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

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SUMMARY OF FINDINGS

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

INTRODUCTION

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

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

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

OBJECTIVES

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

METHODS

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

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

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

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

RESULTS/DISCUSSION

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

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

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

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



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



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



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

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SUMMARY OF FINDINGS

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

INTRODUCTION

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

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

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

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

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

OBJECTIVES

The primary objectives of the pilot study were to

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

STUDY AREAS

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

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

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

METHODS

Identifying SPGR Habitat

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

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

Survey Routes and Listening Points

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

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

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

Cantus Call Surveys

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

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

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

Dog and Pellet Surveys

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

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

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

Radio-marking

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

RESULTS

2014

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

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

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

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

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

2015

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

DISCUSSION

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

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



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



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



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



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



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



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



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



ECOLOGY AND POPULATION DYNAMICS OF BLACK BEARS IN MINNESOTA

David L. Garshelis and Brian J. Dirks

SUMMARY OF FINDINGS

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

INTRODUCTION AND STUDY AREAS

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

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

OBJECTIVES

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

METHODS

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

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

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

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

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

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

RESULTS AND DISCUSSION

Radiocollaring and Monitoring

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

Reproduction

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

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

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

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

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

Mortality

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

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

Camera Trap Photos at Dens

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

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

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

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

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

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

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

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

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

^a Due to radiocollar failure, unreported kill, or long-distance movement.

^b Presumed collar failure, but may have been killed and collar destroyed.

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

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

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

^b Only 1 bear died of "old age".

Voor	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	—	_	_
Overall	179	472	2.6	53%	19%

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

^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	n dens i	n northwestern	Minnesota	during	March.	2007-	2015.
							· · J	,		

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

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

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

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

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

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

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

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

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

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

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

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



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



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



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



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


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



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



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



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



HCBOO HYPERFIRE

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



REPRODUCTIVE ECOLOGY OF FISHERS AND AMERICAN MARTENS IN MINNESOTA

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SUMMARY OF FINDINGS

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

INTRODUCTION

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

Legally, fisher and marten were unprotected in Minnesota prior to 1917, after which harvest season length restrictions were implemented. These protections were removed in the mid-1920s, and remained so until all harvest was prohibited in 1929. Seasons remained closed until 1977 for fisher and 1985 for marten, when limited harvests were reinstated. While marten harvest is now legal in approximately the northern 50% of the state, most harvest occurs in counties bordering Canada, particularly in northeast and north-central Minnesota. Fisher harvest occurs in most of the northern 50% of the state, though harvest is comparatively low in extreme northeast Minnesota (Lake and Cook counties). Over the past 10 years, fisher abundance and harvest have been increasing along the southern and western edge of the 'forest zone' where forest historically transitioned to savanna and prairie and is now characterized by linear forest corridors (e.g., streams, rivers) or smaller forest patches interspersed with agriculture. Conversely, fisher abundance appears to have declined significantly over the same period in the core forested areas of north-central and northeast Minnesota. Peak statewide harvest levels have been near 4,000 and 3,500 for marten and fisher, respectively. However, due to apparent 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 guantified 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, etc.). We note that since birth is never observed, and kits may be moved to new dens within days following birth, distinguishing natal dens from maternal dens can rarely be done with certainty. Hence, we pool natal and maternal dens for purposes of general summaries herein.

We are also collecting more detailed information on vegetative characteristics of the site surrounding each den structure, with a goal of developing a biologically meaningful den site selection model using methods and metrics that should be available from existing and periodically collected forest sampling data (e.g., see Zielinski et al. 2006). Following the United States Forest Service's Forest Inventory and Analysis (FIA) protocol, we quantify vegetative characteristics in a 1-acre (120-ft. radius) area surrounding the den structure by sampling in 4 circular subplots. each being 0.04-acre (24-ft. radius) in size. One subplot is centered on the den structure, with the other 3 subplots centered 120 feet from the den at 360°, 120°, and 240°. Within each subplot, 3 24-ft. coarse woody debris sampling transects are established, originating from the subplot center, and oriented at 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 micro-plot situated at the end of the 3 coarse woody debris sampling transects. Details of vegetation sampling methods within each subplot will be outlined in subsequent years as results become available. Herein, we simply note that we are collecting quantitative data on (1) mean DBH and basal area of live trees, overall and by species; (2) percent overhead (angular) canopy; (3) sapling density; (4) understory cover density; (5) density and volume of snags; (6) volume of coarse woody debris; (7) number of stumps, root masses, and slash piles; (8) distance to improved road; and (9) distance to water. Canopy structure will also be categorized based on number and distribution of canopy layers. Lower-resolution LIDAR data (1 ppm) will also be

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

To better understand any observed fluctuations in reproductive parameters, we are also collecting data on factors that may influence reproductive success, including winter severity and prey fluctuations. In each study area, a temperature monitor was placed on the north-facing side of a tree in each of 6 cover types. Each sensor records temperature every 30 minutes from 1 December to 1 June. At approximately 10-day intervals from 1 December to 1 April, we also recorded snow depth and 2 measures of snow compaction at 3 locations along transects situated in each of 6 cover types. Two snow compaction tools were constructed using PVC pipe, one each with an end-cap similar in diameter to a typical marten and fisher track in the snow. Each pipe length was then adjusted to ensure the pipe-specific load (g/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 prey dynamics. Prey-sampling transects (approximately 125 in each study area) consist of 10 sampling locations (2 parallel lines of 5 stations) spaced 20 m apart, with transects distributed in 6 cover types throughout each study area. Transects are generally oriented perpendicular to roads or trails, with the first plot 30 m off the trail. In spring, we count snowshoe hare pellets in a 1-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, a total of 242 martens (115F, 127M) and 114 fishers (65F, 49M) have been radiocollared. Because tooth aging has not yet been completed for all animals, some of which may be only 1 year of age (i.e., not capable of producing kits), we present results only for animals known to be \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 2015 reproductive status assessment is largely complete.

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

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

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

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

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

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

Table 1. Parturition rate and litter size for radiocollared¹ female fishers and martens in Minnesota from 2008 to 2015.

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

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

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

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

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

Area 2 - Fisher only Area 3 - Fisher only

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

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SURVIVAL AND CAUSES OF MORTALITY FOR FISHERS AND MARTENS IN MINNESOTA

John Erb, Pam Coy, and Barry Sampson

SUMMARY OF FINDINGS

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

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

INTRODUCTION

American marten and fisher are native to Minnesota, but reliable documentation of their historic distribution is limited. Undoubtedly, northeastern Minnesota was a stronghold for the marten population, though notable numbers likely occurred in the northern border areas as far Page 83

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 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 year, York (1996) estimated approximately 67% (males) and 22% (females) annual non-harvest mortality for juvenile fishers in Massachusetts. Kelly (1977, in Paragi et al. 1994) reportedly estimated 18% annual mortality of juveniles and 44% annual mortality for adult fisher in New Hampshire. More recently, Koen et al. (2007) estimated annual mortality rate (including harvest mortality) of fishers in Ontario to be 55-67% for males, and 29-37% for females. While non-harvest mortality of adult fishers is often presumed to be 'low', it has not always proven to be the case. Furthermore, there is limited data on which to assess the amount of geographic or temporal variation in non-harvest mortality of fisher.

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

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

OBJECTIVES

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

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

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

STUDY AREA

Marten research is focused on 1 study area located in northeastern Minnesota (Figure 1; Area 1), though 2 martens have been captured and radio-collared in Area 2 (Figure 1). Area 1 (approximately 700 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.

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

We suspect that 2 broad factors may explain the high mortality of kit-rearing female fishers during late-winter and spring: increased activity and increased vulnerability (independent of activity level). Given the potential for negative energy balance during parts of winter, compounded in early spring by the added energy demands of gestation and lactation, female fishers may need to increase activity in spring to meet energy demands. Combined with the need to locate suitable (and multiple) natal or maternal dens, this activity, much of which may be in localized areas near den trees and hence more predictable and detectable to other carnivores, may increase predation risk. Preliminary data from temperature data loggers attached to radiocollars suggests that fishers do spend increasing amounts of time (compared to winter) outside of den and rest sites during late-winter and spring. Secondly, independent of their activity level, fishers may be more vulnerable in spring because concealment cover is diminished (i.e., before 'green-up') and interspecific competition may be high due to potential prey for carnivores being at the low point in Page 87

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

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

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



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



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

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

Table 1. Number and status of fishers and martens radiocollared¹ in Minnesota from 2007 to 2015.

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

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

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

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

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

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



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

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SUMMARY OF FINDINGS

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

INTRODUCTION

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

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

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

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

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

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

OBJECTIVES

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

METHODS

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

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

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

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

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

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

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

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

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

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

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

RESULTS

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

^b Data from McGraw et al. (2014).

2015-05-15 05:00:02

25 Collar 12567 U



Last location: 2015-05-15 01:05:44

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

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



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

and calf bone fragments at the cluster in the middle of the map, which was also the calving site. We estimated the calf was born on 1 May 2015 and was about 2 days old at time of death.



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



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



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



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



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



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

Glenn D. DelGiudice and William J. Severud¹

SUMMARY OF FINDINGS

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

INTRODUCTION

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

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

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

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

OBJECTIVES

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

STUDY AREA

Calf captures were conducted on 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. This is the Northern Superior Upland region (MNDNR 2015), characterized by a variety of wetlands, including bogs, swamps, lakes, and streams; lowland stands of northern white cedar (*Thuja occidentalis*), black spruce (*Picea mariana*), and tamarack (*Larix laricina*); and uplands of balsam fir (*Abies balsamea*) and pines (*Pinus spp.*), often intermixed with trembling aspen (*Populus tremuloides*) and white birch (*Betula papyrifera*).

Most recently, the wolf (*Canis lupus*) density in northern Minnesota was estimated at 3.1 wolves/100 km² (Erb and Sampson 2013). Mean black bear (*Ursus americanus*) density in Bear Management Unit 31 (most of the study area) in 2008 was 23 bears/100 km² (Garshelis and Page 110

Noyce 2011). White-tailed deer share most of the study area with moose, are managed at prefawning densities of <4 deer/km², and are primary prey of wolves (Nelson and Mech 1986, DelGiudice et al. 2002, MNDNR 2011). Black bears and wolves also are a major source of mortality of deer neonates throughout summer (Kunkel and Mech 1994, Carstensen et al. 2009).

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

METHODS

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

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

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

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

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

RESULTS

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

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

Hematological and serum constituent values can range rather widely and serve as reference values when related to individual neonate and birth characteristics (Tables 1 and 2). Red blood cell (RBC) and white blood cell (WBC) counts, packed cell volume (PCV), and hemoglobin (Hgb) concentration were positively related to BM of neonates at capture (Figures 4 and 5). Hind foot lengths of these neonates were not significantly (P > 0.05) related to these hematological characteristics. Values of hematological characteristics were not related to time to death within 30 days of capture/release. Hematological characteristics that differed significantly ($P \le 0.05$) between neonates that died within 32 days of birth versus those that survived beyond 32 days, included segmented neutrophils (81.6 ± 1.3 , range = 78.0-84.1 versus 70.4 ± 3.4 , range = 52.1-78.0% [x 10^3 /uL]), lymphocytes (13.2 ± 2.1 , range = 6.9-19.1 versus 23.3 ± 2.8 , range = 16.1-36.0% [x 10^3 /uL]), and platelets (421 ± 17.6 , range = 364-460 versus 650 ± 93.7 , range = $357-1,065 \times 10^3$ /uL).

The most meaningful associations of serum constituents included significant relationships between age at capture and free thyroxine (T₄; y = -2.157x + 25.460, $r^2 = 0.381$, P = 0.011) and free triiodothyronine (T₃; y = -1.700x + 15.407, $r^2 = 0.260$, P = 0.044), as well as marginally significant relationships of BM to serum urea nitrogen (SUN, y = 1.683x - 10.403, $r^2 = 0.218$, P = 0.068) and cortisol (y = 50.809x - 683.6, $r^2 = 0.207$, P = 0.077) concentrations. Focusing on the 7 neonates that died within 32 days of age yielded a stronger relationship of BM to SUN (y = 3.638x - 39.364, $r^2 = 0.591$, P = 0.043) and to cortisol (y = 128.8x - 1851.1, $r^2 = 0.649$, P = 0.029) than when all were included. Sorbitol dehydrogenase was the only serum characteristic that differed significantly between neonates that died within 32 days of birth (17.0 \pm 1.6, range = 11–23 U/L) versus those that survived beyond 32 days (12.3 + 1.2, range = 6.0–16.0 U/L).

DISCUSSION

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

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

The most notable of neonates dying within 30 days of capture, male singleton no. 520, initially was assigned "capture-related mortality" as the cause of death. This newborn was not abandoned, and died only 4-5 hours (first clue of a preexisting condition) after an uneventful capture and processing. Its dam was the most aggressive towards the handlers, and unlike most, did not flee with their approach (DelGiudice et al. 2015). She remained close during the capture and was with the dead calf when the handler returned to recover it. An immediate necropsy at the Veterinary Diagnostic Laboratory (University of Minnesota-St. Paul), including macroscopic and microscopic examinations, reported an empty gastrointestinal tract (starvation) as the most probable proximate cause of death and no signs of physical injury. Why this calf was not nursing or not being nursed even prior to handling is unknown, but this case is indicative of unknown pre-existing factors that can influence the fate of neonates whose mortalities may appear to be capture-induced due to temporal juxtaposition, but that were actually unrelated. Neonate 520 was 1 of the 2 largest (20.5 kg, 48.0 cm HFL) of the 16 neonates blood-sampled (and 49 total), was near average age (2.6 days) at capture, and had an average rectal temperature (100.9°F). However, physiological assessment by hematological and serum profiles subsequently revealed several anomalies about this animal's unique condition. Briefly, this neonate had the highest RBC, Hgb, and PCV values of all of the neonates, strongly indicative of hemoconcentration due to severe dehydration (Benjamin 1981, DelGiudice et al. 1990b). Additionally, this animal exhibited a leukocytosis, segmented neutrophilia, and lymphopenia, likely reflective of the stress associated with his condition (Benjamin 1981). Most notable from its serum profile, neonate 520 exhibited seriously elevated serum urea nitrogen (SUN, 44 mg/dL) and cortisol (1,068 nmol/L) concentrations, >2-3 and 25 Page 113

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

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

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Characteristics ^a	Mean	n	SE	Min	Max
RBC (x10 ³ /uL)	6.1	13	0.17	5.1	6.8
Hgb (g/dL)	9.6	13	0.3	8.2	11.8
PCV (%)	31.4	13	1.1	25.7	38.9
WBC (x10 ³ /uL)	5.4	13	.37	3.9	7.8
Neutrophil segs % (x10 ³ /uL)	74.4	13	2.5	52.1	84.1
Lymphocytes % (x10³/uL)	20.0	13	2.3	6.9	36.0
Monocytes % (x10^3/uL)	3.1	13	0.81	0.0	9.1
Èosinophils % (x10³/uL)	2.0	13	0.49	0.0	5.9
MCV (fL)	51.8	13	1.2	45.9	57.2
MCH (pg)	15.9	13	0.3	14.1	17.6
MCHC (g/dL)	30.7	13	0.3	28.7	31.8
Platelets (x10 ³ /uL)	550	13	58.3	357	1065
Fibrinogen (g/dL)	0.5	13	0.03	0.3	0.7

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

^aRBC = red blood cells, Hgb = hemoglobin, PCV = packed cell volume, WBC = white blood cells, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, and MCHC = mean corpuscular hemoglobin concentration.

Characteristics ^a	Mean	Ν	SE	Min	Max
SUN (mg/dL)	17.9	16	2.0	10	44
Creatinine (mg/dL)	0.73	16	0.048	0.5	1.2
SUN:Creatinine	24.2	16	1.7	14	36
Ca (mg/dL)	10.1	16	0.18	8.8	11.4
P (mg/dL)	8.9	16	0.30	5.9	11.0
Mg (mg/dL)	1.7	16	0.04	1.5	2.0
Total Protein (g/dL)	4.2	16	0.17	3.3	5.9
Albumin (g/dL)	2.1	16	0.06	1.7	2.6
Globulin (g/dL)	2.1	16	0.17	1.0	3.7
Na (mmol/L)	141	16	0.8	137	150
Chloride (mmol/L)	95.3	16	0.5	92	100
K (mmol/L)	5.1	16	0.11	4.4	5.9
Bicarbonate (mmol/L)	22.3	16	1.0	15.2	32.6
Osmolality	283	16	1.7	276	304
Anion gap	28.5	16	1.0	22.0	39.0
Total Bilirubin (mg/dL)	0.39	16	0.02	0.3	0.7
Alkaline phosphatase	294	16	21.1	170	436
GGT (U/L)	52.0	16	6.3	21	101
Sorbitol dehydrogenase (U/U)	14.6	16	1.1	6	23
Aspartate transferase	63.0	16	4.8	42	110
Creatine kinase (U/L)	135	16	22.2	52	399
Glucose (mg/dL)	108	16	8.7	8	145
TT ₄ (nmol/L)	96	16	6.2	64	156
TT ₃ (nmol/L)	4.2	16	0.16	3.0	5.4
FT ₄ (pmol/L)	19.3	16	1.2	11	27
FT ₃ (pmol/L)	10.6	16	1.12	5.4	21.2
Cortisol (nmol/L)	172	16	60.6	42	1068

Table 2.	Mean (+ SE)	values of serum	characteristics of	f moose neonates	(1.4-6.0 days of	d) in northeastern	Minnesota, 8-	-
12 May 2	2013.							

^aSUN = serum urea nitrogen, Ca = calcium, P = phosphorous, Mg = magnesium, Na = sodium, K = potassium, TT₄ = total thyroxine, TT₃ = total thyronine, FT₄ = free thyroxine, and FT₃ = thyronine.



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



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



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



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



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



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

Glenn D. DelGiudice and William J. Severud¹

SUMMARY OF FINDINGS

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

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

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INTRODUCTION

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

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

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

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

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

OBJECTIVES

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

STUDY AREA

The 6,068-km² study site for this research (Figure 1) is the same as that of the Environmental and Natural Resources Trust Fund (ENRTF)-supported research addressing survival and cause-specific mortality of adult moose in northeastern Minnesota (Butler et al. 2011). This area has been classified as the Northern Superior Upland region (Minnesota Department of Natural Resources [MNDNR] 2007). The MNDNR assesses winter severity (1 Nov–31 May) by a winter severity index (WSI), calculated by accumulating 1 point for each day with a temperature $\leq 0^{\circ}$ F (–17.8° C, temperature-day) and 1 point for each day with snow depth \geq 15 inches (38.1 cm, snow-day), for a potential total of 2 points per day. Maximum WSI values were 120–139, \geq 180, and 100–119 depending upon location for winters 2013, 2014, and 2015, respectively (National Oceanic and Atmospheric Administration [NOAA] 2015). Additional details of the study area are provided elsewhere (Severud et al. 2015).

METHODS

We collected fresh snow-urine specimens of moose during 9 January–26 March 2013– 2015. We conducted snow-urine sampling according to a random design. In winters 2013– 2015 our field team drove (by truck or snowmobile) a route of approximately 201 km (125 miles), which was divided into 4 legs to distribute the sampling throughout the study area (Figure 1). However, the annual teams were not restricted to this route, and could deviate, particularly on foot, as dictated by the presence of fresh moose sign (e.g., tracks, urine specimens, pellets). Each field team used handheld GPS units loaded with several land coverages (R. G. Wright, Minnesota Information Technology @ Minnesota Department of Natural Resources, Section of Wildlife) and a Superior National Forest map (U. S. Forest Service) to navigate in the field. To be able to associate urine chemistry data of randomly collected snow-urines and nutritional assessments with specific temporal periods, sampling generally was conducted within 7 days of a fresh snowfall, but most often within 2–4 days. Upon observing fresh moose sign, the team tracked the individual(s) on foot as necessary until they came to a fresh specimen(s). The objective for the random collections was to sample primarily adult (>1 year old) moose (indicated by track and bed size). This was not particularly challenging, because by this time of the year calves comprised only 13–15% of the population (DelGiudice 2015). We focused primarily on the adult age class to facilitate optimum comparability of data.

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

Perhaps the ultimate value to management of assessments of nutritional status of 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 ($r^2 = 0.970$) population estimates of moose from the annual aerial survey (Figure 6). What is most clear is that although restriction varies among the 3 winters, elevated UN:C values suggest a level of nutritional deprivation not supportive of positive population performance, population stability, or growth. Further, variation in winter conditions is not directly responsible (DelGiudice et al. 1997). We have also documented that variation in the level of severe nutritional restriction of moose is inversely related (r = -0.91) specifically to the variation of natural survival rates of winter and winter-to-summer (Figure 7). Importantly, because these relationships are consistent with association of assessed severe nutritional restriction with the population estimates, it suggests that the current study cohort of GPS-collared moose is indeed representative of the free-ranging population in northeastern Minnesota. While such relationships do not substantiate cause-and-effect, presently they provide the best preliminary Page 127

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

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

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



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



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



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



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



Percent of snow-urine samples with UN:C >3.5 mg:mg

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



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



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



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



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



MONITORING MOVEMENT BEHAVIOR ENHANCES RECOGNITION AND UNDERSTANDING OF CAPTURE-INDUCED ABANDONMENT OF MOOSE NEONATES¹

Glenn D. DelGiudice, William J. Severud², Tyler R. Obermoller, Robert G. Wright³, Thomas A. Enright, and Veronique St-Louis

ABSTRACT

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

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

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USING GPS COLLARS TO DETERMINE PARTURITION AND CAUSE-SPECIFIC MORTALITY OF MOOSE CALVES¹

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ABSTRACT

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

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

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POTENTIAL VERTICAL TRANSMISSION OF WINTER TICKS (*DERMACENTOR ALBIPICTUS*) FROM MOOSE (*ALCES AMERICANUS*) DAMS TO NEONATES¹

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ABSTRACT

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

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