



## Using movement ecology to investigate meningeal worm risk in moose, *Alces alces*

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

Key words: animal movement, brain worm, climate change, disease ecology, disease risk, environmentally transmitted pathogen, white-tailed deer

Human modification of the landscape and climate change alter interspecific interactions, both directly, by driving geographic range shifts, and indirectly, by influencing behavior; these changes can have profound consequences for the ecology of wildlife diseases (Gilman et al. 2010). Although it is challenging to forecast how particular host-parasite systems may be altered by future climatic conditions (Harvell et al. 2002; Tylianakis et al. 2008; Mills et al. 2010; Altizer et al. 2013), climate change can influence range shifts, population density, prevalence of infection, and pathogen loads (Mills et al. 2010). In particular, populations living along the thermal boundary of a species' range likely will face the most significant ecological changes, due to increased physiological stress and the heightened risk of disease from prolonged seasonal transmission (Lafferty 2009; Lenarz et al. 2010).

In North America, the parasitic meningeal worm (*Parelaphostrongylus tenuis*), also known as brain worm, is forecast to shift its range northward further into the boreal forest ecoregion (Pickles et al. 2013). This northward advancement is tracking favorable climatic conditions (Wasel et al. 2003; Lankester 2018) and the range expansion of their definitive hosts, white-tailed deer (*Odocoileus virginianus*, hereafter "deer"—Dawe and Boutin 2016). Deer have taken advantage of anthropogenic alterations to the landscape and a more suitable climate to expand northward, leading to a higher rate of interspecific interactions between deer and other ungulate species (Fagan and Cosner 1999). With favorable weather conditions from the changing climate, numerous geographically widespread generalist species of gastropods that act as intermediate hosts and complete the life cycle of the meningeal worm will continue to expand northward (Lankester 2018). Slug and snail species (namely *Deroceras laeve*, *Discus cronkhitei*, and *Zonitoides* spp., among others) become infected through contact with deer pellets containing L1 larvae, the first developmental stage of the parasite. Deer become infected by meningeal worm by ingesting infected gastropods in which the L3 larvae (final developmental stage) develop (Lankester and Samuel 2007). The worms reproduce within the deer, which then passes L1 larvae in feces to begin the cycle again. The meningeal worm is nonpathogenic in white-tailed deer, but can cause morbidity and mortality in dead-end hosts, including other cervids such as moose, mule deer (*Odocoileus hemionus*), elk (*Cervus canadensis*), and caribou (*Rangifer tarandus*—Trainer 1973) by infecting the nervous system, sometimes resulting in increased mortality risk (e.g., inability to defend against predators; unaware of anthropogenic risks such as vehicles) and neuromotor diseases (Lankester and Samuel 2007).

The geographic expansion of meningeal worm and deer has important management and conservation implications for several northern ungulate species, including caribou (Turgeon et al. 2018), elk (McIntosh et al. 2007), and moose (Wünschmann et al. 2015). In particular, moose population declines have been linked to high rates of meningeal worm infection, and more generally, range overlap with white-tailed deer (Whitlaw and Lankester 1994; Lankester 2010). Meningeal worm and liver fluke (*Fascioloides magna*) infection, in

combination with presumed stress from climate change, has led to population declines and local extirpations of moose populations along the southern extent of moose range (Murray et al. 2006; Lenarz et al. 2009; Weiskopf et al. 2019). While it is unclear if meningeal worm infection can or will have as dramatic demographic consequences in other northern cervids (Turgeon et al. 2018), assessing the drivers of risk for infection is important because of the northward shift of the parasite (Pickles et al. 2013). Climate alterations may particularly impact the life cycle of the meningeal worm, given its reliance on environmental factors and multiple stages of the life cycle. Therefore, it is increasingly important to understand how land cover and environmental conditions affect transmission risk to northern ungulate species.

In northeastern Minnesota, United States, deer populations and ectoparasitism on deer both have experienced long-term increases (D'Angelo and Giudice 2015; Wünschmann et al. 2015; Norton and Giudice 2018). In contrast, long-term winter severity (i.e., snow depth and temperature) and the duration of seasonal snow cover have decreased and are predicted to further decline (Notaro et al. 2014). In addition, the moose population in northeastern Minnesota has declined by more than 50% in the last decade (ArchMiller et al. 2018; DelGiudice 2018). In this population, meningeal worm was the primary cause of mortality in 14% of collared moose, and a contributing factor (e.g., predisposition to predation) in an additional 11% of mortalities (Carstensen et al. 2017). Although meningeal worm infection rates could be as high as 45% in parts of northern Minnesota (Wünschmann et al. 2015), not all infections of moose are fatal (Lankester 2002). Studies have been conducted in Minnesota using bottom-up approaches to estimate the abundance of meningeal worm in the L1 stage (within deer pellets—VanderWaal et al. 2015), the L3 stage (infected gastropods—Cyr et al. 2014), and across the landscape at broad spatial scales (Escobar et al. 2019). However, time of year and the vegetation type (herbaceous, aquatic, shrub) where moose are ingesting the gastropods, or which combination of conditions (deer density, habitat types, conditions) result in the greatest infection risk, are factors that remain unknown.

To better quantify exposure and transmission risks, the field of disease ecology is increasingly incorporating aspects of movement to account for individual heterogeneity in space use and contact rates with potential pathogens at fine scales (Salkeld et al. 2016; Dougherty et al. 2018). Here, we use a top-down approach using habitat selection and spatial overlap of moose movements and their potential risk of exposure to *P. tenuis*, to explore what fine- and broad-scale environmental variables are most associated with increased risk of meningeal worm infection in the northeastern Minnesota moose population.

We tested several competing hypotheses derived from previous studies. We hypothesized that infection risk would be greater for moose with home ranges that contained higher predicted deer space use (Lankester and Peterson 1996; VanderWaal et al. 2015) and density (Lankester 2010), moose with longer exposure to open ground (no snow—Lankester 2010; Lankester 2018), and habitat conditions associated with

greater gastropod abundance or higher rates of gastropod infection in the region (Lankester 2010; Cyr et al. 2014; Cyr 2015; VanderWaal et al. 2015; Escobar et al. 2019). Further, we hypothesized that space use in spring and autumn would be most strongly associated with infection risk, because of the seasonal activity patterns of the commonly infected slugs and snails (Lankester 2018) and because moose forage for herbaceous plants or among the fallen leaves in spring and autumn (as opposed to summer, when moose primarily forage in the shrub layer where gastropods less frequently occur—Cyr 2015). The use of a top-down approach to assess environmental factors of the disease ecology of meningeal worm infection in moose, by linking their movement ecology with known infection status of individuals, provided a different way of discerning seasonally riskier habitats to moose from this indirectly environmentally transmitted pathogen.

## MATERIALS AND METHODS

**Study area.**—Our study area was located in northeastern Minnesota and characterized by a mid-continental climate, with marked differences between the north shore of Lake Superior and the interior. Summers were warm, with peak average daily temperatures occurring in July (July average daily high: 26.3°C interior and 24.6°C near the lakeshore), and a mean total annual precipitation of 75.8 and 80.7 cm for the interior and along Lake Superior, respectively (includes Snow Water Equivalent). Winters were cold and dry; January exhibits the coldest daily low temperatures (averages of -21.5°C in the interior and -17.2°C along the lakeshore), and snow cover typically occurred from November through March (annual snowfall of 164.1 cm in the interior and 228 cm near Lake Superior). Reported climate norms (1981–2010 averages) were measured by the National Oceanic Atmosphere Administration's National Climatic Data Center ([www.ncdc.noaa.gov](http://www.ncdc.noaa.gov)) for the Ely, Minnesota weather station (interior values; 47.9239°N, 91.8586°W), and Wolf Ridge weather station (lakeshore values; 47.4500°N, 91.2167°W).

Northeastern Minnesota marked the transition from the hardwood forests to the Canadian boreal forest. The area has limited geographic relief with only a few rocky outcrops along lakeshores. Aspen (*Populus* spp.), white spruce (*Picea glauca*), and paper birch were the dominant canopy species (~45% of forested land cover) and there were extensive woody wetlands (~33%) primarily composed of alder (*Alnus* spp.), black spruce (*Picea mariana*), and northern white cedar (*Thuja occidentalis*). The remainder of the land cover composition was primarily shrub/scrub (~8%), open water (~6%), and herbaceous wetlands (~4%—Ditmer et al. 2017).

Moose abundance in the region over the previous 5 years (2013–2018), which includes the duration of this study, was estimated at ~4,000 individuals (2018 estimate: 3,030, 90% confidence interval [CI]: 2,320 to 4,140), which equates to an average density of ~0.20 moose/km<sup>2</sup> (DelGiudice 2018). This represents a significant reduction over the past 12 years (2006 abundance: 8,840, 90% CI: 6,790 to 11,910—DelGiudice

2018). American black bears (*Ursus americanus*) and wolves (*Canis lupus*) were the primary predators of moose calves (Severud et al. 2015), and wolves accounted for 30% of the mortalities among GPS-collared adult moose (moose annual survival rate: 81–88%, mean survival rate 86%—Carstensen et al. 2017). There has been no state moose hunting season since 2012 due to the declines in abundance; however, limited harvest by tribal hunters occurs annually. Within the study area, white-tailed deer density increases from north (~2.0 deer/km<sup>2</sup>) to south (~4.0 deer/km<sup>2</sup>—Norton and Giudice 2018), and from west to east (along the shores of Lake Superior; based on deer harvest data within each Minnesota Department of Natural Resources' (MNDNR) Deer Management Unit—see below for further details). However, the population was semimigratory and moved to areas with less snow during the winter season (Nelson 1995; Fieberg et al. 2008; McGraw 2019).

**Moose GPS locations and home range creation.**—During the winters 2013–2015, the MNDNR GPS-collared 173 adult moose as part of a larger study to understand cause-specific mortality in the northeastern Minnesota population (Carstensen et al. 2017). Each moose was outfitted with a GPS-Iridium collar (Vectronic Aerospace GmbH, Berlin, Germany) programmed to record locations every 1.25–4.25 h. Animal capture and handling protocols met American Society of Mammalogists recommended guidelines (Sikes et al. 2016) and were approved by the University of Minnesota Animal Care and Use committee (Protocol Numbers 1309-30915A for deer and 0912A75532 for moose). Collars transmitted a mortality signal if they were stationary for 6 h. Upon receiving a mortality notification, a team of investigators promptly examined the location (65% of mortality signals were investigated ≤ 24 h—Carstensen et al. 2017) to determine the cause of death via a field or lab necropsy (the former was conducted only when the carcass could not be removed from the field). When possible, whole carcasses were brought to the University of Minnesota Veterinary Diagnostic Laboratory for full necropsy and thorough examination of the spinal cord and brain for the presence of *P. tenuis* (Wünschmann et al. 2015). We classified all mortalities with either a 0 (no meningeal worm) or a 1 (meningeal worm present), regardless of the proximate cause of death. We also determined the sex (SEX; see Table 1 for a full list of variables) and exact age (AGE) of each moose. Exact age was determined by cementum annuli (Matson's Laboratory, Manhattan, Montana—Boertje et al. 2015).

We assessed potential factors that were associated with the risk of meningeal worm infection in moose by creating metrics representing ecological and environmental conditions that were spatially explicit and specific to each individual collared moose. To delineate the spatial aspects of moose exposure to hypothesized risk factors, we used GPS locations of collared moose to 1) delineate seasonal home ranges for each moose (dynamic Brownian bridge), and 2) calculate the centroid of each seasonal home range for extracting model variables that do not vary within an animal's home range. We overlaid moose home ranges or centroids on spatially explicit estimates of deer space use, deer density, snow cover duration, habitat types

**Table 1.**—Hypotheses about the risk of meningeal worm infection in moose and the associated model sets, variables names and descriptions, that we considered when modeling the risks among individual GPS-collared moose living in northeastern, MN.

| Hypothesis                           | Model set                            | Variable name | Description                                                                                                         |
|--------------------------------------|--------------------------------------|---------------|---------------------------------------------------------------------------------------------------------------------|
| Individual information <sup>a</sup>  | Broad spatial/moose demo             | HRarea        | Seasonal home range areas (km <sup>2</sup> )                                                                        |
|                                      | Broad spatial/moose demo             | SEX           | Sex of the moose                                                                                                    |
|                                      | Broad spatial/moose demo             | AGE           | Moose age at death                                                                                                  |
| Exposure to open ground <sup>b</sup> | Broad spatial/moose demo             | MINsnow       | First day of year without any snow cover—based on moose GPS locations                                               |
|                                      | Broad spatial/moose demo             | MAXsnow       | Last day of year without any snow cover—based on moose GPS locations                                                |
| Deer density <sup>c</sup>            | Broad spatial/moose demo             | Ycent         | Latitude (UTM15) of seasonal moose home range centroid                                                              |
|                                      | Broad spatial/moose demo             | LSdist        | Nearest Euclidean distance to the shore of Lake Superior                                                            |
|                                      | Deer space use and density estimates | DEERden       | Average deer density (2012–2014) based on # hunter-killed deer/area (km <sup>2</sup> ) of Deer Management Unit Unit |
| Deer space use <sup>d</sup>          | Deer space use and density estimates | DEERuse       | Average space use of deer based on raw % of habitat cover within seasonal moose home range                          |
|                                      | Deer space use and density estimates | DEERrsf       | Predicted average deer resource selection within seasonal moose home range (see <a href="#">McGraw 2019</a> )       |
| Habitat: cover type <sup>e</sup>     | Hab Model 1                          | DEVEL%        | % developed within moose seasonal home range                                                                        |
|                                      | Hab Model 1                          | EMWET%        | % emergent wetlands within moose seasonal home range                                                                |
|                                      | Hab Model 1                          | WOODY%        | % woody wetlands within moose seasonal home range                                                                   |
|                                      | Hab Model 1                          | WATER%        | % open water within moose seasonal home range                                                                       |
|                                      | Hab Model 1                          | GRASS%        | % grassland within moose seasonal home range                                                                        |
|                                      | Hab Model 2                          | CONFRST%      | % conifer within moose seasonal home range                                                                          |
|                                      | Hab Model 2                          | DECIDFRST%    | % deciduous forest within moose seasonal home range                                                                 |
|                                      | Hab Model 2                          | MIXFRST%      | % mixed forest within moose seasonal home range                                                                     |
|                                      | Hab Model 2                          | REGFRST%      | % regenerating forest within moose seasonal home range                                                              |
| Habitat: soil moisture <sup>f</sup>  | Habitat conditions                   | WETforest     | % wet (based on topographic wetness index) forest in moose seasonal home range                                      |
|                                      | Habitat conditions                   | UPconifer%    | % upland (based on topographic wetness index) conifer forest and shrub in moose seasonal home range                 |
|                                      | Habitat conditions                   | WETarea%      | % of moose home range above median wetness values (based on topographic wetness index)                              |
|                                      | Habitat conditions                   | WETregen%     | % wet (based on topographic wetness index) regenerating forest (forestry/fire) in moose seasonal home range         |
|                                      | Habitat conditions                   | WETcover%     | % wet habitat cover (WATER%, EMWET%, WOODY%) types in moose home range                                              |

<sup>a</sup>Individual moose characteristics may influence infection risk ([Ezenwa et al. 2006](#); [Lankester 2010](#)).

<sup>b</sup>More time spent in open ground with access to gastropods increases risk ([Lankester 2010](#)).

<sup>c</sup>Higher deer densities have been associated with higher infection rates ([Karns 1967](#); [Behrend and Witter 1968](#); [Whitlaw and Lankester 1994](#); [Slomke et al. 1995](#); [Peterson et al. 1996](#); [Wasel et al. 2003](#); [Maskey 2008](#); [Lankester 2010](#)).

<sup>d</sup>Areas where deer spend more time should result in more pellets and thus more L1 larvae ([Lankester and Peterson 1996](#); [Vanderwaal et al. 2015](#)).

<sup>e</sup>Habitat types may influence gastropod abundance and gastropod infection rates ([Lankester and Anderson 1968](#); [Cyr et al. 2014](#); [Cyr 2015](#); [Vanderwaal et al. 2015](#)).

<sup>f</sup>Areas within certain habitats with differing moisture levels may influence gastropod abundance and gastropod infection rates ([Prior 1985](#); [Hawkins et al. 1997](#); [Nankervis et al. 2000](#); [Maskey 2008](#); [Lankester 2010](#); [Vanderwaal et al. 2015](#)).

and conditions which were based on our knowledge of moose ecology or results of previous studies ([Table 1](#)).

To process the movement data, we removed errant GPS locations that resulted in maximum velocity of > 30 km per hour, and those associated with a mortality event. To avoid using movements potentially influenced by the impacts of meningeal worm or injuries/illness associated with other end of life events (e.g., injury from predator attack, liver fluke-induced hepatitis), we removed the last 2 months of locations before time of death. We made this simplifying assumption based on an experimental study that showed young moose displayed neurologic conditions as soon as 4 weeks after receiving a low dose of L3 *P. tenuis* larvae ([Lankester 2002](#)) and estimated meningeal worm infection responses in adult moose (~60 days—[Anderson 1964](#)). We believe our 2-month buffer period is conservative

and should avoid including moose with movements modified by infection with meningeal worm.

We standardized and limited the duration of the GPS data used in the analysis to one full year. We made the assumption that infected individuals had become infected during the year prior to the start of the 2-month window before death (i.e., GPS locations from 14 to 2 months prior to death) for better comparison across individuals and because nonmigratory moose populations generally show home range fidelity ([Cederlund and Sand 1992](#)). We assigned locations to each of three seasons: 1) spring = 15 March to 15 June, 2) summer = 15 June to 15 September, and 3) autumn = 15 September to 15 December. We excluded locations associated with winter conditions because gastropods are no longer available for consumption by moose ([Lankester and Samuel 2007](#)).

We used moose GPS fixes to create dynamic Brownian bridge movement models (Kranstauber et al. 2012) for each moose based on the assigned seasons and extracted the 90% contour lines using the “move” package (Kranstauber et al. 2017) in program R (R Core Team 2018) to create seasonal home ranges. We chose the dynamic Brownian bridge approach to delineate home ranges because it is based on the animal’s movement path (i.e., sequential locations) and more realistically creates use distributions based on changes in behavior as measured by movement characteristics compared to traditional estimators (Kranstauber et al. 2012). We used a raster with a resolution of 30 m, a location error of 15 m, and assigned the “time.step” argument within the “brownian.bridge.dyn” function to the time difference between GPS locations (in minutes) divided by 15 as per the default value. If the GPS locations for a season were collected during different years (e.g., GPS data collected in both the autumn of 2013 and 2014), we delineated the seasonal home range separately by year (i.e., a spring home range for Year 1 and a spring home range for Year 2) and combined all resulting 90% contours to create a final seasonal home range.

*Statistical modeling overview.*—We used a multistep process to model meningeal worm infection in moose, regardless of the cause of death, as a function of several explanatory variables representing hypotheses of infection risk that consisted of both seasonal spatial metrics and individual demographic characteristics. Our process, as described in further detail below, was designed to model the same response variable for each moose, whether the moose was infected with meningeal worm or not, as a function of seasonally changing spatial metrics based on the movements of the same collared moose. We assessed which covariates fit each seasonal model best and the overall fit of the top seasonal models. We aimed to determine both the most influential variables on infection, and if moose movements and corresponding exposure to environmental conditions during a specific season(s) were most associated with infection risk. Each model was created using a logistic generalized linear model (GLM) structure with meningeal worm infection of mortalities as the response variable (binary [0/1]) using the “glm” function in the package “lme4” (Bates et al. 2015). All continuous numeric covariates were standardized.

Our approach to model selection incorporated all possible combinations of demographic and seasonal spatial covariates within independent, seasonal model sets (see Table 1 for an overview of models used in the analyses). The most supported covariates from each seasonal model set (variable importance weight  $\geq 0.6$ ) were then retained in a second round of model selection. We used the same models but now only considered combinations of the retained variables to determine our final top models. Relative model fit within model sets was evaluated using a number of metrics described below.

#### *Modeling Process and Fit Assessments*

*Step 1: initial model sets.*—All initial variables were assigned to: 1) deer space use metrics; 2) estimated deer density; 3) broad spatial patterns; 4) habitat types; and 5) habitat

conditions (Table 1). The seasonal GLMs considered additive effects only and used all combinations of explanatory variables within a model set with a maximum of three explanatory variables per model. Prior to evaluating seasonal model sets, we assessed collinearity among the variables by season and included only covariates where the variance inflation factor (VIF) was  $\leq 2$ . For variables related to proportional habitat within a home range, we assigned them to two independent models because of the inherent collinearity among proportional data. We used the R package “MuMIn” (Barton 2019) to calculate the relative variable importance weights within each set of seasonal model sets (model sets were balanced). Any explanatory variable within seasonal model sets that received a relative variable importance weight  $\geq 0.6$  was retained and used in modeling step #2.

*Step 2: retained model sets.*—Retained explanatory variables from Step 1 (variable importance weight  $\geq 0.6$ ) were then combined to create new model sets for each season. The same modeling process was repeated for each seasonally retained model set, with a maximum of three additive explanatory variables included in each model. All covariates were again checked for collinearity (VIF  $\leq 2$ ). We ran all combinations of the GLMs and calculated the relative variable importance weights, and Akaike’s information criterion corrected for small sample size (AICc) values (Burnham and Anderson 2002).

We hypothesized that the relationships between explanatory variables and meningeal worm infection could change based on deer density. Thus, after running the additive models, including the retained variables from Step 1, we explored interactive effects by creating GLMs using the retained variables and broad metrics of deer density (Ycent, LSdist, DEERden—see “Broad spatial scale/deer density” for detailed description).

*Step 3: assessing model fits and effect sizes.*—We ranked the additive and interactive models by season based on AICc (Burnham and Anderson 2002). Among the top models explaining a similar amount of variation (within  $\Delta 2$  AICc of lowest AICc—Arnold 2010), we examined the model fit metrics to determine the best-fitting model(s). We used R package “rms” (Harrell 2018) to assess model fit in all top seasonal models with Nagelkerke  $R^2$  index (assesses predictive ability; values 0–1 and higher values mean greater predictive ability—Nagelkerke 1991), and the C-index (area under the ROC curve [AUC]), an assessment of model specificity and sensitivity which provides a metric to distinguish between infected and noninfected individuals. We also assessed the relationship between individual covariates and meningeal worm within the best-fitting models based on how consistently coefficients were among top models within season, 95% CI overlap with zero, and a visual assessment of effects plots with raw data points overlaid on them. To create these effect plots, we used the “effects” package (Fox 2003) in program R and generated model-based predictions of meningeal worm infection risk with associated 95% CIs. When generating plots, we used the single best-supported model among top models (based on model metrics) where the covariate was included and plotted points representing the values of the associated explanatory variable for each moose and meningeal worm infection on the x-axis.

*Snow cover duration.*—We uploaded all seasonal home range locations to MoveBank ([movebank.org](http://movebank.org)) and extracted values for snow cover using the ENV-Data system (Dodge et al. 2013) from the MODIS Snow Terra Snow 500m 8d Snow Cover data (8-day, 500-m resolution). We recorded the first day of the year without any recorded snow cover (MINsnow), as well as the last day of the year with any recorded snow cover (MAXsnow) to account for late season snowfall after previous snow cover had melted for each moose during the year of data examined.

*Habitat types.*—We used a multitemporal composite of Landsat 8 imagery and LiDAR data (<https://gisdata.mn.gov/dataset/base-landcover-minnesota>) and created the following variables based on the percent coverage of each cover type within the seasonal home ranges: water (WATER%), developed (DEVEL%), emergent wetlands (EMWET%), woody wetlands (WOODY%), conifer forest (CONFRST%), deciduous forest (DECIDFRST%), mixed forest (MIXFRST%), grasslands (GRASS%), and regenerating forest (REGFRST%). Most regenerating forest in this area of Minnesota is the result of forestry activities and, to a lesser extent, forest fires. Finally, we combined WATER%, EMWET%, and WOODY%, to create a variable representing total cover of wet habitat types (WETcover) because studies have found greater abundances of gastropods in wet environments (Lankester and Anderson 1968), including in our study area (Cyr 2015; Table 1).

*Habitat conditions.*—We hypothesized that moose exposed to relatively wetter habitat conditions may experience a higher rate of meningeal worm infection. While cooler and more moist conditions have been associated with L1 larval survival (Nankervis et al. 2000; Lankester 2010), other studies have associated upland conifer habitats with L1 presence in deer feces (VanderWaal et al. 2015). To estimate several hydrological conditions for our region, we processed a 30-m resolution digital elevation model (DEM) with hydrology functions in ArcGIS 10.5 (ESRI 2018) to produce a normalized topographic wetness index (TWI—Sørensen et al. 2006). We first calculated flow direction (“flowdirection” function), and flow accumulation (“flowaccumulation” function). Using the DEM layer, we took the tangent of any cell with a positive slope (converted to radians) and assigned “0.01” to negative values. The TWI was calculated as the log of the product of the flow from the accumulation layer (+1) and the raster cell size, divided by the tangent of the slope layer. We standardized the TWI values in the study area and calculated the percentage of each moose’s seasonal home range that contained a TWI > 0.5 (above the mean TWI value; WETarea). We used the same TWI threshold and extracted cells with any type of forest (conifer, deciduous, mixed; WETforest), and recently disturbed areas (WETregen) to test whether wet forests were associated with meningeal worm risk (Cyr 2015). We also calculated the percent area within each seasonal home range that contained either shrub or conifer forest habitat and was associated with a TWI wetness value < 0.5 (UPconifer) based on the findings of VanderWaal et al (2015).

*Broad spatial scale/deer density.*—To quantify estimates of deer density within each moose seasonal home range we

calculated: 1) the total area (km<sup>2</sup>; HRarea); 2) the latitude associated with the centroid (Ycent) of each seasonal home range (because deer density decreases from south to north in our study area—Norton and Giudice 2018); and 3) the Euclidean distance between the shore of Lake Superior and each seasonal home range centroid (LSdist).

We used reported deer harvest, delineated by deer permit areas and divided by the size (km<sup>2</sup>) of each deer permit area to create indices of deer density (provided by the MNDNR; available <https://gisdata.mn.gov/dataset/env-mn-deer-harvest>). Within 48 h, deer hunters in Minnesota were required to register their harvest and report the sex (male or female), age (fawn or adult), and deer permit area, where the animal was killed. Legal harvest is the largest source of mortality for deer in the study area (Grovenburg et al. 2011) although wolf predation and occasional severe winters have had significant influences in some years (Nelson and Mech 1991). The hunter-reported harvest data are used by the MNDNR for modeling population trends and management decisions (Norton and Giudice 2018). We used the deer harvest data from the years 2012–2014 to create the average (DEERden) deer density estimates per permit area. We overlaid and extracted the DEERden values based on the centroid coordinates of each moose seasonal home range.

*Deer space use.*—We used deer GPS locations ( $n = 33$  collared deer) to estimate deer habitat use and deer habitat selection. Habitat selection estimates were derived from resource selection functions (RSFs) developed by McGraw (2019) to assess the relative resource selection of deer in our study area (see McGraw 2019 for model values). In contrast, deer space use was solely based on the proportion of time spent in the major land cover types. See [Supplementary Data SD1](#) for seasonal means and 95% CIs. Deer were captured and fit with GPS collars (Vectronic Aerospace GmbH, Berlin, Germany) in two locations within the study area (Ely, Minnesota site latitude/longitude: 47.88/–91.99; Isabella site latitude/longitude: 47.62/–91.42; see McGraw 2019 for complete capture and collar details). We made the simplifying assumption that deer space use from these study sites was representative of deer throughout the study area. We estimated deer space use by averaging the percentages of land cover used by individual deer and creating a population mean for each land cover category.

In the first method, we assigned deer GPS locations (collected every 2 h) to the same seasons used in the moose analysis and associated each location with a land cover type (same categories as the multitemporal composite used for moose analyses). We then averaged the frequency of the GPS locations that overlapped with each land cover type by individual deer, and took the average of the individual means from each deer for each cover type (individual deer were the sampling unit—Murtaugh 2007). We assigned each raster cell a value based on the land cover type and the associated deer average use (DEERuse). The moose seasonal home ranges were overlaid on the raster layer and we calculated the mean of extracted DEERuse values.

We then used results from McGraw (2019) to predict the relative probability of use of resource units using the same

GPS-collared deer. The seasonal RSFs used 2011 NLCD land cover classes (same as was used in *Habitat types* section above) and LiDAR-derived metrics representing canopy height (75th percentile of canopy height), percent canopy cover (% returns > 3 m), and understory (% returns 1–3 m in height—McGraw 2019). RSFs were created separately based on whether or not deer were semimigratory (see McGraw 2019 for more details). We made seasonal spatial predictions using the resulting RSF values for both migrators and nonmigrators separately because the coefficients were different between the groups. We overlaid and extracted predicted RSF values using the moose seasonal home ranges and calculated a mean for both the migrators and nonmigrators. We created an overall average of deer resource selection within each moose's seasonal home range (DEERrsf) by weighting the predicted deer use from the migratory and nonmigratory deer by the percentage classified as each (migratory:  $n = 11$ , 33%; nonmigratory:  $n = 22$ , 67%).

*Post hoc analysis.*—Based on the results from our spring model of meningeal worm infection risk, we combined mixed (MIXFRST%) and conifer (CONFRST%) forests because they had similar negative relationships with risk. We hypothesized that the presence of conifer species of trees and shrubs may influence the abundance of gastropods, or the rate of gastropod infection, in a manner that is similar among the two land cover types. We also added areas of upland MIXFRST% to the variable UPconifer% and refit the best-fitting autumn model with the new values. We assessed the combined mixed and conifer forest variables based on the same metrics and values used in “Step 2: retained model sets.”

## RESULTS

We evaluated the meningeal worm infection status of 24 GPS-collared moose (16 female; 8 male) from northeastern Minnesota between 2013 and 2017, ranging in age from 3 to 15 years (mean = 8.3; Fig. 1). Of the 24 moose, 9 (37.5%) were infected with *P. tenuis* either in the spinal column or in the brain. Infected moose were primarily female ( $n = 8$ ), whereas only one male moose was positive. Of the 24 moose, five necropsies concluded that meningeal worm infection was the primary cause of mortality (21%). However, at least three additional moose that were killed by wolves may have been predisposed to predation because of meningeal worm infection (meningeal worm was not primary cause of death).

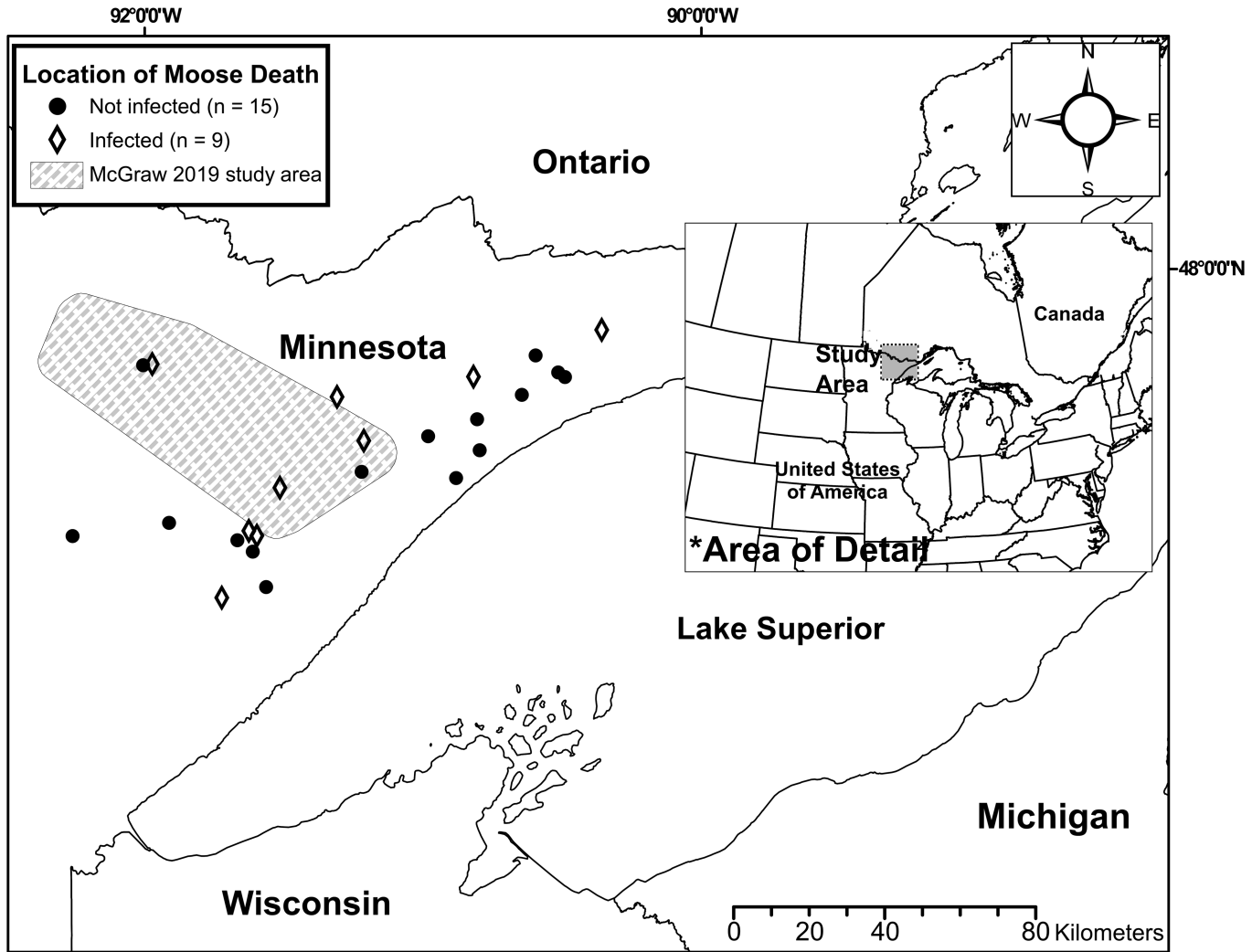
All 24 moose collected enough GPS locations during the spring period to delineate dynamic Brownian bridge seasonal home ranges. Based on the variable importance weight threshold (> 0.6), we retained five variables from our initial spring model sets for inclusion in the retained variable spring model set (MIXFRST%, CONFRST%, WOODY%, DEERuse, WETcover%). Five spring models were within  $\Delta 2$  AICc (Table 2); we considered the first spring model, which included deer space use (DEERuse), conifer forest (CONFRST%), and mixed forest (MIXFRST%), to have the best overall fit based on model metrics (Table 2). All five final top spring models included a negative coefficient for the percent of mixed forest

cover (MIXFRST%; Model 1:  $\hat{\beta} = -2.09$ , 95% CI = -3.89 to -0.28; Fig. 2A). Conifer forest cover (CONFRST%) was included in three of five top spring models and all coefficients were negative. Although the 95% CI overlapped 0, the distribution was highly skewed toward negative values ( $\hat{\beta} = -2.07$ , 95% CI = -4.33 to 0.19; Fig. 2B), suggesting that use of conifer forests generally is associated with lower infection risk during spring. Deer space use (DEERuse) was included in the top spring model (Table 2) and was negatively associated with infection risk, but it was only included in one top spring model and largely overlapped with zero (DEERuse  $\hat{\beta} = -1.32$ , 95% CI = -3.28 to 0.62). Although the percentage of woody wetland (WOODY%) was only included in two top spring models (Models 4 and 5), it had a negative association with meningeal worm infection and a 95% CI that did not overlap zero (WOODY%; Model 5:  $\hat{\beta} = 1.44$ , 95% CI = 0.10 to 2.77; Fig. 2C). Spring was the only season when an interactive effect using a broad spatial pattern was included in a top model (Model 2: MIXFRST%;  $\hat{\beta} = -1.95$ , 95% CI = -3.89 to -0.28; LSdist:  $\hat{\beta} = -2.86$ , 95% CI = 0.25 to 5.46). Model 2 indicates that meningeal worm infection risk is greater for moose living further from Lake Superior during the spring except in areas with greater percentages of mixed forest (MIXFRST%  $\times$  LSdist:  $\hat{\beta} = -2.27$ , 95% CI = -5.13 to 0.59).

The initial summer models of infection risk supported the variables for sex (SEX), deer space use (DEERuse), percent of developed areas (DEVEL%), and water (WATER%) within summer home ranges. Similar to the spring model set, within the top summer model (Model 2; Table 2), hydric land cover types were positively associated with more infection risk (WATER%), but the relationship was generally weak ( $\hat{\beta} = 1.05$ , 95% CI = -0.66 to 2.77) and appeared to be driven by two observations. Developed land also was included in the top summer models (DEVEL%) and had a negative influence ( $\hat{\beta} = -4.11$ , 95% CI = -8.58 to 0.37), but was relatively rare in most moose home ranges (Supplementary Data SD2; Fig. 3). The sex of the necropsied moose, which was included in all seasonal models, was only included in the summer's top retained variable model. Female moose were more likely to be infected within our study relative to male moose (SEX[male]:  $\hat{\beta} = -3.20$ , 95% CI = -6.66 to 0.26).

During autumn, meningeal worm infection was associated with upland conifer (UPconifer%), deciduous forest (DECIDFRST%), and the distance to Lake Superior (LSdist) within the initial models. The best-fitting autumn model (Model 1; Table 2) contained positive associations for UPconifer% and LSdist. Upland conifer (UPconifer%) was included in both top models but had 95% CIs that slightly overlapped zero ( $\hat{\beta} = 2.45$ , 95% CI = -0.44 to 5.35; Fig. 4A). Distance from Lake Superior during autumn had a positive association with meningeal worm but the relationship appeared to be driven by a few moose close to the coast that were not infected (LSdist:  $\hat{\beta} = 1.47$ , 95% CI = -0.38 to 3.32; Fig. 4B).

*Post hoc results.*—Combining the spring land cover classes of MIXFRST% and CONFRST% into one variable resulted in the lowest AICc score among all spring models ( $\Delta$ AICc from

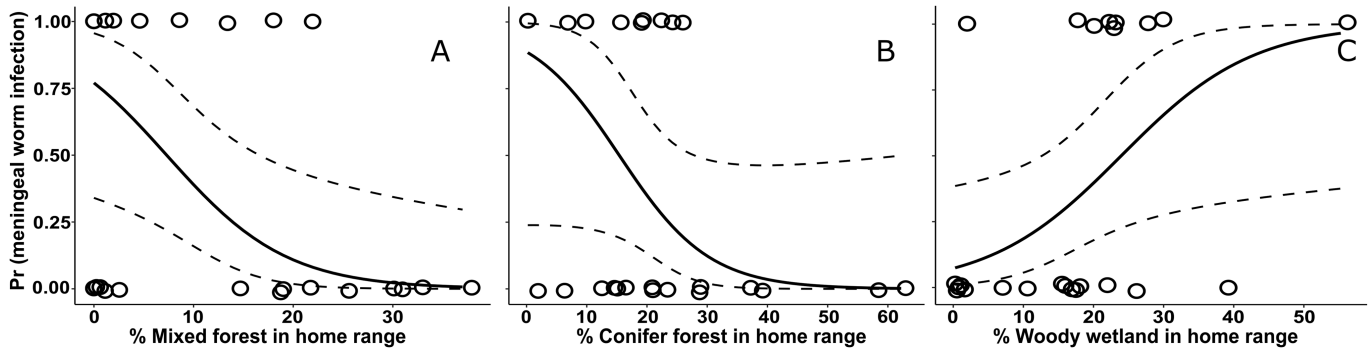


**Fig. 1.**—Last location transmitted by the GPS collars for moose where meningeal worm infection status was verified through necropsy ( $n = 24$ ), in northeast Minnesota, 2013–2017. The study area from McGraw (2019) is also indicated. That study area was defined by the movements of GPS-collared deer used in our analysis.

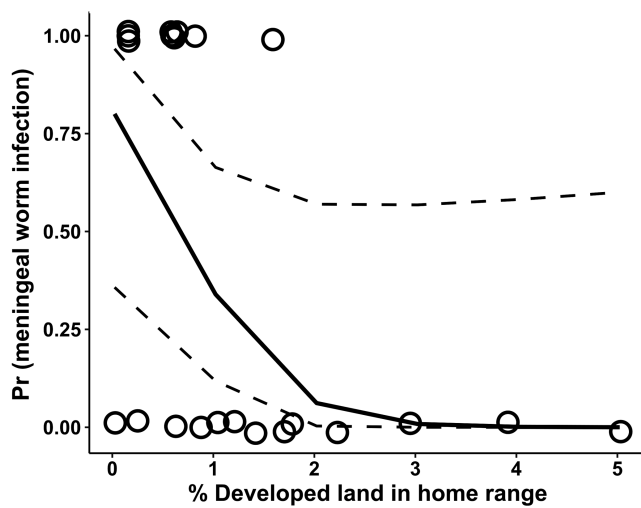
**Table 2.**—Top models from retained seasonal variable model sets for the relationship between meningeal worm infection (MW; present at time or necropsy [1] or free of meningeal worm infection [0]) of moose in northeastern Minnesota, and spatial and individual characteristics of GPS-collared moose. We used logistic additive models for all covariate combinations, considered a limited number of interactions with top covariates and ranked all variables based on AICc. We also included metrics of logistic model fit describing the area under the ROC curve and Nagelkerke  $R^2$ .

| Season | Model # | Model                              | $\Delta AICc$ | C-index (area under ROC curve) | Pseudo- $R^2$ |
|--------|---------|------------------------------------|---------------|--------------------------------|---------------|
| Spring | 1       | MW ~ DEERuse + CONFRST% + MIXFRST% | 0.00          | 0.88                           | 0.61          |
|        | 2       | MW ~ MIXFRST% * LSdist             | 0.61          | 0.89                           | 0.59          |
|        | 3       | MW ~ CONFRST% + MIXFRST%           | 0.97          | 0.84                           | 0.48          |
|        | 4       | MW ~ WOODY% + CONFRST% + MIXFRST%  | 1.39          | 0.90                           | 0.56          |
|        | 5       | MW ~ WOODY% + MIXFRST%             | 1.92          | 0.84                           | 0.44          |
|        | NULL    | Null (MW ~ 1)                      | 6.30          | 0.50                           | 0.00          |
| Summer | 1       | MW ~ SEX + DEVEL%                  | 0.00          | 0.92                           | 0.59          |
|        | 2       | MW ~ SEX + DEVEL% + WATER%         | 0.34          | 0.95                           | 0.67          |
|        | 3       | MW ~ DEERuse                       | 1.52          | 0.84                           | 0.43          |
|        | NULL    | Null (MW ~ 1)                      | 7.44          | 0.50                           | 0.00          |
| Autumn | 1       | MW ~ UPconifer + LSdist            | 0.00          | 0.84                           | 0.56          |
|        | 2       | MW ~ UPconifer                     | 1.47          | 0.72                           | 0.33          |
|        | NULL    | Null (MW ~ 1)                      | 5.80          | 0.50                           | 0.00          |





**Fig. 2.**—Predicted mean and 95% CIs of the logistic relationship between the probability of meningeal worm infection for moose in north-eastern Minnesota and the best-fitting covariates during the spring season (15 March to 15 June; A) % mixed forest within moose home range [MIXFRST%], B) % conifer forest within moose home range [CONFRST%], C) % woody wetland within moose home range [WOODY%]. The covariate values are also plotted along the x-axis and placed at a probability value of 1 if the individual was infected or 0 if they were not. Covariate values were based on the space use of GPS-collared moose and the response variable (meningeal worm infection) was based on necropsies of the same individuals.



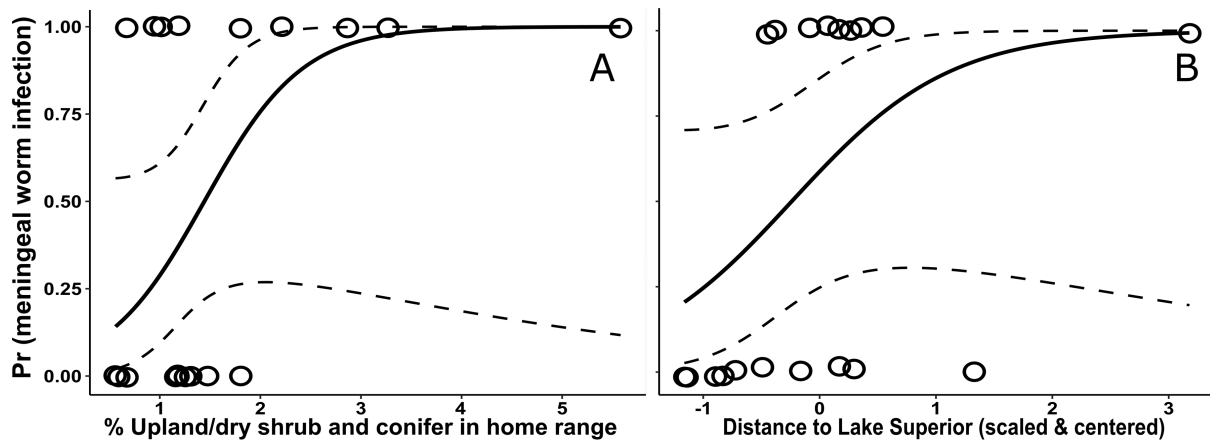
**Fig. 3.**—Predicted mean and 95% CIs of the logistic relationship between the probability of meningeal worm infection for moose in north-eastern Minnesota and the best-fitting covariates during the summer season (15 June to 15 September; % developed lands within moose home range [DEVEL%]) initial set of hypothesis-specific model sets. The covariate values are also plotted along the x-axis and placed at a probability value of 1 if the individual was infected or 0 if they were not. Covariate values were based on the space use of GPS-collared moose and the response variable (meningeal worm infection) was based on necropsies of the same individuals.

spring Model 1 =  $-1.65$ ), a negative coefficient and 95% CI ( $\hat{\beta} = -1.87$ , 95% CI =  $-3.42$  to  $-0.32$ ; Fig. 5A), a similar C-index (0.87) but lower pseudo- $R^2$  (0.47) than Model 1. However, adding upland MIXFRST% to the UPconifer% variable in the top autumn model (Model 1) did not improve model fit ( $\Delta\text{AICc}$  from autumn Model 1: 0.45; pseudo- $R^2 = 0.52$ ; C-index = 0.84), and a CI that still overlapped zero ( $\hat{\beta} = 1.83$ , 95% CI =  $-0.24$  to 3.90; Fig. 5B).

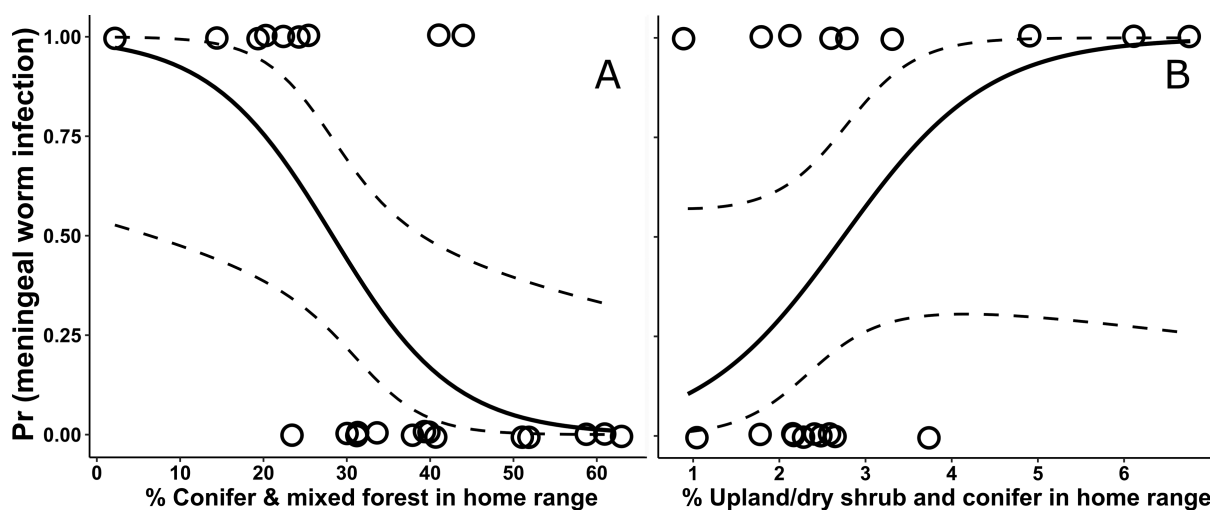
## DISCUSSION

We believe our top-down approach, which integrated movement and disease ecology, yielded novel insights into the biological

and ecological aspects contributing to the likelihood of meningeal worm infection in moose. Tools to analyze the movements of individuals are increasingly being used in disease ecology because of their ability to provide fine-scale estimates of infection risk exposure (Dougherty et al. 2018). Habitat types were more closely associated with meningeal worm infection risk than other environmental conditions (e.g., snow cover duration), individual moose characteristics (i.e., sex and age-class), and deer habitat use and density, based on the relationship between space use of individual GPS-collared moose and whether they became infected. Our findings reveal that it is critical to recognize that the space use and environmental conditions experienced by dead-end hosts, such as moose, may play a significant role in the variation of meningeal worm risk among individuals. While the baseline risk of meningeal worm infection in an area is positively associated with deer density, managers attempting to prevent the spread or increase of meningeal worm infection also should consider that risk, at least in regions with generally low or minimal spatial variation in deer density, may be more frequency-dependent (related to extent or frequency of contact between moose and high-risk environments) than density-dependent (driven by deer density), as previously was assumed. Moose space use during spring, and to a lesser extent autumn, provided the strongest associations between habitat types or conditions and infection risk. In general, habitats associated with wet or moist conditions increased risk, although during autumn, upland conifer habitats were associated with added risk. While we acknowledge that our methodologies were exploratory, that our sample size was relatively small ( $n = 24$  necropsied moose, nine confirmed infected with *P. tenuis*), and our findings were correlative in nature, we believe our approach uniquely integrated several sources of data to test competing hypotheses about meningeal worm risk and related them to known outcomes through the use of necropsies, thus providing additional insights and a novel test of several hypotheses of risk developed through bottom-up approaches (e.g., gastropod and deer pellet sampling). We propose that going forward, greater consideration be given to the possibility of frequency dependence of meningeal worm transmission and



**Fig. 4.**—Predicted mean and 95% CIs of the logistic relationship between the probability of meningeal worm infection for moose in northeastern Minnesota and the best-fitting covariates during the autumn season (15 September to 15 December; A) % upland/dry shrub and conifer within moose home range [UPconifer%], B) distance to Lake Superior [scaled and centered; LSdist]) initial set of hypothesis-specific model sets. The covariate values are also plotted along the x-axis and placed at a probability value of 1 if the individual was infected or 0 if they were not. Covariate values were based on the space use of GPS-collared moose and the response variable (meningeal worm infection) was based on necropsies of the same individuals.



**Fig. 5.**—Predicted mean and 95% CIs of the logistic relationship between the probability of meningeal worm infection for moose in northeastern Minnesota and covariates from top spring and autumn models containing conifer habitat (% conifer forest within moose home range [CONFRST%], and % upland/dry shrub and conifer within moose home range [UPconifer%]). We combined CONFRST% with % of mixed forest (MIXFRST%) because the covariates had similar estimates in the spring model and we hypothesized that conifer species may have the same influence on meningeal worm infection risk regardless of whether it was classified as all conifer or a mixed-conifer forest. The first panel (A) shows the relationship between the combined CONFRST% and MIXFRST% in spring moose home range and the probability of meningeal worm infection. The second (B) uses the variable UPconifer% and adds MIXFRST% contained within each autumn home range.

further, that managers consider land management practices in addition to deer management efforts (e.g., reducing population size to benefit moose; see Lankester 2010).

Our finding that land cover types associated with more hydric soils or wet conditions increase the likelihood of meningeal worm infection, likely because of the favorable conditions they provide to intermediate gastropod hosts, concur with conclusions of previous studies (Prior 1985; Hawkins et al. 1997; Maskey 2008; Lankester 2010). In particular, Lankester and Anderson (1968) reported a 6-fold increase in infected gastropods in wet forests relative to dry upland areas, and the three most commonly infected gastropods in Minnesota prefer wet

conditions (marsh slug, *D. laeve*; and the woodland snails *D. cronkhitei* and *Zonitoides* spp.—Lankester 2001; Cyr et al. 2014). However, studies in nearby Voyageurs National Park (Minnesota, United States) found the lowest infection risk in northern shrub swamps (woody wetlands in this study), based on the percent use of habitat types by moose, gastropod density (Cyr et al. 2014), and sampled deer scat infected with L1 larvae (VanderWaal et al. 2015). Notwithstanding, within our study area of northeastern Minnesota, moose home range contained 17–20% woody wetlands (Supplementary Data SD2) across seasons compared to just 7% in Voyageurs National Park (Cyr et al. 2014), and we found infection risk was positively

associated with woody wetlands along with other wet habitats. A study in northeastern Minnesota found no difference in slug density among habitat types, with only slightly higher snail density in regenerating forests (although surveys were not conducted in developed areas—Cyr 2015). Importantly, no infected gastropods were found in mixed and conifer forests (Cyr 2015;  $n = 1,621$  total gastropods for both habitat types), habitats in which our results indicated a negative relationship between space use and infection risk, whereas deciduous and regenerating forests had rates of infection in gastropods of 0.16% and 0.21%, respectively. Upland conifer stands have been associated with increased rates of infected deer pellets in Voyageurs National Park (Vanderwaal et al. 2015), which our best autumn model also indicated have a positive influence on infection rates of moose, albeit with a high amount of variability.

Deer abundance in northern Minnesota increases from north to south (D'Angelo and Giudice 2015; Norton and Giudice 2018), and within our study area, the semimigratory nature of deer populations (due both to weather and to supplemental feeding) also may play a role in the spatial patterns of meningeal worm risk by creating areas with high densities of infected gastropods in areas where deer congregate (“deer yards”—Lankester and Peterson 1996). Although some deer living inland from Lake Superior move further westward, many individuals in the eastern portion of the study area move eastward to the shores of Lake Superior where nonmigratory deer also are present. Our findings showed weak and contrasting links between deer space use and infection risk; estimated deer density was not supported in any of our top models. Our spring and autumn models included a positive relationship between infection rate and distance from Lake Superior that may reflect broad spatial patterns of deer density. Locations of moose deaths (Fig. 1) of individuals infected with meningeal worms generally were further from shore (the nearest infected moose to shore was ~19 km while eight moose < 19 km from the shore were not infected of a total of 15 noninfected), but moose generally avoid the shoreline where lightly developed areas provide foraging (including recreational feeding) for overwintering deer (closest location of moose death = 4.3 km from Lake Superior). In addition, deer living closer to Lake Superior may in general have lower infection rates (Escobar et al. 2019).

Although deer density has been positively associated with infection rates in deer populations (Karns 1967; Behrend and Witter 1968; Slomke et al. 1995; Peterson et al. 1996; Wasel et al. 2003), the threshold frequently used in deer management, where it is believed deer populations begin to negatively impact moose population growth (5 individuals per km<sup>2</sup>—Whitlaw and Lankester 1994; Lankester 2010) may be too high. Indeed, this value appears less certain after reexamination using different statistical approaches (McGraw 2019). Throughout most of northeastern Minnesota, deer densities have not historically nor presently reached the 5 deer/km<sup>2</sup> threshold. Despite this, infection rates of meningeal worm were relatively high and the moose population, while currently stable, has undergone a ~66% reduction in the past decade (DelGiudice 2018). Previous

studies show deer populations in northeastern Minnesota have high rates of *P. tenuis* infection (69%—Vanderwaal et al. 2015; 82%—Slomke 1995), which may compensate for low deer densities if habitat conditions and spatial overlap between moose and deer are present. Space use by GPS-collared deer (McGraw 2019) and moose generally were similar in highly used habitats, primarily forested and woody wetland habitats, although they diverged in their use of emergent wetlands (Supplementary Data SD1 and SD2). In North Dakota (United States), deer density and moose population growth exhibited a negative relationship, but the relationship was only present in areas with certain environmental and climatic conditions (Maskey 2008). In addition, modeled relationships have found the rate of meningeal worm infection risk in moose is more sensitive to levels of gastropod abundance than deer density (Schmitz and Nudds 1994).

Our results shed light on the temporal components of infection risk from meningeal worm in moose, which historically have been difficult to measure and understand. Deer shed the L1 larvae year-round, with a peak in spring (Slomke et al. 1995). Many gastropod species remain in the soil or leaf litter during early spring (Lankester and Peterson 1996; Cyr et al. 2014; Cyr 2015), although the marsh slug (*D. laeve*), a commonly infected species, often is the first gastropod species active in spring and remains so through autumn (Lankester and Anderson 1968; Lankester 2001). Seasonal foraging strategies of moose may drive the rate of gastropod consumption, regardless of relative availability of gastropods. Throughout their range, moose primarily forage on foliage attached to shrub or tree branches during late spring through early autumn (Renecker and Schwartz 2007). However, moose also forage on the ground for fallen leaves during late autumn and early spring (after snow melt; forbs and graminoids also are important food items during spring—Renecker 1986; Renecker and Hudson 1989; Renecker and Schwartz 2007). Foraging among the leaf litter and forest floor vegetation in early spring and late autumn may be the activity during which moose incidentally consume the most gastropods. Our findings are consistent with this hypothesis: the strongest relationships and largest effect sizes between covariates and infection risk were identified during spring, and to a lesser extent, the autumn. Gastropods rarely climb above the litter layer (0.13/m<sup>2</sup> density climbing above leaf litter to 3 m vs. 9.9/m<sup>2</sup> on/in leaf litter or ground—Cyr 2015); it thus is unlikely that moose are consuming many gastropods while foraging on foliage still attached to branches. Habitats least associated with infection risk, mixed and conifer forest cover (spring models), have relatively little leaf litter available and relatively fewer forbs and graminoids during spring and autumn compared with other environments.

Numerous bottom-up studies have been critical to determining *P. tenuis* abundance through assessing infection rates in gastropods, deer, and deer pellets, but these surveys are laborious, and the complexity of accurately assigning risk to moose is difficult because of the extremely low rates of infection in gastropods (e.g., 0.1%—Cyr 2015;

0.04–0.16%—Lankester and Peterson 1996) despite deer pellet infection rates that can average 76% (Vanderwaal et al. 2015). Although our modeling approach also required us to make several simplifying assumptions about the timing of infection (1-year window) and the period from neuromotor impacts to death (2-month buffer prior to death), we believe our top-down approach was a novel way to investigate meningeal worm infection risk because it enabled us to pull together several sources of data to test existing hypotheses and relate them to known outcomes determined from necropsies. We believe that incorporating both top-down and bottom-up approaches into models of disease transmission risks will provide the most complete understanding of meningeal worm infection risk. Methods that better discern foraging behaviors of moose through the year may better isolate when moose are more likely consuming gastropods, an aspect of understanding meningeal worm risk that has been ignored in most studies. Indeed, the strength of our correlative relationship between space use and infection risk was strongest during the spring, a period when moose often forage on the forest floor. Future studies can build on our findings by further using the improved capabilities of GPS collars that collect ancillary data, such as accelerometers, to identify the amount of time foraging among habitat types (Ditmer et al. 2017; Kröschel et al. 2017) and use movement characteristics to determine when infected individuals become symptomatic. GPS on-board video observation of diet (Thompson et al. 2014) may provide additional insights, especially in conjunction with sampling of *P. tenuis* abundance in gastropods and/or deer pellets within moose movement paths or home ranges (e.g., Portinga and Moen 2015).

In our study area along the southern bioclimatic edge of moose range, the parasitic burden on moose is high, and can lead to malnourishment and morbidity (Wünschmann et al. 2015). With the predicted geographic expansion of *P. tenuis* (Pickles et al. 2013) and lengthening periods of transmission risk associated with more moderate winters (Lankester 2018), developing a better understanding of meningeal worm risk is paramount to improve the conservation and management of ungulates. However, determining all of the spatial and temporal aspects of risk remains difficult due to meningeal worm's complex life cycle (Lankester and Samuel 2007). While many studies and management strategies focus on deer density, the relationship appears to be more complex (McGraw 2019) and likely will require management strategies more conducive to limiting risk associated with frequency dependent diseases (e.g., targeted harvest efforts at broad spatial scales—Jennelle et al. 2014). This could include a combination of deer and land management practices (e.g., larger tracts under prescription) designed to limit contact rates in susceptible populations. Our results agree with those of several studies that highlight the important role that habitat and environmental conditions play in infection risk of *P. tenuis* for moose (Maskey 2008; Vanderwaal et al. 2015; Escobar et al. 2019; McGraw 2019). Although the interactions among deer density, habitat conditions, and moose

behavior, in influencing the risk of *P. tenuis* infection in moose remain incompletely understood, it is clear that more attention should be paid to the frequency of contact between moose and high-risk environments, rather than the density of deer and deer habitat selection alone, particularly during times of year when moose are foraging on the ground.

## ACKNOWLEDGMENTS

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## SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Mammalogy* online.

**Supplementary Data SD1.**—Average and 95% confidence interval for the percent of locations per land cover types for GPS-collared white-tailed deer living in northeastern Minnesota. Data were collected as part of a larger study (see McGraw 2019 for details). We used individual deer as the sampling unit and seasons were delineated as: spring = 15 March to 15 June, summer = 15 June to 15 September, and autumn = 15 September to 15 December.

**Supplementary Data SD2.**—Average and 95% confidence interval area (%) of land cover types within seasonal dynamic Brownian bridge home range estimates of GPS-collared moose living in northeastern Minnesota. We used individual moose as the sampling unit and seasons were delineated as: spring = 15 March to 15 June, summer = 15 June to 15 September, and autumn = 15 September to 15 December.

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