperatures. Vaginal implant temperatures were on average 2.12 °C lower than paired MIT temperatures in these three moose, with VIT temperatures often being as low as 19.78 °C. Differences in VIT temperatures are suspected to be due to the design of each device. Temperature loggers in ATS VITs are placed adjacent to the base of the transmitter wire, this close proximity to the external portion of the VIT allows for possibility of greater exposure to ambient temperatures.

Drinking behaviors were found to have a significant effect on MIT temperatures during all four seasons (results not show; Figure 3; Figure 4; *t*=-3.99, *df*=67, *p*=<01). Winter MIT temperatures were less affected by drinking behaviors than the other three seasons (average temperature decrease: 0.17 °C; standard deviation: 0.24; Table 2). Drinking behaviors elicited the greatest decrease in MIT temperatures during the fall season (average temperature decrease: -5.34 °C; standard deviation: 1.18; Table 2). The mean time it took for MIT temperatures to return to within one standard deviation of pre-drinking MIT temperatures ranged from 1,152 seconds during the spring season to 2,284 seconds during the fall season (Table 2). These lasting temperature effects of drinking behaviors over time and space can be visualized in Figure 4. Empirical cumulative distribution functions using 99% thresholds resulted in an average threshold of 37.33 °C needed for censoring low MIT temperatures (Figure 5). After censoring low temperatures, MIT temperatures were found to be 0.03 °C lower than paired VIT temperatures

for the eight moose implanted with ATS VITs (95% CI -0.57-0.55; mean MIT: 38.14 °C; mean VIT: 38.17 °C; n=760439; figure 6). Moose implanted with Vectronic VITs had MIT temperatures on average 0.31 °C higher than paired VIT temperatures for the two moose implanted with Vectronic VITs (95% CI -0.13-0.73; mean MIT: 38.14 °C; mean VIT: 37.84 °C; n=55495). Ruminating behaviors were found to have no significant effect on MIT temperature during any of the four seasons (*t*=0.32, *df*=208, *p*=0.75).

The relationship between MIT and VIT temperatures using linear mixed-effect models can be seen in Table 1. On average, seasonal models were very comparable (<10% RMSE difference) between 15 minute and 90 minute averaged models (Table 1). Differences were more apparent between 15 minute or 90 minute) models and daily-averaged models, with some seasons seeing up to a 40% reduction in RMSE from 15 minute averaged to daily averaged models (Winter: 15 min=0.33, Daily=0.13). After accounting for the offset between ATS and Vectronic VITs, the difference between the daily predicted VIT temperatures and VIT temperatures during the winter was on average 0.14 (95% CI 0.03-0.24 ; Figure 6). During the summer the average temperature difference was 0.34 (95% CI 0.13-0.55; Figure 6). For 15 minute averages, the average difference between predicted VIT and VIT temperatures was 0.11 (95% CI -0.16-0.34; Figure 7) and 0.28 (95% CI -0.11-0.66; Figure 7) for winter and summer respectively. Greater temperature differences in the summer could be due to more variability in both MIT and VIT temperatures during those months. This increase in variability during summer could have arisen from a greater range of daily minimum and maximum ambient temperatures experienced by the moose, change in time spent foraging per day, drinking behaviors, etc.

Future work will include an evaluation of model performance during time periods of high VIT-recorded body temperatures, and sensitivity analysis of ECDF MIT temperature thresholds. This study demonstrates that, with minimal censoring and data correction, minimally invasive MITs can provide unprecedented data related to an animal's physiological response to its environment. This method has direct applications for understanding moose physiological and behavioral responses to increasing ambient temperatures in Minnesota.

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Table 1. Coefficient estimates from linear mixed-effect models relating VIT-measured to MITmeasured temperatures. Separate models for 15 minute, 90 minute, and daily averaged VITmeasured and MIT-measured temperatures over four seasons. Models were built using data for eight captive adult female moose at the Moose Research Center, Kenai, Alaska during 2015.

				Sd			
Model	Season		Estimate	Error	t-value	P-value	RMSE
15-min							
avg	Winter	Intercept	38.2013	0.1143	334.0996	<0.001	0.2566
		Centered- MIT	0.1958	0.0575	3.4087	0.0007	
	Spring		38.1905	0.1224	311.947	<0.001	0.2893
	Spring		0.1752	0.1224	2.7960	0.0052	0.2093
	0						0.0547
	Summer		38.2843	0.0315	1216.2320	< 0.001	0.2517
			0.1741	0.0482	3.6159	0.0003	
	Fall		38.0836	0.1175	323.9909	<0.001	0.3286
00 ·			0.1381	0.0659	2.0948	0.0362	
90-min	Winter	Intercent	38.2134	0.1145	333.7053	<0.001	0.2426
avg	VVIIILEI	Intercept Centered-	30.2134	0.1145	333.7033	<0.001	0.2420
		MIT	0.2083	0.0574	3.6319	0.0002	
	Spring		38.1946	0.1246	306.4937	<0.001	0.2682
	1 0		0.1880	0.0674	2.7891	0.0053	
	Summer		38.2323	0.0403	947.7906	< 0.001	0.2230
			0.2109	0.0537	3.9258	8.65 E-05	
	Fall		38.0881	0.1183	321.9268	< 0.001	0.3184
			0.1469	0.0644	2.2810	0.0255	
Daily avg	Winter	Intercept	38.2655	0.0891	429.4465	< 0.001	0.1973
,		Centered-	00.2000				0
		MIT	0.2346	0.0269	8.7133	<0.001	
	Spring		38.2775	0.0629	608.1345	<0.001	0.1579
			0.2903	0.0157	18.4753	<0.001	
	Summer		38.1695	0.0450	848.3559	<0.001	0.1314
			0.2399	0.0133	18.0554	<0.001	
	Fall		38.1048	0.0995	382.9494	<0.001	0.2817
			0.1578	0.0217	7.2790	3.36 E-13	

Sd error represents the standard error associated with each coefficient estimate. T-value represents the t-statistic associated with the estimated parameter. P-value represents the p-value associated with the estimated parameter. RMSE represents the overall root mean squared error of all cross validations for the corresponding season.

Table 2. Summary statistics of drinking behaviors for eight captive adult female moose over four seasons of behavioral observations conducted in 2015 at the Moose Research Center, Kenai, Alaska.

Season	N _d *	Mean T _d	MinT	Mean Time
			-0.17	
Winter	11.71 (<u>+</u> 2.04)	42.93 (<u>+</u> 61.72)	(<u>+</u> 0.24)	1678.92 (±1864.98)
			-1.58	
Spring	5.67 (±2.59)	33.86 (±37.26)	(<u>+</u> 1.58)	1152.42 (±1041.02)
	· · /	. , ,	-4.27	
Summer	4.00 (±2.83)	53.42 (<u>+</u> 26.28)	(<u>+</u> 0.61)	2234.50 (±1242.15)
			-5.34	
Fall	3.50 (<u>+</u> 0.71)	82.79 (<u>+</u> 33.29)	(<u>+</u> 1.18)	2284.50 (<u>+</u> 1800.29)

 ${}^{*}N_{d}$ represents the mean number of drinking behaviors. Mean T_d represents the mean time spent drinking during a six hour observation time period. MinT represents the mean temperature drop associated with one drinking bout. Mean Time represents the time (seconds) it takes for the Mortality Implant Transmitter (MIT) temperature to return to within one standard deviation of the MIT temperature at the time of the preceding drinking behavior.

Figure 1. Map of the Kenai Moose Research Center in Game Management Unit 15A, Kenai Peninsula, Alaska.

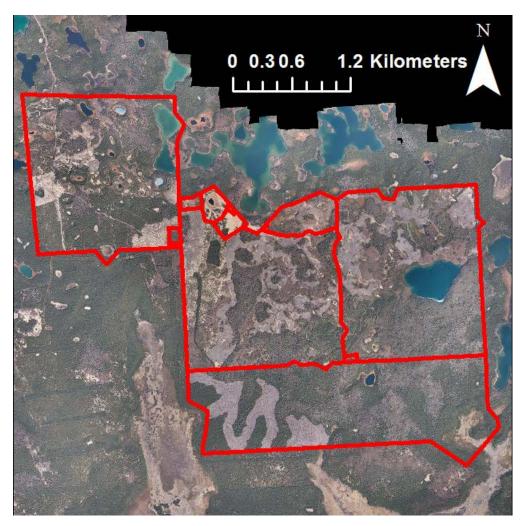


Figure 2. Oral deployment of a Mortality Implant Transmitter during the December 2014 immobilizations, Moose Research Center, Kenai, Alaska.



Figure 3. Variability in Mortality Implant Transmitter body temperature and Vaginal Implant Transmitter body temperature across seasons at the MRC during 2015 and Minnesota (MN) during 2013 and 2014.

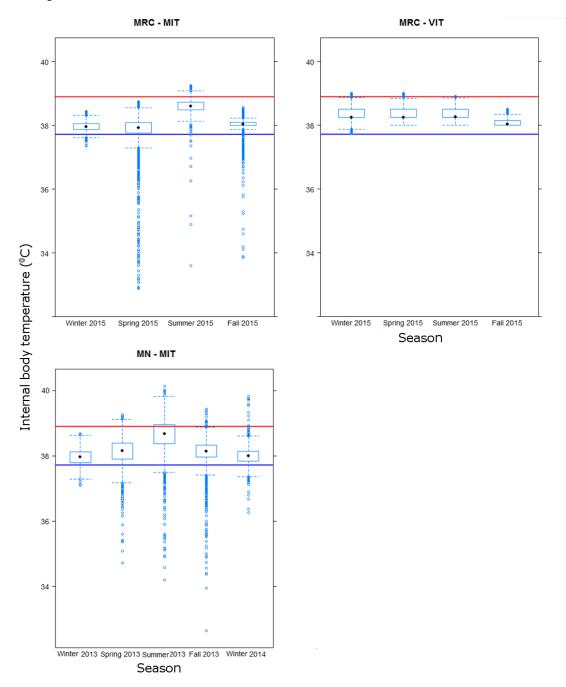
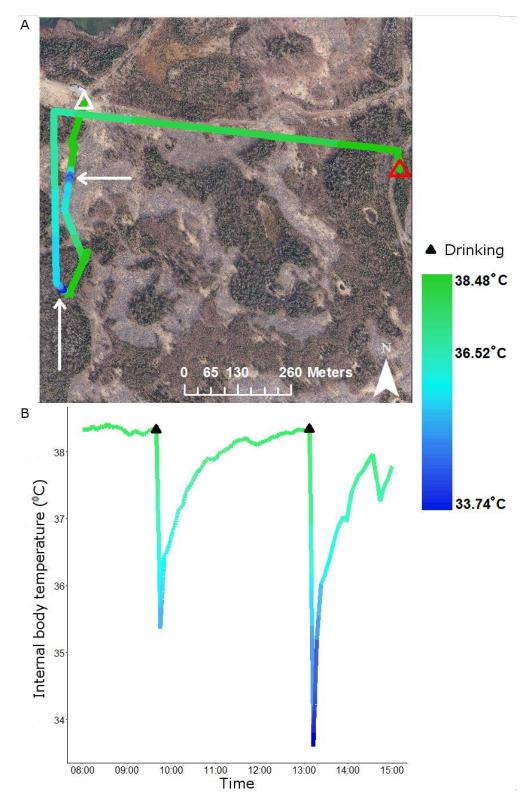
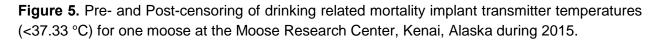
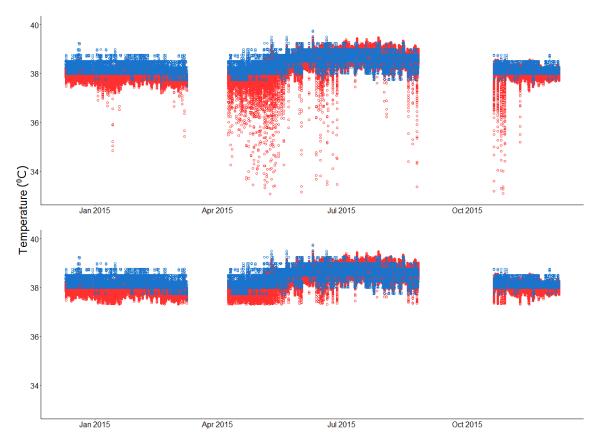
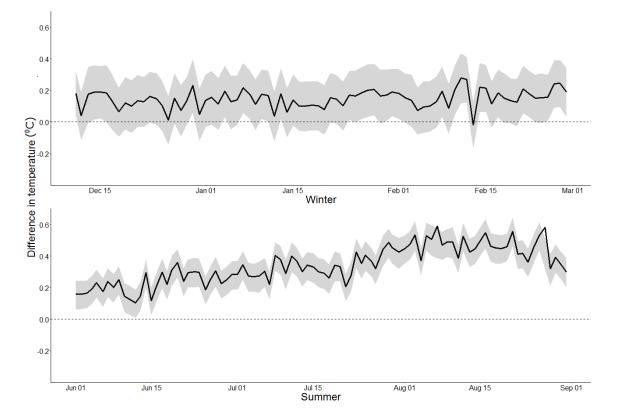


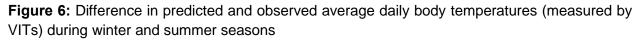
Figure 4. Visualization across space (A) and time (B) of mortality implant transmitter temperatures paired with drinking behaviors during one 6-hour observation period in April 2015.











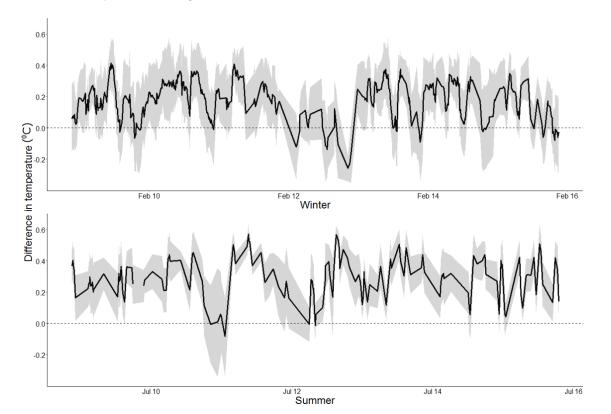


Figure 7: Difference in predicted and observed averaged 15 minute body temperatures (measured by VITs) during winter and summer.

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FALL MOVEMENTS OF MALLARDS IN MINNESOTA

Bruce E. Davis

SUMMARY OF FINDINGS

Distribution of waterfowl during fall migration and concurrent hunting seasons is affected by numerous factors including weather, habitat conditions, and hunting seasons. Understanding the chronology of immigration and emigration events and the factors affecting those events is important to waterfowl hunters and informed management of waterfowl habitat and regulations.

During August and September of 2015, we marked 116 Mallards (*Anas platyrhynchos*) with backpack transmitters. Due to difficulty capturing Mallards in some zones, we did not attain the even distribution of marked birds among the duck hunting zones that we proposed and marked 60% of the sample in the North Zone.

To date, 19 birds with tracking units have been shot and reported by hunters (16% of available marked birds were recovered); 15 of these were returned to our office. Neither the GPS/Argos, nor GPS/data logger units performed as expected, and very few viable locations were obtained. The company we purchased the units from determined it was likely that all of the units would have similar issues and has offered free replacement of the entire deployed sample.

Because of the catastrophic failure of electronics for the entire sample of marked birds, we were unable to evaluate most of the objectives outlined in the proposal. We will attempt to mark 120 Mallards with the replacement units in 2016. We expect delivery of the replacement units in August 2016 and will attempt deployment of the new units at that time.

INTRODUCTION

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

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

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

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 (in order of priority) and some specific questions that could be answered for each are:

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

During the pilot-year of this study, we seek 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. We will attempt to mark birds uniformly across the state in multiple areas of the state and across the 3 the hunting zones.

METHODS

GPS Data logger or Argos backpack transmitter units receive satellite signals to estimate highly accurate locations; precision of locations is accurate to within a few meters. These units are suitable for estimating detailed parameters associated with habitat use, use of refuge areas, local movements, and major migration events.

We attached 38 GPS-Argos backpack units (Lotek Wireless Inc., Newmarket, Ontario, Canada) to adult female Mallards. These units were under development by Lotek Engineering and similar units from this manufacturer (differing in size and attachment type) have been successfully deployed on shorebirds. These units log GPS data and then transmit that data

back to the Argos system upon completion of their duty cycle. These units weigh 12-15 g and are able to record about 100 GPS fixes. Additionally, we marked 78 hatch year male Mallards with a GPS-archival backpack unit (Lotek Wireless Inc.). These units record GPS location data at a user specified interval, but must be recovered to acquire data. We selected 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 is 18% and an additional 6% are expected to be recovered in the 2nd hunting season after recovery (USGS, Gamebirds data set). These units are small (9-11 g) and are configured as backpack type transmitters.

We attempted to mark equal numbers of birds in each of the 3 hunting zones throughout the state. As birds migrate through the state, we expect to have some birds from the north duck hunting zone stop in zones south of them. Mallards were marked in conjunction with MN DNR's operational banding program. In 2014 and 2015, departmental duck banding crews captured and banded ducks at many locations throughout the state. These crews captured sufficient numbers of Mallards such that we could mark the sample of birds in conjunction with our current banding effort. We originally proposed a reward of \$100 for hunters returning transmitters from shot birds to boost reporting rates to nearly 100% (Royle and Garrettson 2005); however, due to administrative concerns we ended up labeling the transmitters with text noting a generic reward and contact information. We paid a \$50 reward incentive for hunters returning tracking units.

The location data will be overlaid on GIS data layers to gather information on use of habitats, distances moved, and all other parameters needed to obtain data to inform the above named objectives. Data layers including agricultural land cover, designated feeding and resting areas, refuge areas, and hunting zones are readily available in the DNR's database. During marking, morphological measurements and weights will be recorded to generate body condition indices for use as covariates in subsequent analyses.

Several options for data analyses are available. Habitat use could be estimated, for example, through compositional analysis of the location data (Aebischer et al.1993). Retention rates and impacts of factors affecting these rates could be estimated using methodologies normally used in analysis of survival data; several potential methodologies may be appropriate for this. Proportional hazards regression analysis (Allison 1995) allows estimation of hazard ratios which give comparisons of factors affecting the shape of the retention curve; Program MARK (White and Burnham 1999) or a logistic exposure model (Shaffer 2004) may also be appropriate for analysis of retention data. Movement parameters including stopover duration and distance of movements can be analyzed using repeated measures mixed model ANCOVA, the propensity of marked birds to leave the state or hunting zone and factors affecting this outcome can be examined using repeated measures logistic regression. We will work with the DNR biometricians to choose further appropriate analysis techniques.

RESULTS

During August and September of 2015, we marked 116 Mallards with backpack transmitters. Due to difficulty capturing Mallards in some zones, we did not attain the even distribution of marked birds among the duck hunting zones that we wanted. We marked 78 HY-M Mallards with GPS-Archival tracking units. We marked 49 of these birds in the North Duck Zone, 9 in the Central Duck Zone, and 20 in the South Duck Zone. We marked 38 AHY-F Mallards with GPS-Argos tracking units (21 in the North Duck Zone, 2 in the Central Duck Zone, and 15 in the South Duck Zone).

To date, 19 birds with tracking units have been shot and reported by hunters (16% of available marked birds were recovered); 15 of these were returned to our office. We expected all GPS-Argos units to have uploaded their location data by now, but only 1 unit uploaded its data as scheduled. We were able to recover only very limited data from the tags that have been returned to us. We returned several of these units to Lotek for diagnosis of the problem and data recovery. Lotek engineers discovered failed water seals on the returned units and damage

to the internal electronics. Lotek deemed it likely that all of the units we deployed would have similar issues and has offered free replacement of the entire deployed sample.

Because of the catastrophic failure of electronics for the entire sample of marked birds, we were unable to evaluate most of the objectives outlined in the proposal.

DISCUSSION

We did not achieve the planned distribution of Mallard captures and over ½ of the birds were marked in the North Zone. Capture of Mallards in the Central Duck Zone was particularly difficult and we did not allow enough trap days in this zone to capture our sample; we expect that this problem will be remedied by allowing ourselves more trapping and marking days in the Central Duck Zone.

We marked hatch year male Mallards with GPS-Archival units for the pilot work to obtain the most recoveries and 18% of these units were reported recovered to date. Previous banding data indicated 14% would be recovered the 1st hunting season. This cohort is most likely to be harvested and we had more transmitters returned which allow us to troubleshoot problems with the units. After successful data are gained in this pilot work, a larger sample representing additional cohorts could be marked in subsequent years.

We will mark 120 Mallards with the free replacement units from Lotek in 2016. We expect delivery of the replacement units in August 2016 and will deploy the new units at that time.

ACKNOWLEDGEMENTS

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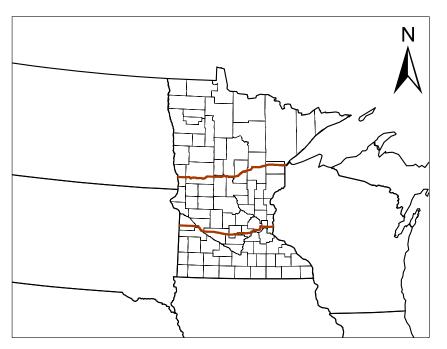


Figure 1. Minnesota waterfowl hunting zone boundaries depicted by heavy brown lines separating the north, central and south zones.

SHALLOW LAKES IN MINNESOTA: CAN WE PREDICT FUTURE CONDITIONS?

Kelsey Vitense¹, John Fieberg¹, Nicole Hansel-Welch², Mark A. Hanson, Brian R. Herwig³, and Kyle D. Zimmer⁴

SUMMARY OF FINDINGS

Shallow lakes are key resources in Minnesota, sustaining regional wetland wildlife, providing critical habitat links between breeding and wintering areas for continental waterfowl, and affording opportunities for outdoor enthusiasts who may live long distances from deeper lakes traditionally considered as recreational destinations. Roadways, agriculture, and extensive anthropogenic development often encroach on shallow lakes, leading to external nutrient loading, sedimentation, and drainage from ditches, fields and impermeable surfaces. It is not surprising that shallow lakes in many areas typically have poor water quality and provide substantially less valuable habitat than they did historically. Lake scientists have shown that nutrient loading, high water levels, and dense fish populations are key triggers in shallow lakes, and combinations of these factors cause rapid transitions to turbid conditions characterized by extreme algal blooms, sparse submerged vascular plants, and poor habitat quality. Research also suggests that turbid-state conditions in shallow lakes were historically uncommon but now appear to be widespread, probably due to combinations of factors noted above. Lake management is especially difficult because turbid conditions are often resilient, and lake rehabilitation frequently only leads to short-term improvements. Presently, lake managers need decision tools to help guide and prioritize future lake projects. We are developing models to identify combinations of factors responsible for lake deterioration, to assess management potential of individual lakes, and to help gauge the relative risk of state transitions for shallow lakes. Preliminary models have identified fish abundance, lake depth, and landcover in watersheds as major factors contributing to phosphorus concentrations in shallow lakes. We also have developed a modeling framework that allows classification of lake states and estimation of state-dependent relationships between measures of turbidity (Chla) and nutrients (TP). We plan to use these and other study products to develop an interactive decision support tool capable of integrating available information in a framework that will help managers identify lakes needing special protection, fine-tune management needs of individual lakes, and rank lakes as candidates for future lake management efforts.

INTRODUCTION

Shallow lakes generally conform to one of two alternative stable states: a clear state with primary production dominated by submerged aquatic vegetation (SAV) and a turbid state with phytoplankton dominating over SAV (Scheffer *et al.* 1993). Excessive nutrient inputs from current and historical land use, food web-mediated influences and sediment disturbance caused by planktivorous and benthivorous fish, and wind all drive transitions to, and affect the resilience of, turbid states (Scheffer 2004). Shallow lakes with high nutrient levels are prone to explosive, unhealthy phytoplankton "blooms," especially when phosphorus (P) is readily available (Scheffer 2004). Submerged aquatic vegetation, which sustains the diverse invertebrate communities that provide important food sources for waterfowl, is reduced in this turbid, algae-dominated state (Hargeby *et al.* 1994). Parasites associated with amphibian malformations likely have higher prevalence in turbid lakes (Johnson & Chase 2004), and nitrogen may accumulate at higher

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rates (Zimmer et al. 2003). It is not surprising that key goals for shallow lake management are to prevent shifts from clear to turbid states, to induce shifts from turbid to clear states, and to maintain the natural resilience of clear-water shallow lakes.

Complex ecological and physical mechanisms are responsible for maintaining the stability of each alternative state, such as competition between primary producers. When SAV declines, phytoplankton abundance typically increases, limiting light reaching the lake bottom and further restricting SAV in a positive-feedback loop (Scheffer *et al.* 1993). Additionally, when SAV is sparse, sediments are easily disturbed by benthivorous fish and waves. Suspended sediments further increase turbidity, and mobilized P stimulates even higher phytoplankton growth rates (Scheffer 2004). In contrast, in clear-state lakes, SAV remains widely distributed and helps maintain water clarity by stabilizing sediments and taking up nutrients (Søndergaard et al. 2003). Charophytes (*Chara*) often accompany clear-water conditions in Minnesota lakes and are believed to release algal toxins (Berger & Schagerl 2004) and provide refuge for zooplankton, which may further reduce the phytoplankton population and help stabilize clear-water conditions.

Shallow lakes are notoriously difficult to restore after shifting from clear to turbid states, with turbid conditions frequently returning within 5-10 years following lake management (Søndergaard *et al.* 2007; Hanson *et al.* in press). Theoretical models are useful for understanding how nutrients influence whether lakes will tend to turbid or clear water states in the long run. For example, Figure 1 shows a bifurcation diagram derived from a model describing shallow lake dynamics similar to those in Scheffer and Carpenter (2003) and Scheffer (2004). At low nutrient levels (left of "flip down!" threshold in Figure 1), lakes can only exist in the clear stable state. At high nutrient levels (right of the "flip up!" threshold in Figure 1), lakes only exist in the turbid state. In between these two thresholds, the system exhibits hysteresis in which two different steady states are possible under the same nutrient conditions, depending on whether the initial turbidity levels lie above or below the unstable state in this region of bistability (dashed line in Figure 1).

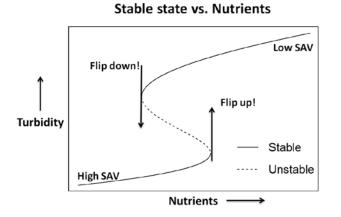


Figure 1. Bifurcation diagram from a theoretical model describing shallow lake dynamics.

The bifurcation diagram is also useful for understanding temporal dynamics and shifts between stable states. If a lake is in the clear state with high SAV (lower solid line) and nutrient input increases beyond the "flip up" bifurcation point, the lake will likely transition quickly to the

turbid state with low SAV (upper solid line). Once SAV is lost, the internal loading of nutrients increases and becomes hard to control, and external nutrient loading must be substantially reduced to the lower "flip down" bifurcation point to reverse the state shift (Scheffer & Carpenter 2003). In practice, such drastic nutrient reduction may not be possible or may only be accomplished over long time periods. Alternatively, managers may attempt to induce a state shift by forcing the system across the unstable point, e.g., by decreasing the planktivore and benthivore populations with rotenone (if nutrients can at least be reduced to the region of bistability) (Jeppesen et al. 2009). These resulting transitions are typically short-lived, however, since perturbations to the system (e.g., fish colonization, destruction of submerged vegetation) can force the lake back to the turbid state. For instance, Lake Christina, a large shallow lake in Minnesota, has been rehabilitated with fish toxicants three times in recent decades in an effort to improve habitat quality for migrating waterfowl. In each case, improved water quality and clear-state characteristics followed lake management, but the lake persistently transitioned back to turbid conditions 5-10 years after treatment (Hanson & Butler 1994; Hansel-Welch et al. 2003; Hobbs et al. 2012). Clear water conditions in Danish and Dutch lakes have also been observed to start deteriorating five years following biomanipulation (Meijer et al. 1994). Similarly, Hanson et al. (in press) showed that 8 shallow lakes in Minnesota did not transition to stable clear-state conditions during a period 2-4 years after management. Returns to turbid conditions following biomanipulation suggest that some shallow lakes may have nutrient levels beyond the "flip up!" threshold in Figure 1 where only the turbid state is possible, or that observed clear states may have little ecological resilience such that small perturbations easily push the lakes back into the basin of attraction of the turbid state. These patterns are also consistent with paleolimnological findings of Ramstack Hobbs et al. (in press) who suggested that some shallow Minnesota lakes never recovered after crossing from clear- to turbid-state ecological regimes.

Failed attempts to manage turbid lakes illustrate that managers need better tools to predict whether their efforts will maintain clear conditions in high quality lakes, whether clear lakes are approaching thresholds and thus are likely to transition to turbid conditions, or if management will succeed in improving highly deteriorated lakes. Theoretical models and empirical studies suggest that we need to more accurately predict implications of changing nutrient levels and biological community features, and how they in turn are influenced by geographic location, land use practices, and lake depth, in order to identify attracting states and to assess the likelihood that lakes will flip to turbid states. Such information will help managers prevent undesirable state shifts in shallow lakes, identify lakes that are good candidates for rehabilitation, and inform future conservation strategies for both lakes and adjacent watershed areas.

OBJECTIVES

The overarching objectives of our research are:

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

2. Develop a modeling framework that allows classification of lake states and estimation of state-dependent relationships between measures of turbidity (Chla) and nutrients (TP) (similar to Figure 1).

3. Extend the model in Objective 2 to allow for temporal dynamics, with state transitions modeled as a function of varying nutrient levels and biological variables (e.g., zooplankton size, fish community types and densities).

4. Using results from Objectives 1-3, develop a tool to compare the relative risk of state transitions for different lakes. Conceptually, this objective can be viewed as attempting to determine where lakes "sit" in Figure 1, and for lakes falling within the region of bistability, assessing the likelihood of the lake transitioning as the result of (possibly management-induced) perturbations (e.g., fish colonization or extirpation).

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

We have completed preliminary models predicting TP levels in shallow Minnesota lakes to address Objective 1. The current best model is a linear mixed effects model for the logarithm of TP with random intercepts and three fixed explanatory variables: percent woodlands and shrubs in the upstream watershed, the logarithm of benthivore mass (average kg per gill net), and average lake depth (see Vitense *et al.* 2014 and data description below). The next steps for this analysis are to investigate the feasibility and usefulness of incorporating soils data from the Soil Survey Geographic Database and/or surface water connectivity information compiled by the MDNR into the model. Additionally, we hope to develop a similar model for TN.

For this interim summary, we focused on Objective 2 in the Methods and Discussion sections below and described anticipated work to be completed for Objectives 3 and 4 during the next two years.

METHODS

Data

We currently have access to two datasets provided by the Minnesota Department of Natural Resources (MDNR) to address the above objectives. First, the MDNR Wildlife Research Unit compiled a "research lakes dataset" (hearafter, research lakes), based on a sampling of 132 lakes surveyed once in July during each of three consecutive years, 2009-2011. Measures of TP, total nitrogen (TN), turbidity, depth, chlorophyll a concentration (hereafter, Chla), as well as relative abundances of SAV, fish (planktivores, benthivores, piscivores), and invertebrates (cladocera and copepods) were obtained in each year. Land cover data in the upstream watershed of each research lake were derived by summarizing manually-delineated cover type polygons that were created using on-screen digitizing procedures in ArcGIS. Color air photos from 2008 were used as the primary interpretive reference for distinguishing cover types, with 2001 National Land Cover Database and 1991 GAP land-cover used to corroborate air photo interpretations as needed. A second similar set of water quality and land cover data was developed from 330 additional lakes using data provided by the MNDNR Shallow Lakes Program. Preliminary modeling has focused on data from research lakes, but program lake data may also be incorporated into the analyses.

State classification and estimation of Chla/TP relationships

To address Objective 2, we developed a framework using Bayesian latent variable regression (BLR) models to identify critical total phosphorus (TP) thresholds, classify attracting lake states, and estimate steady-state relationships between TP and chlorophyll a. We described relationships between the natural logarithms of TP and Chla with linear models with

normally distributed errors and state-dependent intercepts and slopes (equations 1 and 2). In the BLR model, lake state (*S_i*) is a latent variable following a Bernoulli distribution (equation 3). The probability that lake *i* is in the turbid state (denoted by *S*=1) depends on both its TP and SAV values (equation 4). If the lake's TP level falls below the lower TP threshold (π_1 on the log scale), its probability of being turbid is 0; i.e., the lake is classified as clear. If the lake's TP level falls above the upper TP threshold (π_2 on the log scale), its probability of being turbid is 1; i.e., the lake is classified as turbid. If the lake's TP level falls between the thresholds, logistic regression is used to model its probability of being turbid as a function of SAV abundance.

$$\log(Chla_i) \sim N(\mu_i, \sigma^2)$$
 (eq. 1)

$$\mu_{i} = a_{0} + \tau S_{i} + b_{0}(1 - S_{i})\log(TP_{i}) + b_{1}S_{i}\log(TP_{i})$$
(eq. 2)

$$S_i \sim Bern(p_i), S_i = \begin{cases} 0, & \text{if lake } i \text{ is clear} \\ 1, & \text{if lake } i \text{ is turbid} \end{cases}$$
(eq. 3)

$$p_i = P(\text{turbid}) = \begin{cases} 0, & \text{if } \log(TP_i) < \pi_1 \\ \log \text{it}^{-1}(\gamma_0 + \gamma_1 \times SAV_i), & \text{if } \pi_1 \le \log(TP_i) \le \pi_2 \\ 1, & \log(TP_i) \ge \pi_2 \end{cases} \quad (eq. 4)$$

We chose priors that ensured the slopes describing the relationships between Chla and TP were positive and that the probability of a lake being turbid decreased as its abundance of SAV increased. All other priors were weakly informative:

$$\begin{aligned} a_0 &\sim \mathcal{N}(0,100); & \tau \sim \mathcal{N}(0,10); & b_0 \sim Unif(0,10); \ b_1 \sim Unif(0,10) \\ \gamma_0 &\sim \mathcal{N}(0,100); \ -\gamma_1 \sim \ln \mathcal{N}(0.5,1); \ \log(\pi_i) \sim Unif(0,7); \ \sigma \sim Unif(0,20) \end{aligned}$$

Finally, we included a constraint to force the line connecting the turbid line at $\log(TP_i) = \pi_1$ to the clear line at $\log(TP_i) = \pi_2$ to have a negative or flat slope to reflect the "S"-shape of Figure 1:

$$(\tau + b_1 \pi_1 - b_0 \pi_2) / (\pi_1 - \pi_2) \le 0$$

We evaluated the method using data simulated from a stochastic differential equation model, and we also applied the modeling framework to three years of data for 127 shallow lakes from the research lakes dataset. We ran the models in JAGS (Plummer 2003) using the R package 'R2jags' (Su and Yajima 2015) and examined convergence using trace plots and the Gelman-Rubin convergence statistic (Gelman & Rubin 1992). We discarded simulations if the Gelman-Rubin convergence statistic was greater than 1.3 for either slope parameter to strike a balance between computation time, number of simulations with convergence, and adequate representation of the sampling distributions. We classified a lake as turbid (clear) if over half of the sampled states from the Monte Carlo Markov (MCMC) chains were turbid (clear) for that lake. We estimated regression coefficients and TP thresholds using medians and modes of the

posterior distributions, respectively. Finally, we computed 95% credible intervals for the regression coefficients and TP thresholds.

RESULTS

For the simulated datasets, the BLR model provided an accurate approximation to the bifurcation diagram generated by the true simulation model, especially at sample sizes and noise levels similar to the research lakes dataset (Figure 2). Our approach also resulted in more accurate state classifications and threshold estimates compared to *k*-means clustering, with the added advantage of providing estimates of uncertainty for these classifications and thresholds. The BLR model produced reasonable fits to each of the three years of research lakes data (Figure 3, Table 1).

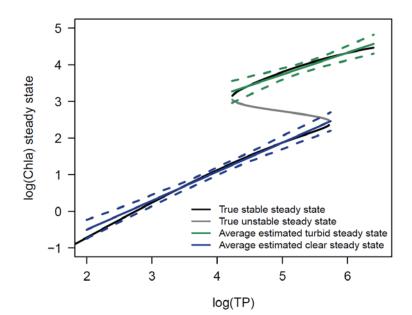


Figure 2. Average BLR estimated stable steady state relationships between log(TP) and log(Chla) across 100 simulated datasets (with n = 100 lakes sampled at "moderate" noise levels). Solid (dashed) green lines depict the average (2.5th, 97.5th quartiles) of the estimated turbid steady state regression lines across the 100 simulations. Solid (dashed) blue lines depict the average (2.5th, 97.5th quartiles) of the estimated clear steady state regression lines across the 100 simulations. Black solid lines (gray line) depict stable (unstable) steady state relationships associated with the simulation model.

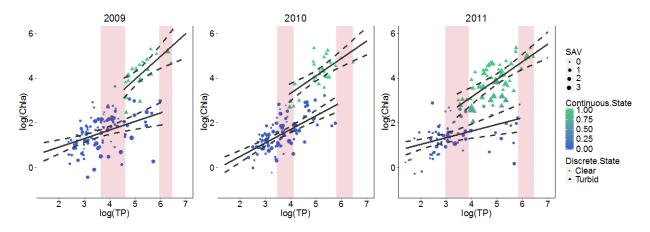


Figure 3. Bayesian latent variable regression (BLR) estimated steady state (SS) relationships between total phosphorus (TP) and chlorophyll *a* (Chla) for three different years of data obtained from the Minnesota Department of Natural Resources. Black solid (dashed) lines represent average (2.5th, 97.5th quantiles) estimated SS relationships across all MCMC samples. Pink bands represent 95% credible intervals for TP thresholds. These thresholds determine which lake states are possible for a specific value of TP (only the clear state is possible to the left of the lower threshold, only the turbid state is possible to the right of the upper threshold, and either state is possible for TP values between the two thresholds). Triangular points represent lakes classified as clear (>50% of MCMC sampled states were clear), and circular points represent lakes classified as turbid (>50% of MCMC sampled states were turbid). The average MCMC sampled state for each lake is shown on a blue to green color gradient (0=clear, 1=turbid). Point size is proportional to submerged aquatic vegetation (SAV, units: average kg/sample).

Table 1. Estimated total phosphorus (TP) thresholds with 95% credible intervals from the fit of Bayesian latent variable regression models to three different years of shallow lake data collected in Minnesota, USA. These thresholds determine which lake states are possible for a specific value of TP (only the clear state is possible to the left of the lower threshold, only the turbid state is possible to the right of the upper threshold, and either state is possible for TP values between the two thresholds).

Year	Lower TP threshold (µg/L)	Upper TP threshold (µg/L)
2009	94.79 (38.44, 101.27)	437.03 (385.12, 641.68)
2010	50.91 (32.21, 62.80)	350.72 (330.02, 633.53)
2011	30.27 (19.44, 48.81)	368.71 (342.21, 635.86)

DISCUSSION

Our TP threshold estimates and state classifications provide important first steps toward a framework that will help managers make decisions about whether and how to treat different shallow lakes. For example, shallow lakes with TP levels below the lower estimated critical TP threshold may be deemed high priority clear lakes, with efforts focused on protecting their pristine conditions. On the other end, lakes with TP levels above the upper TP threshold could be considered low priority, virtually unrecoverable turbid lakes. The internal P loads are so great in these lakes because of historically high inputs that the lakes will persistently (and often quickly) return to turbid water conditions following management actions. Only lakes of unique importance (e.g., critical waterfowl habitat) may be worth the likely perpetual efforts to maintain higher water clarity, reduced algal abundance, and more robust SAV communities. Finally, lakes in between the two thresholds are those for which active management is likely to be most practical. These lakes can be forced from the turbid to clear stable state through biomanipulation of fish stocks, for example, and lakes can be prioritized in terms of their relative resilience and likelihood of successful rehabilitation. Our expectation is that these lakes might often show persistent improvements following lake management, especially if fish communities, water depth, and perhaps external nutrient loading concerns can be addressed.

FUTURE WORK

State transitions in shallow lakes

To address Objective 3, we will incorporate higher trophic levels and temporal components to model how both nutrients and biological variables influence transitions between clear and turbid states in shallow lakes. We propose to use a Hidden Markov Model with a bivariate observation vector for Chla and SAV and transition probabilities modeled as a function of TP, fish, zooplankton, and/or surface water connectivity. We expect that this framework will allow us to compare the relative resilience of different lakes using transition probabilities and/or expected return times.

Management tool

The statistical models we are developing use landscape-level characteristics and within-lake data to predict nutrient levels in shallow lakes, estimate critical nutrient thresholds that determine shallow lake dynamics, and identify important factors that influence the likelihood that lakes will deteriorate, or conversely, that they can be managed for clear conditions. Our goal is to translate the results of the research described above into an interactive decision support tool capable of handling varying levels of information availability that will allow lake managers to:

- 1. Assign shallow lakes to one of three management categories (Figure 4, top panel):
 - a. Protect: highly resilient clear lakes with nutrient levels below the flip down point $(N_1 \text{ in Figure 4})$;
 - b. Actively manage: vulnerable clear lakes that are susceptible to state shifts (lakes with nutrient levels between N_1 and N_2 and algal abundance below A_{crit} in Figure 4) and "opportunistic" turbid lakes that can be restored to clear conditions with appropriate management actions (lakes with nutrient levels between N_1 and N_2 and algal abundance above A_{crit} in Figure 4); and
 - c. High cost: highly resilient turbid lakes with nutrient levels above the flip up point (N₂ in Figure 4) that require costly, drastic actions (e.g., sediment removal) to restore them to clear conditions.
- 2. Prioritize lakes for management based on their current status, the likelihood of

transitioning to or from desired states, and the chance of achieving clear conditions in the long term.

- 3. Explore appropriate management actions that integrate consequences of a lake's current status, its connectivity to other lakes, and regional factors that may reflect historical land-use legacies (e.g., long-term nutrient loading).
- 4. Help identify uncertainties resulting from data gaps for particular lakes, evaluate the benefits of collecting additional information, and prioritize lakes for future sampling.

Ultimately, this project will result in a product that can be used by a diverse audience (local and state governments, lake associations, natural resource agencies) to prioritize shallow lakes for management. We have tentative plans to organize several regional workshops to illustrate the tool in a series of "case study" lakes. Specifically, we plan to explore how the decision tool may be used to prioritize lakes for management attention and to evaluate the likelihood of successful lake manipulations (Figure 4, bottom panel).

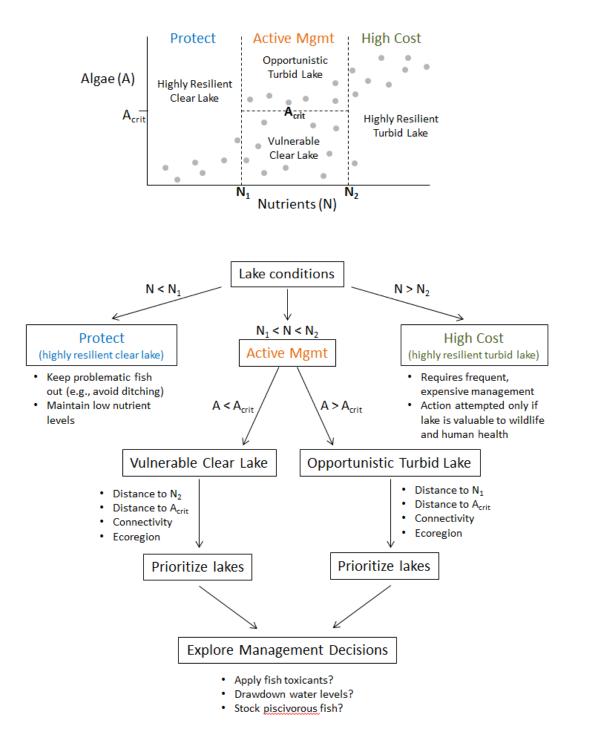


Figure 4. We will translate results of recently developed statistical models into an interactive tool that can be used to assign lakes to one of three management categories (top panel): a) protect (highly resilient clear lakes); b) actively manage (clear lakes that are vulnerable to state shifts and "opportunistic" turbid lakes that can be returned to clear conditions with appropriate management actions); and c) high cost (turbid lakes that are highly resilient and require costly, drastic to return them to clear conditions). We will illustrate, using a series of workshops, how the tool can be used to prioritize lakes for management and explore appropriate management actions (bottom panel).

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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

Some terrestrial and aquatic habitats used by wood ducks (*Aix sponsa*) in Minnesota and the upper midwest have been altered substantially. The potential effect of these changes on the wood duck population has caused some concern among Minnesota Department of Natural Resources (MNDNR) managers. Consequently, we initiated a study in which the overall goal is to develop 2 methodologies to monitor spatiotemporal changes in wood duck habitats used during their pre-breeding to brood-rearing life cycle phases.

First, we will use Light Detection and Ranging (LiDAR) technology to identify terrestrial and aquatic habitats of interest in northern Cass County, Minnesota. The MNDNR Division of Forestry, Resource Assessment Program (RAP) chose this location as a study area in which forest structural characteristics will be examined with high density (8–12 pulses / m²) LiDAR data. The primary goals of RAP's pilot project, entitled "Development of Innovative Cost-Saving Methodology for Forest Inventory", are to (1) develop a stand-based forest inventory (i.e., species composition, detailed vegetation and forest structure, and other characteristics associated with wildlife and ecological suitability), and (2) estimate several individual tree-based metrics (e.g., height, diameter-at-breast-height [DBH], crown size, age, basal area, biomass, volume) associated with wildlife and ecological suitability. The primary interest of this LCCMR-funded pilot project is to characterize forested habitats, but many aquatic habitats will be included within the LiDAR survey area. Our field research will begin during July 2016.

Second, we will use recently developed statistical methods to analyze Forest Inventory and Analysis (FIA) data to ascertain the spatiotemporal changes that have occurred since the 1970s with regard to attributes of forest composition and structure that characterize wood duck nesting habitat in Minnesota.

INTRODUCTION

Some terrestrial and aquatic habitats used by wood duck hens and broods during the pre-nesting, nesting, and brood-rearing life-cycle phases have been altered substantially in Minnesota and the upper midwest. 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

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2002 (Dexter 2002, p. 52), both of which were used by wood duck broods (see McGilvery 1968, Bellrose and Holm 1994). Although the number of potential nesting trees for wood ducks was projected to increase both in Minnesota (Jaakko Pöyry Consulting, Inc. 1994) and the upper midwest (Denton et al. 2012), there has been recent concern among MNDNR managers that harvesting relatively large-DBH trees of economically valuable species (e.g., aspen) in northern Minnesota will reduce the availability of cavity trees frequently used for nesting by some waterfowl species (R. A. Norrgard and D. P. Rave, MNDNR, personal communication).

The ultimate goal of this project is to develop methodologies that can be used to predict the locations of habitats that are likely to be used by wood ducks during their pre-breeding to brood-rearing life cycle phases. These methodologies should have (A) flexibility to identify both forested and non-forested habitat components that occur at different spatial scales, (B) accuracy to reliably quantify spatiotemporal changes in the characteristics (e.g., areal extent) of habitat components, and (C) efficiency to collect habitat data over large spatial scales. It also would be beneficial to develop a method to analyze habitat data that were collected in long-term standardized surveys and that likely will be performed in the future.

Meeting all of these needs with 1 methodology or existing dataset probably is not possible. Consequently, we will develop 2 independent methodologies for obtaining better knowledge regarding spatiotemporal changes in wood duck breeding-habitat components. We will use LiDAR methodology to identify multiple habitat components and to monitor changes in these components from the contemporary period forward. We also will provide better historical context regarding spatiotemporal changes in nesting habitat by analyzing FIA data with a recently developed quantitative method. FIA data can be used to characterize some nesting habitat attributes that have not yet been examined with LiDAR and for a longer time period than LiDAR has been available (i.e., since the 1970s). The development of this methodology also will provide database queries that can be used in future analyses, and an insight of whether the predicted trend in the abundance of tree cavities (i.e., Denton et al. 2012) is accurate.

RAP will conduct a Legislative-Citizen Commission on Minnesota Resources (LCCMR)funded pilot project to associate LiDAR data with ground-survey data of forest habitats. More specifically, the goals of this investigation are to (1) develop a stand-based forest inventory (i.e., species composition, detailed vegetation and forest structure, and other characteristics associated with wildlife and ecological suitability), and (2) estimate several individual tree-based metrics (e.g., height, diameter-at-breast-height [DBH], crown size, age, basal area, biomass, volume) associated with wildlife and ecological suitability. This study will be conducted in 202,342 ha of northern Cass County, and the LiDAR data will be obtained at a density of 8–12 pulses/m² during September-October 2016. Further, RAP will collect ground-level forest-plot data ($n \approx 300$) during the summer of 2017. The focus of RAP's study will be on characterizing forest habitats in this study area, but many wetland sites will be included in the LiDAR survey.

OBJECTIVES

Meeting the primary project goal will require that we (1) identify the location and areal extent of breeding-habitat components in the main study area, (2) validate the algorithms developed to predict the locations of wood duck habitats with independent, empirical data from other sites, and (3) quantify the spatiotemporal trends in potential nesting trees in Minnesota over the long term. Our specific objectives are to:

- Develop and evaluate spatially explicit predictive models of habitat components that are important to breeding wood ducks (i.e., tree species [alternatively deciduous v. coniferous], DBH, crown size, stand type, wetland type, water depth) based on LiDARgenerated metrics or other sources of spatial data (e.g., existing geographic information system [GIS] layers, aerial photographs). This evaluation will include determining the accuracy with which each component can be predicted with LiDAR-cloud data.
- 2) Assess the influence of pulse density on the accuracy of our classification of each habitat component thought to be important to wood ducks.

- 3) Determine the generalizability of the LiDAR method for predicting the locations of habitat components by applying algorithms developed from data collected in the main study area (Cass County, Laurentian mixed 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., other MNDNR LiDAR 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 detected changes in FIA data.

STUDY AREAS

The study will be conducted primarily at the same study site as the LCCMR-funded LiDAR project, in the Laurentian mixed forest ecological province of northern Cass County. To ascertain how well algorithms developed from data collected at the main study site predict vegetation patterns consistent with our understanding of wood duck habitats, we will apply these algorithms to LiDAR-cloud data collected at other sites, including MNDNR fisher and marten study sites (Hoyt Lakes and eastern Cass County, J. Erb, personal communication). High pulse-density LiDAR-cloud data were obtained at the Hoyt Lakes site, and ground-level vegetation data were collected at both sites. Low pulse-density LiDAR-cloud data was obtained statewide for the MNDNR elevation measurement effort.

METHODS

Cavity-Tree Surveys

The overall objectives of tree-cavity surveys are to collect empirical data that can be used to ascertain the proportion of tree species and DBH-classes with suitable nest cavities and estimate the detection probability of this habitat component. Although some variables examined by RAP during field surveys likely influence use by nesting wood ducks, additional survey effort is needed during leaf-off, when tree cavities are most observable.

Wetland Surveys

The overall objectives of the wetlands surveys are to predict the locations of habitat components (i.e., feeding and loafing habitats) based on vegetation structure and classify aquatic habitats from LiDAR-cloud data. We will select and survey wetland plots because the focus of the LCCMR-funded project is to characterize forest habitats only. We will use existing GIS layers (e.g., National Wetland Inventory) to select a stratified random sample of wetland plot locations. We will select plots in both frequently used and infrequently used habitats.

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

Gino J. D'Angelo

SUMMARY OF FINDINGS

Minimizing damage caused by white-tailed deer (Odocoileus virginianus) is an important consideration for managing deer densities in Minnesota. I am conducting an ongoing study, which will be completed in 2016, to assess the effectiveness of localized management of deer (i.e., targeted removal of deer in a limited area) to reduce damage to agricultural crops in southeast Minnesota. The objective of this study is to evaluate the effectiveness of localized management for reducing fine-scale deer abundance and to examine whether damage caused by deer to agricultural crops is reduced on properties where deer densities are lowered. Two field seasons of the study were completed during 2014-2015 in southeast Minnesota. Baited infrared camera surveys were used to estimate deer abundance on focal properties. Yields of corn in fenced and unfenced plots were evaluated to estimate the impacts of browsing by deer. Corn yield loss was seemingly low on most properties, and there was no difference in corn damage between properties where localized management was utilized versus normal sport-hunting. Corn damage could not be explained solely by deer abundance at the property level. However, extra deer harvest opportunities were utilized when requested. Deer management was >2 times as intensive on properties where integrated management was used versus normal sport-hunting. The final field season is being conducted in 2016. The results of this study will provide a basis for improving the framework for future application of localized management in agricultural regions.

INTRODUCTION

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

Minimizing damage caused by deer is an important consideration in managing their populations in Minnesota. In many deer permit areas in Minnesota, deer are managed at or near population goals annually. However, complaints of deer damage from agricultural producers are common. During years 2003-2012, wildlife managers fielded an average of 130 complaints annually about damage caused by deer. Complaints of depredation by deer in Minnesota include consumption of forage stored for livestock, damage to specialty crops (e.g., produce, Christmas trees, nursery stock), row crops (corn [*Zea mays*] and soybeans [*Glycine max*]), alfalfa (*Medicago*

sativa), and forest stands. Deer damage is reported throughout Minnesota, but a distinct cluster of complaints occurs in the southeast region of the state (Nelson and Engel 2013).

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

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

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

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

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

OBJECTIVES

- 1. To evaluate the effects of localized white-tailed deer management techniques-Including shooting permits, and depredation permits–on localized deer densities in southeast Minnesota.
- 2. To quantify the amount of damage caused by white-tailed deer to corn crops relative to localized management in southeast Minnesota.

STUDY AREA

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

Pre-fawn deer densities in these southeast Minnesota averaged 5 deer per km² (Grund 2013), which represents the highest deer densities found in the farmland zone of Minnesota. An average of 1.5 deer per km² was harvested in these southeast Minnesota during 2012, which was nearly twice the statewide average (McInenly 2013).

METHODS

Experimental Design

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

Data Collection

Corn Evaluations-Within each field, I delineated 8 plots, which were stratified into interior (>10 m from the field edge) and edge (0-5 m from the field edge). Each plot included two paired 5-m X 5-m subplots (~6/1000th acre) separated by 5 m and within the same rows of corn. One subplot of each pair was fenced to exclude deer and the other subplot was an unfenced control. Within each pair, the treatment and control were assigned randomly. Square exclosures were constructed with 2-m high heavy-duty plastic mesh attached to four 2.4-m u-posts. Exclosures surrounding subplots were approximately 6 m X 6 m to reduce the effect of fencing on plants within the subplot. Exclosures were installed immediately following planting and herbicide treatment or initial cultivation. When necessary, exclosures were removed for <24 hours to allow farmers to conduct additional field treatments. I evaluated corn crops near the estimated date of plant maturity before senescence (approximately 130 days after planting). Within each subplot I recorded the number of rows, number of plants, and for 30 randomly selected plants. I measured plant height, level of herbivory per plant, and classified the quality of each ear of corn relative to damage caused by deer. I estimated grain yield (total seeds produced per 30 plants) for fenced and unfenced subplots, and calculated the percent corn loss for each fenced and unfenced plot as: ((total seeds in fenced plot minus total seeds in unfenced plot) divided by total seeds in the fenced plot) multiplied by 100. I consulted with the agricultural producer to determine the variety of corn planted in each field.

Deer Abundance Estimates on Focal Properties–To aid in estimating deer abundance and management intensity (i.e., deer harvested per deer available for harvest) on focal properties, I used baited infrared camera surveys to obtain estimates of the abundance of deer at a fine scale in the area of crop fields designated for evaluation. This method of survey was conducted according to previous research by Jacobson et al. (1997) and a pilot study I conducted in southeast Minnesota during 2013 (G. D'Angelo, unpublished data). The abundance of deer in an area can be determined using baited surveys, where bucks can be uniquely identified by antler characteristics and their number used to infer the number of does and fawns visiting repeatedly a bait site. Cameras were placed at a density of one camera per 65 hectares in wooded or brushy habitat immediately adjacent to crop fields. This relatively high density of cameras was intended Page 249 to reduce bias associated with capturing adult bucks at a higher rate at lower camera densities because males have larger home ranges (Jacobson et al. 1997). A bait site was established at each camera location during a 7-day pre-baiting period. During pre-baiting, whole kernel corn and trace mineral salts were placed at each bait site in a quantity sufficient to maintain consistent access by deer 24 hours per day. Following this acclimatization period, an infrared camera was set to record still photographs of deer 24 hours a day at 10-minute intervals during a 14-day survey period. As in the pre-baiting period, bait was provided ad libitum. I generated deer abundance estimates using data pooled from all cameras on a property. Deer abundance estimates were conducted during August. This timing increased the likelihood that: 1) fawns were mobile with their dams and available for survey, 2) antler growth of bucks was sufficient to uniquely identify individuals, 3) deer photographed near crop fields were those that caused damage during the growing season and were available for harvest in the same area, and 4) harvest mortality and disturbance of deer by hunting activities was minimized since the survey preceded deer hunting seasons.

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

RESULTS AND DISCUSSION

The portion of the study described in this summary occurred during April 2014-December 2015. Field work is ongoing and will be completed during 2016. HUNT was used to manage deer on 3 properties and INT was used on 4 properties. In each year, I sampled 112 subplots in corn fields including 56 unfenced subplots and 56 fenced subplots. In 2014, I excluded from analysis 2 pairs of fenced and unfenced subplots (i.e., 4 subplots total) on one property because the growth of corn plants was severely affected by soil erosion. In 2015, I excluded from analysis 2 pairs of fenced and unfenced subplots (i.e., 4 subplots total) on one property because of damage caused by raccoons (*Procyon lotor*).

Deer abundance via infrared camera surveys was similar among HUNT and INT properties during 2014 (Table 1, t = 0.139, df = 5, P = 0.896) and 2015 (t = 0.742, df = 5, P = 0.491). Among HUNT properties, deer abundance was similar between 2014 and 2015 (2014: \bar{x} = 20 deer per camera, SE = 4; 2015: \bar{x} = 25 deer per camera, SE = 2; t = 2.00, df = 2, P = 0.184). Also, among INT properties deer abundance was similar between 2014 and 2015 (2014: \bar{x} = 20 deer per camera, SE = 3; 2015: \bar{x} = 21 deer per camera, SE = 5; t = 0.163, df = 3, P = 0.881).

Agricultural producers on INT properties utilized extra deer harvest opportunities in both years. During 2014, management intensity on INT properties was >2 times the management intensity on HUNT properties (HUNT: $\bar{x} = 0.19$, INT: $\bar{x} = 0.44$, t = -2.393, df = 5, P = 0.097). During 2015, management intensity on INT properties was >3 times the management intensity on HUNT properties (HUNT: $\bar{x} = 0.13$, INT: $\bar{x} = 0.42$, t = 3.504, df = 5, P = 0.017).

Despite increased harvest pressure for deer on INT properties versus HUNT properties during both years, percent corn loss was similar on all properties regardless of the deer management strategy employed during 2014 (HUNT: $\bar{x} = 7\%$, SE = 3%; INT: $\bar{x} = 6\%$, SE = 4%; t = 0.028, df = 52, P = 0.978) and 2015 (HUNT: $\bar{x} = 22\%$, SE = 7%; INT: $\bar{x} = 11\%$, SE = 4%; t = 0.115, df = 52, P = 0.146). Among HUNT properties, percent corn loss was substantially greater in 2015 versus 2014 (2014: $\bar{x} = 7\%$, SE = 3%; 2015: $\bar{x} = 22\%$, SE = 7%; t = 2.120, df = 46, P = 0.039). Among INT properties, percent corn loss was similar between 2014 and 2015 (2014: $\bar{x} = 6\%$, SE = 4%; t = 0.715, df = 58, P = 0.480). For all properties, percent corn loss was relatively low, but slightly higher 2015 (2014: $\bar{x} = 7\%$, SE = 3%; 2015: $\bar{x} = 16\%$, SE = 4%; t = 1.982, df = 106, P = 0.05).

During 2014, there was no difference in percent corn loss between edge and interior plots on HUNT properties (Table 2; t = 0.919, df = 22, P = 0.368) or INT properties (t = 1.160, df = 28, P = 0.256). During 2015 on HUNT properties, percent corn loss on edge plots was on average >4 times that on interior plots (t = 2.194, df = 22, P = 0.039). During 2015 on INT properties, corn damage was similar on edge and interior plots (t = 1.893, df = 28, P = 0.069).

The primary objective of this study was to evaluate the effectiveness of localized management for reducing fine-scale deer abundance and to examine whether damage caused by deer to agricultural crops is reduced on properties where deer densities are lowered. The true effects of integrated deer management conducted during 2014-2016 on deer abundance and crop damage will not be evident until the field season is completed in 2016. Deer abundance was similar among all properties in this study during both years, despite management intensity on INT properties being 2-3 times greater than on HUNT properties. Generally, deer densities in southeast Minnesota were high relative to other regions of the state. Although a higher proportion of deer estimated to be using INT properties were harvested annually, deer on adjacent properties likely filled any voids created by localized management. However, temporary reductions in deer abundance on INT properties may have reduced annual corn losses since deer harvest on these properties occurred throughout the corn growing season. The level of corn damage that may have occurred had localized management not been used is not known. Although not statistically significant, mean percent corn loss on INT properties was half that found on HUNT properties during 2015. Much of this difference could be attributed to plots along the edge of corn fields on HUNT properties experiencing substantial losses of corn. Deer typically cause greater damage on field edges, especially those nearer escape cover (Stewart et al. 2007). Overall, during 2014 and 2015, corn yield loss was seemingly low on most properties. There was no difference in corn damage between properties where localized management was utilized versus normal sporthunting, and the level of corn damage could not be explained by deer abundance at the property level.

However, extra deer harvest opportunities were utilized by landowners when requested. Management was more intensive on INT properties versus HUNT properties. Also, deer were harvested earlier and more continuously throughout the growing season, corn drydown period, and crop harvest seasons on INT properties. Increased deer harvest pressure on INT properties may have prevented corn damage from being worse had additional deer not been harvested. Therefore, extra opportunities to harvest deer should be afforded on properties where landowners consult with MNDNR staff about their concerns for potential deer damage. These concerns are likely legitimate and landowners are basing their concerns on prior experiences and current conditions. The results of this study will provide a basis for improving the framework for future application of localized management in agricultural regions.

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Table 1. Estimates of the abundance of white-tailed deer, management intensity of deer, and corn damage caused by white-tailed deer on 7 privately owned properties in southeast Minnesota, during 2014 and 2015.

		Estimated deer abundance (deer per camera) ²		Management intensity ³		% corn loss ⁴	
Property	Deer management strategy ¹	2014	2015	2014	2015	2014	2015
А	HUNT	26	27	0.16	0.08	7	37
В	HUNT	22	26	0.21	0.23	-1	29
С	HUNT	13	22	0.21	0.07	14	0
D	INT	26	35	0.35	0.28	24	0
Е	INT	21	17	0.39	0.50	-6	23
F	INT	22	18	0.28	0.54	0	11
G	INT	11	12	0.74	0.36	12	16

¹On properties with HUNT management deer harvest was conducted by sport-hunters during the regular season framework. On properties with INT management deer harvest was through integrated methods including by sport-hunters during the regular season framework and using depredation and shooting permits outside of the regular season framework. ²Deer abundance estimated from infrared camera surveys indexed as deer per camera with camera densities of 1 camera per 65 ha on each focal property.3⁴Proportion of the number of deer estimated to be using a property that were harvested.

⁴Negative values indicate higher average yield estimates in unfenced subplots versus subplots fenced to exclude deer.

Table 2. Estimates of corn damage caused by white-tailed deer on edge and interior sampling plots on 7 privately owned properties in southeast Minnesota, during 2014 and 2015. Edge plots were along the field edge and interior plots were >10 m from the field edge.

Year	Deer management strategy ¹	% corn loss						
		Edge			Interior			
		n	\bar{x}	SE	n	\bar{x}	SE	
2014	HUNT	12	0.04	0.04	12	0.09	0.04	
	INT	15	0.11	0.07	15	0.02	0.04	
2015	HUNT	12	0.36	0.12	12	0.08	0.11	
	INT	14	0.19	0.08	16	0.03	0.03	

¹On properties with HUNT management deer harvest was conducted by sport-hunters during the regular season framework. On properties with INT management deer harvest was through integrated methods including by sport-hunters during the regular season framework and using depredation and shooting permits outside of the regular season framework.



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

Nicole Davros

SUMMARY OF FINDINGS

Ring-necked pheasant (*Phasianus colchicus*) responses to the amount of grassland acres in the landscape have been well documented but we lack current information on the individual components of reproductive success (e.g., nest success, brood success, chick survival) that are driving pheasant population dynamics in Minnesota. I radiocollared 67 hens on 2 study sites in southwestern Minnesota to monitor them during nesting and brood-rearing in 2015 and 2016. I collected data on hen survival, nest survival, chick survival, and nest site selection each year. During 2016, I also collected data on brood-rearing habitat selection. Video cameras were used to document nest predation events. Preliminary descriptive findings are described within this report as this study is ongoing and final results are pending. Ultimately, the results will be used to better understand the factors that limit reproductive success of pheasants so that natural resource managers can prioritize their grassland management and land acquisition strategies.

INTRODUCTION

Ring-necked pheasant population dynamics are driven largely by variation in survival rates, and predation is the primary cause of mortality for hens and their young (Peterson et al. 1988, Riley et al. 1998). Predator control efforts can help improve reproductive output over short time periods, but such efforts are economically and ecologically inappropriate at the landscape scale (Chesness et al. 1968, Riley and Schulz 2001). Management of pheasant populations has instead focused mainly on providing abundant nesting cover to minimize the effects of predation and maximize reproductive success to increase populations. As acres enrolled in 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 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) also play important roles in determining susceptibility to nest predation.

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

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

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

OBJECTIVES

My overall objective is to evaluate the relative importance of within-patch diversity (e.g., sites dominated by smooth brome (*Bromus inermis*), warm-season grasses, and high diversity grass-forb mixtures) within Wildlife Management Area (WMA) project areas on pheasant productivity. Specifically, I will

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

Results from a pilot study during the 2015 breeding season allowed me to refine methods and protocols for the study's expansion in 2016, and the 2016 field season was still underway at the time of this report. Therefore, I present preliminary and descriptive results only in this report. A more complete evaluation of results is pending further data analyses.

STUDY AREA

My study is being conducted in the southwest region of Minnesota. Topography ranges from flat to gently rolling. This region is intensively farmed, and corn and soybeans combined account for approximately 75% of the landscape (U.S. Department of Agriculture 2013a, U.S. Department of Agriculture 2013b). Grassland habitats, including those on private land (CRP, Reinvest in Minnesota (RIM), Conservation Reserve Enhancement Program (CREP), and Wetlands Reserve Program (WRP)) and public land (MNDNR Wildlife Management Areas (WMA) and U.S. Fish & Wildlife Service (USFWS) Waterfowl Production Areas (WPA)) account for 6.3% of the landscape in this region (Davros 2016). The southwest region lies within the core of Minnesota's pheasant range, and MNDNR's 2016 August roadside counts indicated 96.0 pheasants per 100 mi driven (Davros 2016).

I selected two WMA project areas as study sites for the 2015 and 2016 field seasons. Each study site is about 9 mi² in size and has extensive amounts of permanently protected habitat. The Lamberton WMA study site (Redwood County) is a large, nearly contiguous WMA complex with >1,100 acres of permanently protected upland and wetland habitats. The Worthington Wells study site (Nobles County) has >1,500 acres of permanently protected habitat that spans multiple WMAs, the Okabena-Ocheda Watershed District, and USFWS lands.

METHODS

My crew and I captured hen pheasants throughout each study site during 3 time periods: 2 February – 15 April 2015, 7 October – 11 November 2015, and 11 January – 29 April 2016 (hereafter referred to as spring 2015, fall 2015, and spring 2016, 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 the right tarsus to the nearest 0.5 mm, banded her with a unique combination of 3 plastic and 1 aluminum leg bands, and fitted her with a 16.0-g necklace-style VHF radiotransmitter with integrated mortality switch (Advanced Telemetry Systems (ATS), Isanti, MN) before release.

We began radiotracking hens 3-5 times per week in late April each year to determine the onset of incubation. We assumed that incubation had begun when the radio signal was projected from the same location for several consecutive days. We flushed hens from their nests between incubation day 5-16 to determine clutch size and floated a subset of eggs to estimate hatch dates (Westerskov 1950, Carroll 1988). We marked the location of nests using a global positioning system (GPS) receiver. We also placed flagging within 5 m of nests to aid relocation efforts. If a hen began making large daily movements prior to being flushed, we assumed her nest failed and we waited for her to re-localize and begin incubating her next nest before attempting to flush her again. We used the homing technique on radiocollars emitting a mortality signal to retrieve the collars. We used the condition of the hen's body (e.g., teeth marks, feathers plucked, intact but frozen) and nearby evidence (e.g., predator scat, den site) to determine a cause of death, if possible.

We placed miniature color video cameras (GE 45231 MicroCam Wired Color Camera, Louisville, KY) at a random subset of nests in an attempt to document nest predation events (Cox et al. 2012). Cameras had infrared light-emitting diodes (LEDs) to allow recording at night and were connected to digital video recorders (Model MDVR14H, Super Circuits, Austin, TX) with SD memory cards and deep-cycle marine batteries housed in waterproof containers >20 m from nests. Video footage was later reviewed in the office and relevant video clips were archived.

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

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

We triangulated hens and their broods 2-3 times daily \geq 3 times per week. We took each bearing from \geq 100 m away to prevent disturbance to the hen and her brood. We then used triangulation software (LOAS, Ecological Software Solutions LLC) to estimate their locations. We continued our monitoring of hens and their broods through the first 4 weeks post-hatching. On day 14 and day 30, we flushed the hen just before sunrise to determine if she still had her brood.

To estimate individual chick survival, we listened for the signal of each radiomarked chick every third day while triangulating its hen. We relied primarily on fluctuation in the chick's signal to determine if it was alive and moving. 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 evidence towards assigning 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 estimated percent canopy cover (Daubenmire 1959) of grasses, forbs, litter, bare ground, woody vegetation, and other (e.g., logs, rocks) using a 0.5 m² sampling quadrat. We estimated percent cover on an overlapping basis using 8 classes: 0%, 0.1-10%, 11-25%, 26-50%, 51-75%, 76-90%, 91-99%, and 100%. We estimated litter depth to the nearest cm and we counted the number of grass and forb species to determine species richness within the quadrat. We also recorded visual obstruction readings (VOR; Robel et al. 1970) in the 4 cardinal directions to determine the vertical density of vegetation to the nearest 0.5 dm around the nest and 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 two random points within 15 m of the nest site.

To evaluate brood habitat selection, we collected vegetation data at 5 estimated brood locations (hereafter, brood points) and 10 random points outside of each brood's biweekly home range until each brood was 4 weeks old. First, we mapped each brood's estimated locations in a Geographic Information System (GIS; ArcMap 10.2, ESRI, Redlands, CA) to estimate their biweekly home range. We defined each biweekly home range as the area bounded within all estimated brood points for that 2-week time period. We placed a 100 m buffer around the home range and used a random point generator in ArcMap to select 10 random points outside of the home range for comparison. We restricted the selection of random points so that they were within the same habitat type (e.g., grassland). Roadsides were considered as available grassland habitat and included in sampling efforts. We then collected vegetation data at each brood point and each random point within 7 days of the biweekly interval. At each brood point, we sampled 1 center point and 3 equidistant points 10 m away to capture the spatial variation of a brood location. We estimated percent canopy cover, litter depth, species richness, VOR, and maximum height of live and dead vegetation using the same methods described above for nest site selection. We repeated this sampling scheme at each of the 10 random points associated

with each brood's biweekly home range. We restricted the sampling of brood habitat selection to field types other than row crops. If a hen and her brood spent more than 50% of their time in a row crop field during the 2-week period of observation, we did not include them in habitat sampling efforts. If more than one hen with a similar-aged brood was using the same habitat patch during the same time period, we only sampled 5 additional random points within that patch. Finally, we did not collect brood habitat data if a hen lost her entire brood within the first week of each 2-week observation window.

RESULTS AND DISCUSSION

We captured 67 hens during the 3 trapping periods across both sites in 2015 and 2016 (Table 1). The baited walk-in traps were not a productive capture technique. I speculate that pheasants were not motivated to use the bait due to mild winter conditions with above-average food availability. Only 3 hens were captured using the walk-in traps (4%) whereas 64 hens (96%) were captured by spotlighting. We ended spotlighting capture efforts at the onset of the breeding season which limited our ability to increase sample size. Although fall 2015 capture efforts did help increase sample size going into the second field season, 10/19 hens (53%) either died or lost their collars prior to the start of the 2016 breeding season. Considering the cost and effort involved in capturing hens via spotlighting relative to the low survival rates of hens through winter, we will not continue fall capture efforts. We will continue to use baited walk-in traps in late winter if weather conditions are severe enough to warrant this method. Winter conditions are considered severe for pheasants when snow is ≥ 6 inches deep and temperatures reach $\leq 0^{\circ} F$.

Due to mortalities (n = 3) and dropped collars (n = 2; unknown causes), we were able to monitor only 15 hens from the end of April through at least one nest attempt during the 2015 breeding season. One unmarked hen was flushed incidentally during field work and her nest was also monitored. We monitored a total of 22 nests from 16 hens. Four nests were abandoned presumably due to our activities; therefore, I excluded them from my analysis of nest success. Twelve of 18 nests hatched successfully (67% apparent nest success). I used the logistic exposure method (Shaffer 2004) to estimate daily survival rates (DSR) of nests using a constant survival model. The 2015 DSR was 0.9406 \pm 0.41 (range: 0.8731-0.9729). Extrapolated to a 23-day incubation period, the nesting success rate was 24.5% in 2015.

We began the 2016 field season with 36 hens on-air at the end of April but early season mortalities and collar drops reduced sample size to 23 hens by the time nesting began. At least 17 hens successfully hatched nests during the season. Fourteen hens successfully hatched their first nest attempts whereas the other hens were forced to re-nest due to flooding or predation events.

We placed video cameras on approximately 40% of nests each year. Hens were extremely tolerant of them and we were able to place the cameras between 1-5 m away from the nest bowl. No predation events were captured on video during 2015 but notable observations included a rooster visiting a hen at her nest during incubation (Figure 1) and a chick appearing on video (Figure 2) prior to the hen leading the brood away from the nest site. Reviewing of video footage from 2016 is in progress and 2 predation events may have been caught on video.

We captured and tagged 10 chicks from 5 broods during summer 2015. As of this report, we captured 23 chicks from 11 broods during summer 2016; however, 3 chicks were too light for transmitters and had to be released unmarked. Two chicks were tagged but their transmitters failed soon after release and they could not be monitored. Therefore, at least 18 chicks were marked with functional radiotags at the time that this report was written.

We monitored 11 of 12 broods during the 2015 field season. One hen lost her chicks immediately after hatching due to a severe thunderstorm. We monitored each brood until they were at least 4 weeks old. By 6 weeks, broods began separating from their hens and they became too difficult to monitor. Two radiomarked chicks were confirmed dead but their cause of death was difficult to determine. Radiotags dropped from 3 chicks but there was no clear evidence whether the chicks died or the sutures failed. Five chicks survived until they were \geq 21 days old at which point battery failure or signal loss likely occurred (Figure 3).

During the 2016 field season, we monitored \geq 17 broods until they were 4 weeks old or until the hen lost her entire brood, whichever came first. Two hens lost their broods within the first week of hatching. Of the 18 chicks that were tagged and monitored in 2016, 8 were either confirmed dead or dropped their tags, 3 had unknown fates, and 7 were monitored beyond 2 weeks old. We recaptured one chick at 2 weeks old and replaced its 0.65 g transmitter with a sutured 1.1-g backpack-style transmitter (ATS, Isanti, MN). Recapturing chicks at this age was easy and seems like a viable option to replace lighter transmitters with heavier ones that have a longer battery life, thereby allowing monitoring of chicks beyond 4 weeks of age in future work.

We collected vegetation data from 19 nest sites in 2015. I calculated means and standard errors (SE) for 2 groups of comparisons: nest sites versus random points (Table 2), and successful versus depredated nests (Table 3). I included all nests regardless of nest fate (e.g., successful, depredated, abandoned, other failure) for the comparison of nest sites versus random points. Hens seemed to use nest sites with slightly less grass cover, lower total species richness, lower grass species richness, shallower litter depth, and reduced VOR compared to random points nearby. Hens that successfully hatched a nest in 2015 appeared to use nest sites with less grass and forb cover but more standing dead vegetation cover, reduced species richness of both grasses and forbs, and reduced VOR. Sample sizes for both of these comparisons are low, however, and more data are needed to make formal comparisons.

Due to time and personnel constraints in 2015, we did not collect vegetation data to examine habitat use by broods. In 2016, we were able to collect brood habitat vegetation data for all broods that hatched and stayed in habitat types other than corn or soybeans. Notably, approximately 50% of hens led their broods into row crop fields each year. Several of these hens took their broods into row crops within the first week of hatching and spent a significant amount of time there.

A third field season is planned for 2017. The final results from this study will relate pheasant survival rates to nesting and brood-rearing habitat selection. Ultimately, the information gained will help managers better understand the factors that may limit pheasant productivity so that they can prioritize their management activities in an era of reduced grassland habitat on the landscape.

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Table 1. Ring-necked pheasant hen captures by season^a and method in southwestern Minnesota, 2015-2016.

	Spring 2015		Fall 2015 ^b		Spring 2016	
	Walk-in trap	Spot-lighting	Walk-in trap	Spot-lighting	Walk-in trap	Spot-lighting
Lamberton	2	8	n/a	8	1	12
Worthington Wells	0	10	n/a	11	0	15

^aSeason dates include: Spring 2015 = 2 February - 15 April 2015; Fall 2015 = 7 October - 11 November 2015; Spring 2016 = 11 January – 29 April 2016.

^bWalk-in traps were not used during fall trapping efforts.

Table 2. 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 southwestern Minnesota. Data are shown for 2015 only.

	Nest Sites (<i>n</i> = 19)		Random Points (n = 19)	
	Mean	SE	Mean	SE
% Canopy cover ^a				
Grasses	3.4	0.30	3.6	0.18
Forbs	0.9	0.22	1.0	0.20
Standing dead	1.6	0.14	1.5	0.14
Species richness				
Total	3.3	0.62	3.6	0.51
Grasses	1.4	0.14	1.7	0.18
Forbs	1.8	0.59	1.8	0.44
Litter depth (cm)	2.8	0.43	3.3	0.46
VOR (dm) ^b	4.9	0.50	5.1	0.42

^aMeans and SEs for canopy cover measurements were transcribed into cover classes for analysis and have not been back-transcribed. Cover classes include: 1 = 0%, 1 = 0.1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-90%, 6 = 91-99%, and 7 = 100%.

^bVOR is the average visual obstruction reading as determined by using a Robel pole.

Table 3. Descriptive statistics for successful versus depredated ring-necked pheasant nests in southwestern Minnesota during summer 2015 only.

	Successful Nests (n = 9)		Depredated Ne	ests (<i>n</i> = 4)
	Mean	SE	Mean	SE
% Canopy cover ^a				
Grasses	3.4	0.44	3.8	0.85
Forbs	0.9	0.20	1.8	0.48
Standing dead	1.7	0.17	1.0	0.00
Species richness				
Total	3.1	0.56	6.8	1.65
Grasses	1.4	0.18	1.8	0.48
Forbs	1.6	0.53	4.8	1.93
Litter depth (cm)	2.3	0.55	2.3	1.41
VOR (dm) ^b	4.3	0.53	6.2	1.95

^aMeans and SEs for canopy cover measurements were transcribed into cover classes for analysis and have not been back-transcribed. Cover classes include: 0 = 0%, 1 = 0.1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-90%, 6 = 91-99%, and 7 = 100%.

^bVOR is the average visual obstruction reading as determined by using a Robel pole.



Figure 1. A ring-necked pheasant rooster visits a hen at her nest during incubation in southwestern Minnesota during May 2015.



Figure 2. A ring-necked pheasant chick appears <1 m from a nest within hours of hatching in June 2015 in southwestern Minnesota. About 2 h later, the video showed the hen leaving the nest with her brood.

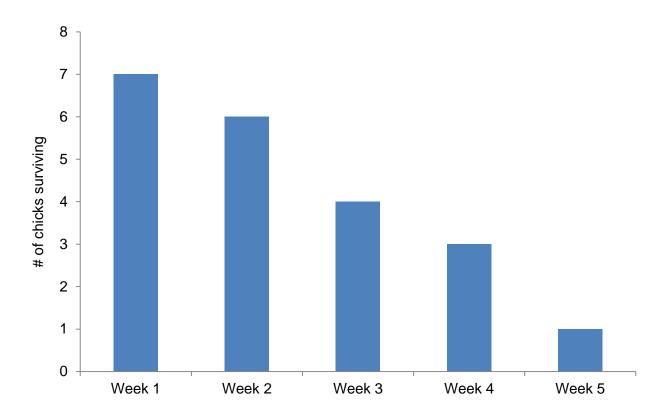


Figure 3. Apparent survival of radiomarked ring-necked pheasant chicks (n = 10) by week during summer 2015 in southwestern Minnesota. Transmitter batteries may have started to fail by the end of week 3.



SEASONAL HOMERANGE MOVEMENTS AND HABITAT SELECTION OF FEMALE ELK IN NORTHWESTERN MINNESOTA

Alicia E. Freeman, Gino J. D'Angelo, Louis Cornicelli, and John D. Krenz

SUMMARY OF FINDINGS

Home range and habitat selection of North American elk (*Cervus elaphus*) have been extensively studied; however, similar research has not been undertaken in Minnesota. Our project will provide foundational ecological data for the only free-ranging population of elk in Minnesota by examining the movement ecology, landscape-level habitat use, and selection of fine-scale habitat features by adult female elk. To accomplish our research objectives, we placed Global Positioning System (GPS) collars on 20 adult female elk in northwestern Minnesota in February 2016. This research summary provides background information, methods, and some preliminary study results.

INTRODUCTION

Elk in North America use a variety of habitats to balance energy intake and expenditures while reducing the risk of predation, and to maintain intraspecific interactions (Ager et al. 2003, Boyce et al. 2003, Anderson et al. 2005a, Beck et al. 2013). Habitat selection can be defined as the use of habitats disproportionate to the availability of those habitats (Arthur et al. 1996, Boyce and McDonald 1999, Boyce et al. 2002, Manly et al. 2002). Cow elk entering parturition isolate themselves and use dense habitats to provide cover for vulnerable calves. Once calves are more mobile, cows aggregate in summer (Hazard 1982, Barbknecht et al. 2011). In summer, elk utilize grasslands and other vegetation with higher nutrient content (Conard and Gipson 2012) and good visibility to enable them to detect predators at greater distances (Anderson et al. 2005b, Pitman et al. 2014). During warmer months and prior to breeding, elk utilize more open areas, but tend to remain close (i.e., <200 m) to cover (Thomas et al. 1988, Baasch et al. 2010). Wintering elk prefer dense thermal cover (e.g., forest and tall shrub) to maximize energy conservation. Agricultural fields and stored forage for livestock are sometimes utilized as food sources for elk and are more likely to be used if near cover (Beck and Peek 2001). In Minnesota, elk occur in an intensively farmed landscape and minimizing elk damage to agriculture is a management priority for the Minnesota Department of Natural Resources (MNDNR); consequently, local biologists work closely with producers to resolve elk-human conflicts (MNDNR 2016).

Elk were numerous throughout Minnesota before European settlement (Hazard 1982). It is believed that by 1900 elk were extirpated due to overharvesting and modifications to the land, especially conversion to agriculture. The first elk reintroduction included 56 elk from Jackson Hole, Wyoming in 1914 into Itasca State Park. Numerous reintroductions using the elk from Itasca State Park were attempted but failed across the northwest Minnesota until 1935 when a herd was successfully established near the town of Grygla (Hazard 1982, MNDNR 2016). Currently, about 130 elk reside in northwestern Minnesota in 4 distinct sub-groups: the Caribou-Vita herd ranging between the Caribou Wildlife Management Area (WMA) and Vita, Manitoba; the Grygla herd, near the cities of Gatzke and Grygla; the Lancaster North group, north of the city of Lancaster and ranging east toward the Skull Lake WMA; and the Lancaster South group, located south of Lancaster and ranging east onto the Percy WMA. Likely, the current Grygla herd is a remnant of the 1935 restocking event, while the other 3 sub-groups formed by elk dispersal from Manitoba Page 268

and North Dakota around the 1980s (Hazard 1982, MNDNR, 2009, 2015). Current management plans are to limit elk sub-group sizes to 65-75 in the Lancaster North and South groups combined, 30-38 elk in the Grygla herd, and 150-200 in the Caribou-Vita herd (MNDNR 2016).

Home range and habitat selection of North American elk (*Cervus elaphus*) have been extensively studied; however, similar research has not been undertaken in Minnesota Our project will provide foundational ecological data for the only free-ranging population of elk in Minnesota by examining the movement ecology, landscape-level habitat use, and selection of fine-scale habitat features by adult female elk in northwestern Minnesota.

OBJECTIVES

The overall objectives for this study are aimed at improving the understanding of movements and habitat use of adult female elk in northwestern Minnesota. Specifically, our objectives are:

- 1. To describe annual and seasonal home ranges and movements of adult female elk. These home ranges will be quantified for individual elk, as well as for each of the 4 sub-groups.
- 2. To characterize the seasonal habitat use of adult female elk at the landscape level. This will be done for the entire year, summer and winter seasons, as well as during the biologically critical seasons (e.g., winter, calving).
- 3. To examine fine-scale habitat structure selected by adult female elk during the growing season (May through July).

METHODS

Study Area

The elk population in northwestern Minnesota is found in an extremely rural area that borders both North Dakota and Manitoba, Canada. The study area is primarily in Kittson, Roseau, and Marshall counties (Figure 1). Three of the 4 sub-groups of elk remain in the US annually (Lancaster North, Lancaster South, and Grygla herds), while the Caribou-Vita sub-group crosses the border with Manitoba freely throughout the year (Caribou-Vita herd). Large Wildlife Management Areas (WMAs), lands owned and managed by The Nature Conservancy, Conservation Reserve Program grasslands, small woodlots, and wetlands comprise much of the natural habitats in the region. Over 50% of the land use in the region is for agricultural purposes which include pasture lands, hay fields, and cultivated crops. The primary crops produced in this region are soy beans, corn, sunflower, wheat, and hay. Other habitat types include: open water, developed land, and barren land (i.e., rocks/sand/clay). There is a small percentage of urban land (0.2%) around the cities of Lancaster, Hallock, and Grygla (Ditmer et al. 2015). The area also has an extensive grid- patterned road network. The region is approximately 330 m above sea level, but there is a lack of elevation gradients as a result of glacial Lake Agassiz, which covered the area 9000-11,700 years ago (Ojakangas and Matsch 1982).

Capture and Handling

We established capture protocols that included handling, biological data collection, limitations on chase times, and health monitoring of restrained animals. Elk were captured from a helicopter (Robinson R-44) using both net guns and darts. One elk was mortally injured during capture with a net gun and the decision was made to switch to immobilizing agents for the remaining captures. Elk captured via net gun were hobbled and blind-folded, whereas elk captured with immobilizing agents were only blindfolded. Tranquilizer darts were loaded with Carfentanil (3.5 mg) and Xylazine (20 mg). Carfentanil was reversed with 350 mg of Naltrexone and Xylazine was reversed with 600 mg of Tolazoline (Stoskopf 2013).

We fitted 20 elk with Global Positioning System (GPS) collars (GPS PLUS Iridium Collars and GPS Vertex Iridium collars, VECTRONIC Aerospace GmbH, Berlin, Germany) and identifying eartags (Orange sheep and goat 2" X 7/8" ear tags, Destron Fearing[™], Dallas, TX). The GPS collars were equipped with a mortality sensor, VHF beacon, and remotely triggered and timed release mechanisms. We monitored rectal temperatures throughout processing, and if temperatures exceeded 105°F, a collar was quickly fitted and the animal was released without further data taken. Blood and hair samples were taken for each elk. The hair will be archived for future genetic studies and blood samples were analyzed for detection of diseases and to evaluate pregnancy status. Pregnancy was determined through testing blood serum for progesterone levels. Elk with progesterone levels >1.0 P4 ng/ml were considered pregnant (Huang et. al, 2000). A wildlife veterinarian was present during all capture operations to prepare tranquilizer darts and to consult the capture crew if an injury occurred. Elk that were darted or those that had visible injuries were administered a dose of antibiotic (10 mL LA 200, Wildlife Pharmaceuticals Inc. Windsor, Colorado).

Monitoring

Capture myopathy was assessed by monitoring the movement patterns of collared elk using hourly locations for 2 weeks post-capture. We censored from analyses locations collected during this time period. To accomplish our objectives, the collars were set to store GPS locations every 4 hours from March through April, every 1 hour from May through July, and returned to the 4-hour schedule from August through March of the following year. After every 11th location was stored, all of the most recent locations were transmitted from the collar to an Iridium satellite. The satellite then transmitted the information to a computer base station at the Carlos Avery MNDNR Office in Forest Lake, MN.

Objective 1: Home Ranges and Movements

We sought to determine the variation in seasonal home ranges and movements of elk in northwestern Minnesota. We used Geographic Information Systems (GIS) software, specifically ArcMAP 10.2, the General Movement Ecology (GME) tool, and the Animal Movements Extension (ArcMET) to estimate home ranges for elk using locations taken every 4 hours. We estimated annual and seasonal home ranges for the 4 groups as well as for each individuals (n = 20). We chose to conduct analyses separately for the 4 sub-groups of elk since they were spatially separated with no apparent interactions among groups (A. Freeman, unpublished data).

We determined home ranges during biologically critical time periods including, preparturition (1 March to 30 April), parturition (1 May to 31 June), post-parturition (1 July to 8 September), breeding (9 September to 30 September), and post-breeding (1 October to 28 or 29 February). We also conducted separate analyses on summer (July through September) and winter (December through February) home ranges for comparison with a simultaneous study occurring in North Dakota.

We used a kernel density estimator to define the 95% home range and created contour lines around the 95% and 50% core areas, and used ad hoc methods to define smoothing parameters. Harmonic means were used to define the geographic centers of the different home ranges. We investigated site fidelity among summer and winter home ranges during 2016 and 2017 by measuring the distance between home range centers. We measured the distances between the center of core areas of the summer and winter seasons and examined the overlap of seasonal home ranges for each elk group to investigate potential migratory behaviors. We also calculated relocation velocities and distances for individual elk for all seasons to detect any differences in movements among seasons.

Objective 2: Landscape-level Habitat Use

We characterized the landscape-level habitat selection of adult female elk during different seasons throughout the year. We define habitat selection as the use of a habitat type disproportionate to its availability in the landscape. Resource Selection Functions (RSF) were used to assess habitat use versus availability. We assessed RSFs across the biologically critical seasons pre-parturition (1 March to 30 April), parturition (1 May to 31 June), post-parturition (1 July to 8 September), breeding (9 September to 30 September), and post-breeding (1 October to 28 or 29 February). We also conducted these analyses for summer (July through September), and winter (December through February) for comparison with a concurrent study being conducted in North Dakota.

To create home ranges for these analyses, we used a 95% Kernel Density Estimator based on 4-hour locations of elk within the aforementioned seasons. Ad hoc methods were used to estimate the smoothing parameters for home ranges. For each season, we created home ranges for each of the 4 sub-groups of elk. Within each home range, we selected GPS collar locations from each individual elk and created random locations equal to the number of elk locations (LaForge et al. 2016, Lehman et al. 2016). Around both the elk and random locations, we created a buffer that was the mean distance traveled between 2 locations calculated from the combined relocations made by all of the elk. The proportion of habitat types within these buffered home ranges was used for the habitat selection calculations. For the RSFs, we generated a global model that pooled all of our variables, including: land cover types, habitat selection, crop type, canopy cover within habitat types, distance to roads, and distance to nearest water source. Backwards selection using the AIC allowed us to find the best fit for the model based on our variables.

Objective 3: Fine-scale Habitat Use

We conducted sampling of fine-scale structural habitat features on areas of elk use, from May through July 2016, to better understand how adult female elk select habitats during the growing season.

We evaluated structural habitat components at locations used by elk from May through July 2016 and at randomly selected points potentially available to elk to examine resource selection in natural habitats during the growing season. We used hourly locations collected during 1 May through 31 July 2016 to delineate sampling areas for 7-day periods (Sunday to Saturday) throughout the 3 months of the growing season.

For each 7-day period, sampling areas for fine-scale habitat evaluations were defined by 4 minimum convex polygons (MCP) (Arthur et al. 1996, Lehman et al. 2016), 1 around each of the different sub-groups of elk (Figures 2, 3), using the hourly locations from all elk in each group. This resulted in 13 sampling areas for each sub-group of elk, for a total of 52 sampling areas for the season. We chose to keep sampling for all of the sub-groups of elk separate since they were spatially separated with no interactions among sub-groups observed to date (A. Freeman, unpublished data).

We generated random points within the sampling area boundaries for each sub-group (i.e., potentially available habitat), using ArcMap 10.2 (Anderson et al. 2005b, 2012, Baasch et al. 2010, Barbknecht et al. 2011, Rumble and Gamo 2011). Using 2011 NLCD and satellite imagery, we censored all known elk locations and random points that occurred within agricultural habitats. We defined strata for sampling as the natural habitat categories where elk locations were found during that time period. For each sub-group we used stratified random sampling to select a sample of known elk locations and random points within the major habitat strata. We repeated this process for each of the elk sub-groups and for each week of sampling. There were 3 random locations selected for each known elk location.

Known elk locations were prioritized for field sampling, and at least one random location was sampled for each known location. If time allowed, additional random locations were sampled. At each sampling location, 2 perpendicular 60-m transects were centered on the coordinates for a known elk location or randomly generated coordinates, and oriented in the cardinal directions (Figure 4). This resulted in 4 30-m sub-transects per sampling point. To determine percent ground Page 271

cover, we sampled within 5 0.25-m² quadrats along each 30-m sub-transect at 5-m intervals on alternating sides of the sub-transect (Anderson et al. 2005b, Barbknecht et al. 2011, Rumble and Gamo 2011, Pitman et al. 2014, Lehman et al. 2016). To estimate canopy cover we used a densitometer at plot center and at points 15 m and 30 m along each sub-transect (Barbknecht et al. 2011, Rumble and Gamo 2011, Pitman et al. 2014). To estimate visual cover, we viewed a Robel pole at 1 m above ground from plot center at 15-m and 30-m distances in the 4 cardinal directions (Nudds 1977, Barbknecht 2008, Pitman et al. 2014, Lehman et al. 2016).

RESULTS

Twenty-one adult female elk were captured 15-19 February 2016 using a net-gun (n=9) and tranquilizer darts (n = 12) fired from a helicopter. One elk was mortality wounded using the net-gun; as a result, the capture operation was halted and a decision was made to switch to immobilizing agents. In all cases, the capture crew adhered to DNR capture protocols, including limiting chase times to under 5 minutes. We collared 3 cows each in the Caribou-Vita and Grygla sub-groups; 9 in the Lancaster North sub-group; and 5 in the Lancaster South sub-group.

As of 30 August 2016, approximately 3,000 locations per elk have been collected, resulting in over >60,000 locations. Based on elk locations collected to date, the primary habitats that elk used were cultivated crops (49%), pasture lands (5%), deciduous forests (22%), woody wetlands (10%), and emergent herbaceous wetlands (10%). Twenty-one percent of elk locations were on WMA and wildlife sanctuary properties owned by The Nature Conservancy. Elk were also observed regularly using an area of land that had undergone prescribed burning 3 weeks prior on the Caribou WMA.

We sent letters by U.S. mail to all properties used by collared elk from February through May to obtain permission to access properties for vegetation sampling. Other means of contact with landowners included phone calls and emails. Of the 455 landowners contacted, 33 letters were returned to sender, and 67 responded. Of the respondents, 48 gave permission, 15 denied access, and 4 were undecided. On parcels where permission was obtained, field sampling of fine-scale habitat features was conducted 16 May-17 August 2016. We sampled habitat structure at 502 locations, including 230 elk locations and 272 random points. Data analysis is ongoing and will be presented in a future report.

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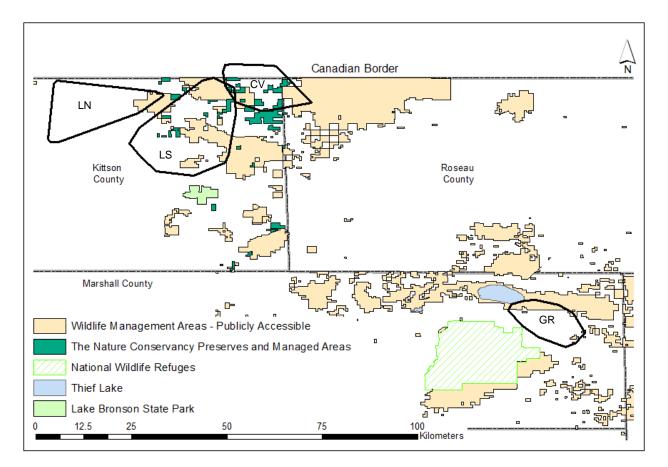


Figure 1. Minimum Convex Polygons (MCPs) around the current known range of the northwestern Minnesota elk population. MCPs were based on locations taken from 20 GPS collars placed on adult female elk in February 2016. The abbreviations for the 4 sub-groups of elk were Caribou-Vita (CV), Grygla (GR), Lancaster North (LN), and Lancaster South (LS).

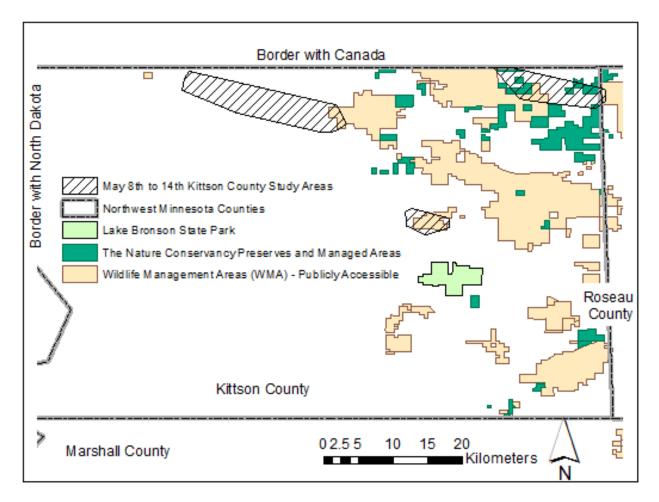


Figure 2. Minimum convex polygons around the Caribou-Vita, Lancaster North, and Lancaster South elk sub-groups designating the sampling areas based on elk locations in northwestern Minnesota taken from 8-14 May 2016.

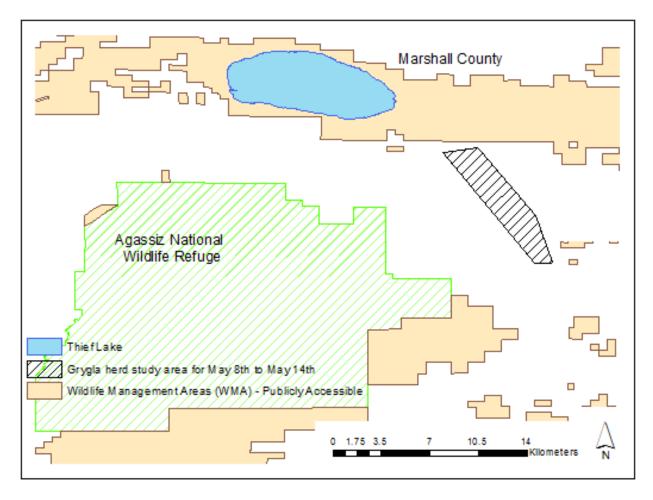


Figure 3. Minimum convex polygon designating the study area around the Grygla sub-group of elk designating the sampling areas based on elk locations in northwestern Minnesota taken from 8-14 May 2016.

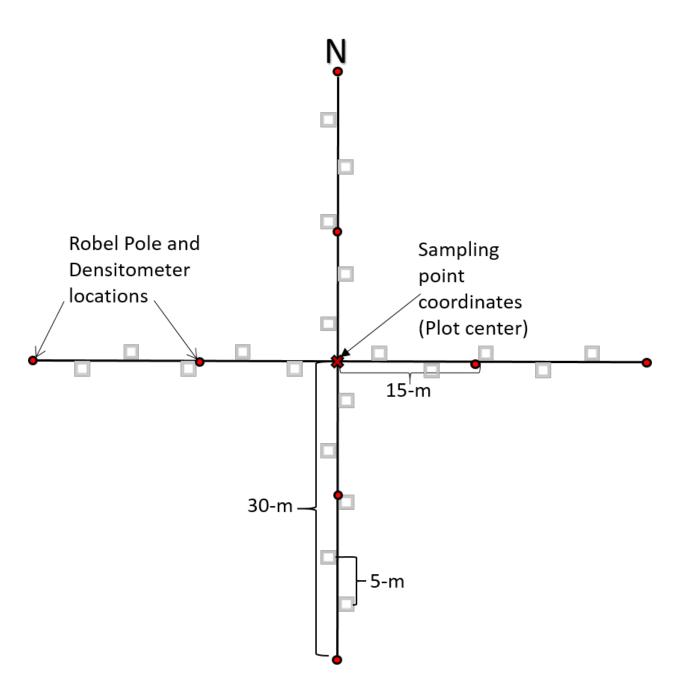


Figure 4. Habitat sampling transect array used to quantify structural vegetation at the locations of GPS-collared adult female elk and at randomly chosen locations in northwestern Minnesota during spring and summer 2016.



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

Ryan G. Tebo and John H. Giudice

SUMMARY OF FINDINGS

Squirrel (Sciurus spp.) hunting is a popular activity in Minnesota, but hunter perceptions of squirrel decline on publicly owned land near the Twin Cities Metropolitan Area of Minnesota (hereafter, metro) has led interested user groups to voice concern over squirrel populations in recent years. To explore these concerns, we are conducting an ongoing study comparing squirrel mortality rates on a heavily-pressured hunted site (Whitewater Wildlife Management Area (WWMA)) to a paired non-hunted site (Whitewater State Park (WSP)) in southeastern Minnesota. From 1 July 2015-19 September 2015 we trapped and radio-collared 51 gray (S. carolinensis) and fox (S. niger) squirrels on WWMA, and 43 gray squirrels on WSP. We failed to find evidence that survival probabilities differed between sites during most of the monitoring period (1 July 2015-12 April 2016). The estimated survival probability for late summer (1 July-18 Sep) on both sites was 0.916 (85% CI: 0.871-0.946) and for the late fall through early spring monitoring interval (1 Nov-12 Apr) was 0.835 (85% CI: 0.752-0.892). However, during the first 6 weeks of the hunting season, 13 squirrels were harvested on the WWMA and the survival probability was estimated to be 0.529 (85% CI: 0.398-0.645). Conversely, survival probability was estimated to be 0.955 (85% CI: 0.929-0.971) on the WSP during the same time period. A second field season is being conducted in 2016, with the intent to capture and radio-collar an additional 100 squirrels on each site. Our current estimates of survival may show a positive bias due to the high proportion of animals with unknown fates (e.g. missing animals, unrecoverable collars in mortality), but with additional data we hope to be able to construct more sophisticated survival models that reflect likely variation in survival probabilities over space, time, and individual covariates.

INTRODUCTION

Small game hunting is a popular recreational activity in Minnesota with approximately 292,000 hunters buying licenses each year since the late 1990s (Dexter 2009, Dexter 2014). Nearly 15% of small game hunters pursue gray and fox squirrels (*Sciurus carolinensis* and *S. niger*, respectively) with an estimated take of 5.1 gray and 3.9 fox squirrels per hunter (Dexter 2009, Dexter 2014). The combined gray and fox squirrel harvest and the number of squirrel hunters has each declined by 13.8% since the late 1990s; however, the combined take per hunter has declined by only 3.3% during this same time (Dexter 2009, Dexter 2014). In an effort to better understand barriers to hunter participation, the Minnesota Department of Natural Resources (MNDNR) conducted a survey of squirrel hunters (Dunbar 2009). More hunters in the metro responded that they believed squirrel populations were declining (51%) as compared to other

hunters statewide (19%). Metro hunters also indicated that they had limited access to private land and heavy hunting pressure existed on publicly-owned land (Dunbar 2009).

Many factors cause squirrel populations to fluctuate naturally (e.g., mast abundance, population density, disease outbreaks; see Barkalow et al. 1970, Nixon et al. 1974, Nixon et al. 1975, Healy and Welsh 1992, Descamps et al. 2009, Vander Haegen et al. 2013), and population dynamics are determined by reproduction, immigration, emigration, and mortality. Although squirrels are considered a game species where hunting mortality is often assumed to be compensatory to natural mortality, previous research suggests hunting mortality can be additive to non-hunting mortality in exploited squirrel populations (Herkert et al. 1992). If so, wildlife managers can alter hunting regulations to adjust the contribution of hunting mortality to overall mortality rates.

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

The MNDNR Section of Wildlife has considered changes to the squirrel season structure in the metro based on the aforementioned survey results. However, because a paucity of information exists with respect to the impacts that the current hunting regulations have on squirrel population growth rates, no changes have been made to date. This study intends to assess the contribution of harvest mortality to overall mortality rates of gray and fox squirrels on public lands in Minnesota. Prior to initiating this large research project, we initiated a pilot study to evaluate squirrel trapping, handling, and tracking methods (see Curtis and Davros 2014).

OBJECTIVE

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

METHODS

Study Area

Our study is being conducted in southeastern Minnesota during 2015-2017. Within the study area, we chose two sites in close proximity to achieve a paired design of one treatment (i.e., hunted) and one control site. Whitewater Wildlife Management Area (WWMA), which receives pressure from squirrel hunters, is the treatment site. Whitewater State Park (WSP) is not open to hunting and is being used as the control site.

Trapping

We trapped gray and fox squirrels during July-September 2015. We used wire box traps (48 x 15 x 15 cm; 2.5 x 1 cm mesh) baited with sunflower seeds, dried corn, peanut butter, and/or black walnuts. Using the MNDNR Forest Inventory layer (where available) or the MNDNR Landcover layer, we selected oak habitat within our sites in ArcGIS 10.2 (ESRI, Redlands, California, USA). We placed a 30-m buffer around all trails and roads. In areas where terrain allowed, we created a grid of points 25 m apart in the area outside the buffer and placed traps at these points. In areas with challenging terrain or insufficient contiguous oak habitat, traps were selectively placed at locations that researchers deemed to be likely squirrel-use areas. We checked traps at least twice per day (i.e., late morning and late evening) to reduce the amount of time squirrels remained in the traps. We closed traps during inclement weather. We also removed traps before the weekends, thereby allowing us to clean and repair traps before changing sites, reducing the risk of theft, and reducing disturbance to WSP and WWMA visitors during peak visitation days.

Upon capture, we identified the sex of each squirrel and determined the reproductive status of females. We weighed squirrels in the trap to the nearest 10 g using a digital hanging scale. We used a modified handling cone to restrain squirrels, which allowed us to handle and radio-collar without sedation (Koprowski 2002). Handling cones were constructed of denim with hook and loop straps to help secure the squirrel and a zipper opening to allow access to the head and neck during collar attachment. Once in the handling cone, a removable plastic funnel was attached around the squirrel's neck to protect handlers from bites during collaring (McCleery et al. 2007). We only collared squirrels weighing ≥300 g in an effort to keep the transmitter weight below 3% of the animal's body mass. Squirrels received a 7-g, 10-g, or 13-g VHF necklace-style radio-collar (models M1525 and M1535, Advanced Telemetry Systems, Isanti, MN) depending on the weight of each individual squirrel. Each transmitter was equipped with an integrated mortality sensor that changed the pulse rate of the signal if an animal did not move for 12 h. Expected battery life was 362 days for the 13-g collar and 302 days for the 7- and 10-g collars. Upon completion of the handling procedure, all squirrels were immediately released. All non-target captures were released immediately. No specimens were collected.

Tracking

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

Data Analysis

Mortality is a continuous time process, but in this study we observed it discretely and incompletely (interval-censored and truncated monitoring data with staggered entry, missing animals, unknown fates, and uneven monitoring intervals). This data-collection design shares many similarities with nest-survival studies (Heisey et al. 2007). Therefore, we used a logisticexposure modeling approach (Shaffer 2004) with interval- or right-censoring of animals with unknown fates (Bunck et al. 1995) to conduct an exploratory analysis of the survival process (based on monitoring data from year 1). We used the glm function in the R programming language (R Core Team 2016) with a user-defined link function (GLM function, Program R) to estimate daily survival rates (DSR) as a function of site, study time (relative to 01 July 2015) or time groups (seasons), and individual covariates (e.g., sex, body mass, collar weight, capture location [x,y], distance from capture location to public roads). For the exploratory analysis we did not attempt to model competing risks (harvest vs. natural mortality) and instead focused on estimating overall survival probabilities on the 2 study sites. However, for analysis purposes, we treated data from the WWMA during the first 6 weeks of the hunting season (19 Sep 2015-31 Oct 2015) separately because all observed harvest mortalities occurred during this period. Thus, we used 2 datasets for survival estimation: 1) WWMA data from the first 6 weeks of the hunting season, and 2) all remaining data from both sites (which generally described the natural mortality process). We used Akaike's Information Criterion (AIC) to select among competing models, including a null model with constant daily survival. For inference, we used the most parsimonious model (fewest model parameters) that was within 2 AIC units of the best-approximating model (lowest AIC value).

An important assumption in this type of analysis is that censoring is independent of fate. Forty-four percent of our study animals had some form of censoring that reflected unknown fates (either interval status or final fate). Further, there were twice as many "missing" animals on the WWMA (vs. the WSP) and half of these animals went missing during the peak of the hunting season. Thus, we were concerned that censoring might not be independent of fate, which can lead to positively biased estimates of survival. We used a replicated imputation algorithm with simulated survival parameters (informed by the data) to construct complete histories for each animal with missing data or unknown fates. We used this procedure to construct 300 replicate monitoring datasets, which we fit to the models selected above (based on the observed data) to produce estimates of daily survival probabilities. We used the estimates from the imputation datasets as our point estimate and the conditional variance formula to compute the variance in the daily survival rate. We then extrapolated both imputed and observed daily survival rates to obtain annual survival probabilities, which we used to quantify potential biases due to censoring. We did not attempt to vary the simulated survival parameters (other than allowing the survival process to vary as a function of binomial variation); thus, differences between imputed and observed annual survival probabilities do not include uncertainty in the simulated survival parameters. However, this approach was sufficient to inform our exploratory analysis and provide guidance on analysis options for the full monitoring dataset (years 2015-2017).

RESULTS AND DISCUSSION

Trapping and Monitoring

We successfully trapped 56 squirrels (49 gray, 7 fox) on WWMA, 51 of which received radio-collars, during the 2015 trapping season. In an effort to distribute captures throughout the WWMA, trapping locations were spread out across the unit (Figure 1). During the entire trapping season on the WWMA (6 July-17 September), the percentage of traps containing a gray or fox squirrel with a body weight sufficient to collar was 1.5% per trap check (3,666 trap checks). Between 6 July-30 August, trap success was very low; only 10 squirrels were captured and 0.34% of traps contained a squirrel per trap check (2,960 trap checks). However, from 31 August-17 September, 46 squirrels were captured and 6.5% of traps contained a squirrel per trap check (706 trap checks). Twenty-six males and 25 females were collared. Based on their lactation status, 8 female squirrels that received collars (32%) were reproductive during the prior breeding season. Weights of collared squirrels on the WWMA ranged from 410-950 g ($\bar{x} = 644$ g).

Forty-nine gray squirrels were captured on WSP, 43 of which received radio-collars during the 2015 trapping season. Trapping locations were spread out across the interior of the WSP (Figure 2). During the entire trapping season on the WSP (4 August-16 September), the percentage of traps containing a gray squirrel with body weight sufficient to collar was 4.5% per trap check (1,079 trap checks). Between 4 August-20 August, trap success was relatively low; only 10 squirrels were captured and 1.7% of traps contained a squirrel per trap check (604 trap checks). However, from 26 August-16 September, 39 squirrels were captured and 8.2% of traps contained a squirrel per trap check (475 trap checks). Twenty-three males and 20 females were collared. Six female squirrels that received collars (30%) had been reproductive during the prior breeding season. Weights of collared squirrels on the WSP ranged from 380-840 g ($\bar{x} = 615$ g).

We attributed the significant improvement in trap success on both the WWMA and WSP to switching the bait type to whole walnuts, rather than peanut butter, corn, and sunflower seeds. All squirrels captured on both sites from 26 August-17 September were captured in traps using whole black walnuts as the bait type. The preference for walnuts may have been in part to a seasonal behavior change, where caching food became the ultimate goal of squirrels once trees began producing the year's walnut crop. Once squirrels began responding to traps baited with walnuts, we ceased using other bait types for the remainder of the trapping season.

We censored 7 of the 51 radio-collared squirrels on the WWMA from the survival study due to capture complications (2) or lack of monitoring data (5). Four of the 44 (9%) remaining squirrels were known to survive until the end of the first monitoring period (12 April 2016). Thirteen (30%) squirrels were harvested by hunters, all within the first 6 weeks of the season (Figure 3). The last observed harvest mortality was 27 October 2015. In addition, 5 (11%) animals were lost to natural mortality events, 5 (11%) dropped their collars, 3 (7%) collars were not recoverable (e.g., in a tree), 1 (2%) collar was recovered but fate could not be determined, and 13 (30%) squirrels could not be relocated. Over half (7) of the 13 missing squirrels on the WWMA disappeared during the first 6 weeks of hunting season, and although it cannot be confirmed, we believe it is very likely some of these squirrels were harvested. In contrast, all 43 radio-collared squirrels on the Whitewater WSP were included in the survival study. Twenty (47%) squirrels were known to survive until 12 April 2016, 7 (16%) squirrels were lost to natural mortality events, 4 (9%) dropped their collars, 5 (12%) collars were not recoverable, 1 (2%) collar was recovered but fate could not be determined, and 6 (14%) Pàge 283 radio-collared squirrels could not be relocated.

Of the 13 recorded harvests on the WWMA, only one was reported despite efforts to inform hunters of the research in progress. Signs placed throughout the WWMA and at popular parking areas asked hunters to report the harvest of any radio-collared squirrels. However, most radiocollars from harvested squirrels were found cut off the animal in the presumed location of harvest, or in parking lots. Other squirrels were found deceased with noticeable gunshot wounds, but were left in the woods or unrecovered by hunters. Ear tags with a call back number will be placed on captured squirrels in the coming field season in hopes of increasing the number of reported harvests.

Nine collars (6 WWMA, 3 WSP) were found to be unrecoverable in trees. Five of these collars continued to fluctuate between normal and mortality signal. Whether this is an indication that the squirrel remained alive and merely slipped its collar, is unknown. However, one collar that had been unrecoverable in a tree for months was found on the ground by researchers in late March, with clear signs that the zip-tie attachment had been chewed through. We will continue to monitor these collars to see if any others can be found at a later date, with the hope that fates may eventually be determined.

Survival Analysis

We failed to find strong evidence that the log odds of survival varied as a function of site (excluding the first 6 weeks of the hunting season on the WWMA), time, or season (again, excluding he first 6 weeks of the hunting season on the WWMA), or the individual covariates we examined. Thus, we used constant-survival models (null models) to make inferences on the survival process, at least for our exploratory analysis. We acknowledge that constant daily survival is unlikely to be true, especially over long periods of time, but given the paucity of monitoring data for some seasons (late summer on both sites and winter-spring on the WWMA) and concern about the amount of censoring in our data, it was a reasonable starting point for the exploratory analysis. Hopefully with additional data we will be able to construct more sophisticated survival models that reflect likely variation in survival probabilities over space, time, and individual covariates (e.g., age, sex, distance). For example we found weak signals that suggested the log odds of harvest decreased with distance from capture site to public roads (i.e., access points for hunters) on the WWMA (Figure 4), and the log odds of survival in the WSP and WWMA (excluding the first 6 weeks of hunting season) was lower during late summer compared to fall, winter, and early spring (Figure 5). The latter might reflect dispersal and greater vulnerability of juvenile squirrels to natural mortality events, but we lacked a sufficient sample size during this time period (due to trapping challenges) to precisely estimate the effect on survival probabilities.

The estimated daily survival rate (DSR) on the WWMA during the first 6 weeks of the hunting season was 0.985 (85% CI: 0.978-0.990). Conversely, the estimated DSR for the WWMA during other times of the year and in the WSP was 0.999 (85% CI: 0.998-0.999). Extrapolating the DSRs to seasonal time intervals resulted in an estimated survival probability of 0.529 (85% CI: 0.398-0.645) for the WWMA during the first 6 weeks of the hunting season, compared to 0.955 (85% CI: 0.929-0.971) for the WSP during the same time period (Figure 6). The estimated survival probability for late summer (1 July 2015-18 Sep 2015) on both sites was 0.916 (85% CI: 0.871-0.946) and for the late fall through early spring monitoring interval (1 Nov-12 Apr) was 0.835 (85% CI: 0.752-0.892).

Not surprisingly, our imputation analysis suggested that censoring was not independent of fate and, thus, survival estimates based on the censored data were positively biased for both sites (Figure 7). The degree of bias should be interpreted cautiously because it was based on one set of survival parameters (assumptions). Nevertheless, it suggests that censoring by itself may not be sufficient to generate accurate estimates of survival given the limitations of our data (e.g., the presence of many animals with unknown fates and the likely lack of independence between censoring and fate). One potential solution is to use a Bayesian integrated survival analysis where the probability of relocation and survival for animals with unknown fates is estimated via a mark-resight approach (Walsh et al. 2015). We will explore this option for analyzing the full monitoring dataset (years 2015-2017). Despite the potential bias in survival estimates caused by unknown fates, it is still clear that squirrels subjected to hunting pressure have much lower survival rates during the first 6 weeks of the season.

All remaining radio-collared squirrels continue to be monitored on both sites. A second trapping season began mid-May 2016, with the intent to capture and radio-collar 100 additional squirrels on each site prior to the beginning of the hunting season in mid-September 2016. Individuals radio-collared in the coming months will be monitored for survival through the spring of 2017. The survival estimates derived from this study will improve our understanding of the extent to which hunter harvest affects overall mortality rates in gray and fox squirrels. Our results will be used by MNDNR's Section of Wildlife to determine if adjustments to squirrel harvest regulations are warranted or if further research on squirrel populations and their habitat is needed.

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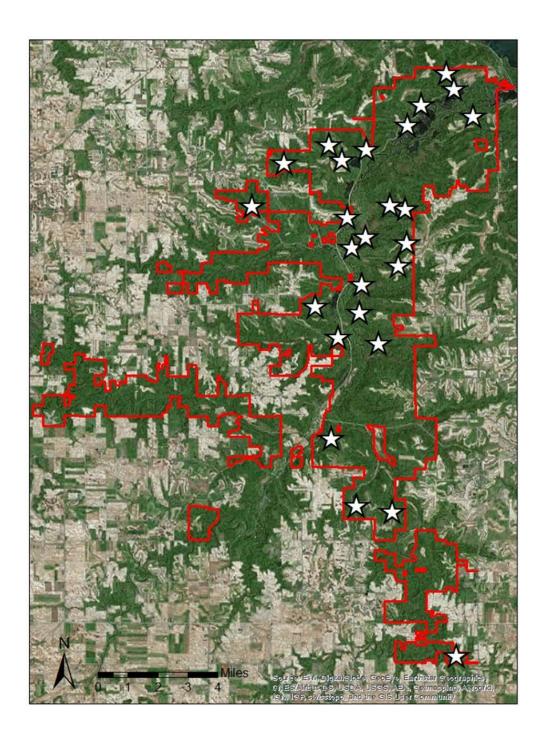


Figure 1. Trapping locations for gray and fox squirrels (as indicated by white stars) used during 6 July-17 September 2015 in Whitewater Wildlife Management Area, Minnesota (outlined in red).

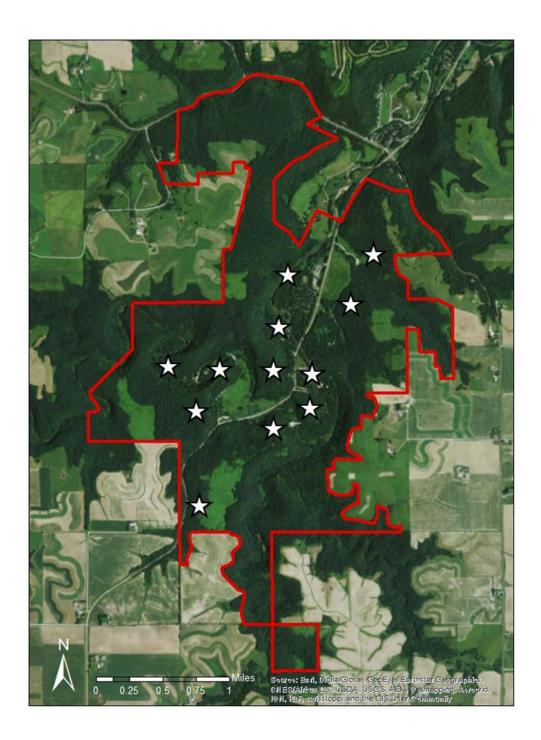
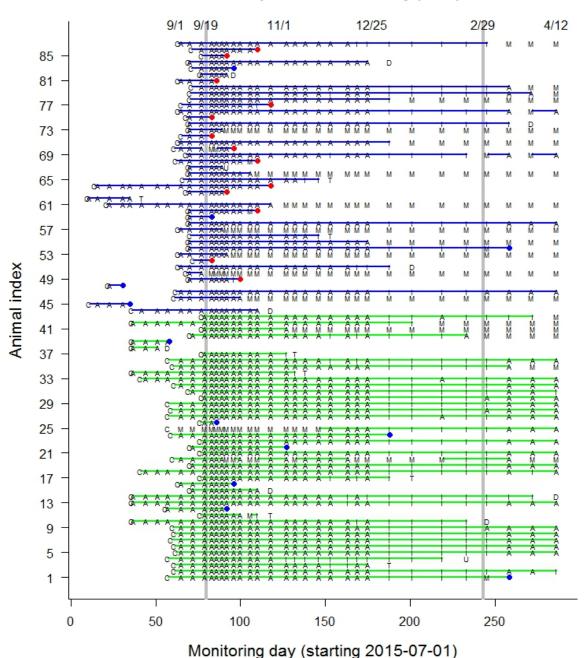
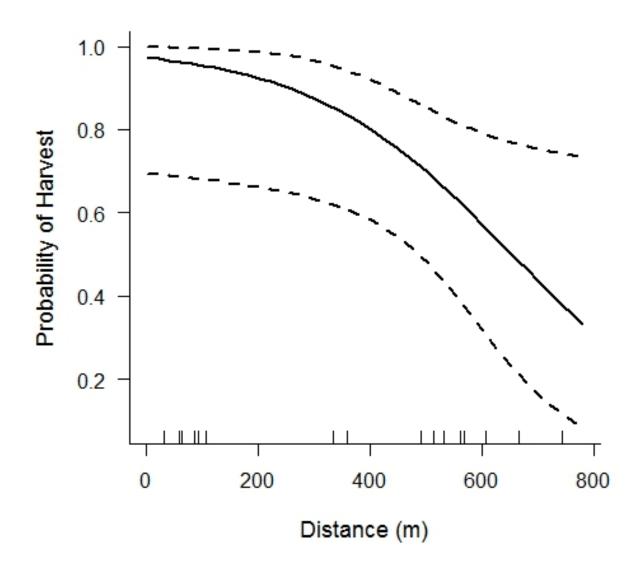


Figure 2. Trapping locations for gray and fox squirrels (as indicated by white stars) used during 4 August-16 September 2015 in Whitewater State Park, Minnesota (outlined in red).



MNDNR Squirrel Survival Study (2015)

Figure 3. Life history diagram of radio-collared gray and fox squirrels at Whitewater Wildlife Management Area, Minnesota (blue lines, n = 44) and Whitewater State Park, Minnesota (green lines, n = 43)-solid lines indicated continued survival across monitoring intervals. Fates of squirrels during the 2015-2016 hunting season are found within the gray vertical lines. Fate codes are as follows: red dot = harvest, blue dot = natural mortality, C = date of capture, A = active alive, I = inactive presumed alive, T = mortality in tree, D = dropped collar, U = undetermined collar loss, M = missing/signal not heard.



WMA 2015

Figure 4. Probability of harvest of gray and fox squirrels (n = 44) in relation to distance from capture site to nearest hunter access point in Whitewater Wildlife Management Area, Minnesota during 19 September 2015-29 February 2016.

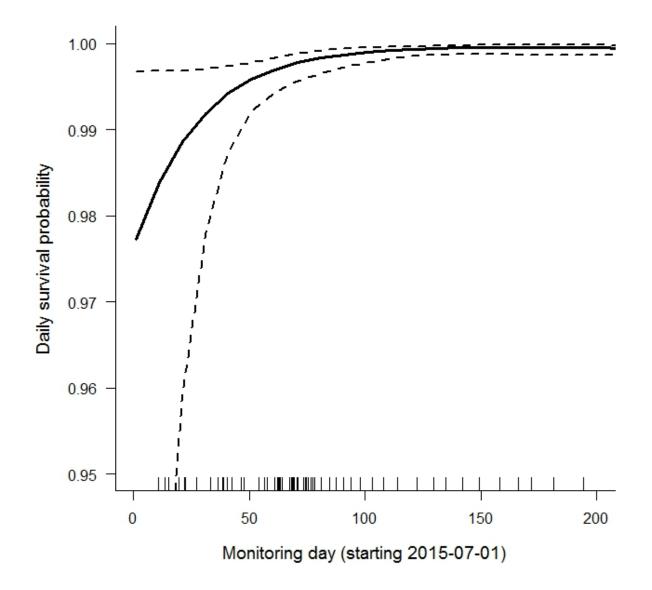


Figure 5. Daily survival probability (excluding the first 6 weeks of hunting season) of gray and fox squirrels in Whitewater Wildlife Management Area, Minnesota (n = 44) and Whitewater State Park, Minnesota (n = 43) during late summer, fall, winter, and early spring 2015-2016.

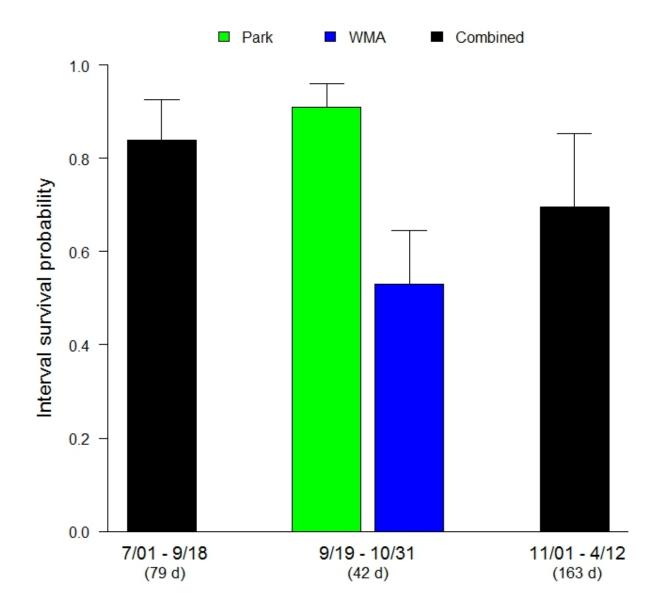


Figure 6. Estimates of combined squirrel survival (black, n = 87) and by site (green = Whitewater State Park, Minnesota (Park, n = 43); blue = Whitewater Wildlife Management Area, Minnesota (WWMA, n = 44) during the pre-hunt time period (1 July 2015-18 September 2015), first 6 weeks of hunting season (19 September 2015-31 October 2015), and winter-spring time period (1 November 2015-12 April 2016).

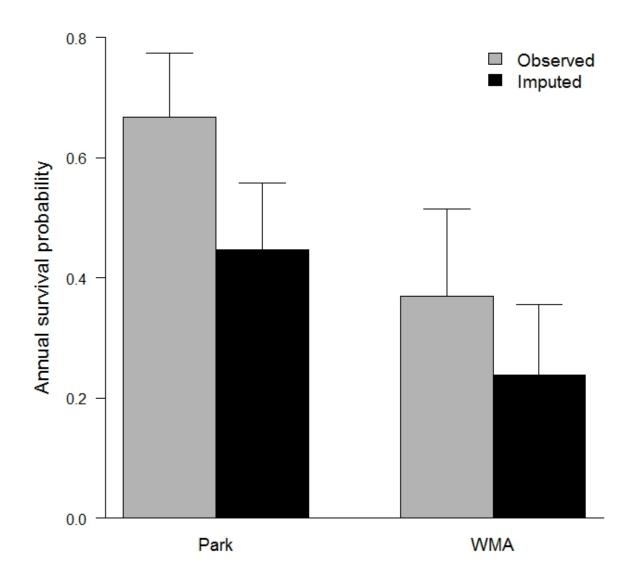


Figure 7. Imputation analysis indicating positive bias of observed annual survival probability due to non-independence of censoring and fate caused by high proportion of gray and fox squirrels with unknown fates. Sixteen of 43 and 22 of 44 squirrels had unknown fates on Whitewater State Park, Minnesota and Whitewater Wildlife Management Area, Minnesota, respectively, during 2015-2016.

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