EASTERN MASSASAUGA RATTLESNAKE (SISTRURUS CATENATUS) DETECTION AND SPACE USE NEAR ROADS IN THE SOUTHERN LOWER PENINSULA OF MICHIGAN, USA

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Fisheries and Wildlife – Master of Science

2024

ABSTRACT

Roads are a mortality source for federally threatened eastern massasauga rattlesnakes (EMR; *Sistrurus catenatus*) in Michigan, yet we lack a clear understanding of EMR space use and habitat selection near roads. Identifying when, where, and why EMR use spaces near roads could inform decisions to reduce risk of road-related mortalities. Objectives of this research were to survey for EMR at sites along roadsides, report on occupancy probability, demonstrate how weather influences EMR visibility during visual encounter surveys, and explore environmental factors resulting in high EMR space use near roads. During 54 surveys (following previously published survey protocol) 35 sites, five EMR were detected at two distinct sites. Individual EMR surveys had low site-level detection probability (mean = 0.16 (SE = 0.11)), but repeated surveys increased detection probability to near 1.00 at some sites, and visual encounter surveys should be conducted during optimal temperature ranges (~30-40° C in this study). The 24 telemetered EMR at occupied sites were relocated 1-5 times per week, and the percentage of body visible and burrow use was recorded. Body exposure and burrow use were modeled with a Bayesian beta regression to discern when EMR are most visible in active season (April-October; 2020-2022). Estimated body exposure ($\overline{x}$ = 42%, SE = 3%, range = 0-100%) increased from ~25% exposure at ~15° C to ~55% at ~40° C. Probability of burrow use was related to Julian day broadly represented the transition from inactive to active seasons, with low probability of use (<0.10) from June to August. Male EMR were less likely ($\overline{x}$ = ~0.02%, range = ~2 - 4%) to use burrows than females ($\overline{x}$ = ~8%, range = ~2 - 42%). Brownian Bridge kernel home ranges for four (3 – female, 1 – male) telemetered EMR with ≥20 relocations were combined into a single spatial layer representing intensity of snake use. A Bayesian beta regression model predicted intensity of snake use based on environmental covariates. At Site 1, probability of use was greatest ≥70m of the road and correlated negatively with canopy cover (use decreased from 45% at 0% canopy cover to 20% at 90% cover). At Site 2, EMR probability of use increased by 5% (42% to 47%) as distance from the road approached 100m, but use declined sharply by 23% (50% to 27%) approaching 300m from the road. Core use areas for EMR were close (e.g., ≥20 m) to roads, likely due to vegetation management (e.g., mowing), and the physical properties of the roadbed (i.e., warmer below ground temperatures).

For Nicholas. And Jax the cat.

ACKNOWLEDGEMENTS

I would like to thank the Michigan Department of Transportation (MDOT) for their collaboration and financial support of this research (OR#19-137). Thank you as well to the U.S. Fish and Wildlife Service (USFWS), Michigan Ecological Services East Lansing Field Office; Michigan Department of Natural Resources (MDNR); and U.S. Forest Service (USFS) for their partnership and collaboration without which this research wouldn't have been possible. Special thanks should also go to the Lansing Potter Park Zoo (PPZ) for providing capture and handling training. I am particularly grateful to Matt Ihnken, Jeffrey Grabarkiewicz (MDOT), Carrie Tansy (USFWS), Shaughn Galloway (USFWS), Dennis Tison (MDNR), Julie Oakes (former MDNR), Mark Boersen (DNR), Phil Huber (USFS), and Eric O'Neil (USFS) for their interest, support, and expert advice. Thank you to Dr. Bruce Kingsbury (Indiana-Purdue University Fort Wayne) and Chris May (The Nature Conservancy) for their expert guidance on EMR habitat and space use. Thank you to Dr. Jennifer Moore for canopy cover methodology advice and collaborating regarding previously monitored EMR populations in proximity to this study's survey sites. My deepest thanks to the private landowners who allowed researchers access to their property to conduct detection and vegetation surveys.

I could not have undertaken this journey without the support of my co-advisors, Drs. Gary Roloff and Henry (Rique) Campa. To Dr. Gary Roloff, thank you for the endless encouragement, patience, and guidance you've shown me as I've worked to grow into an independent, confident researcher. To Dr. Rique Campa, thank you for always being available for guidance, from aiding in search efforts to sharing your breadth of knowledge and invaluable advice. From answering the smallest questions to providing support in difficult times, you've both taught me to approach bumps in the road with creativity, compassion, and humor, for which I am forever grateful. I owe a lot of gratitude to my committee member, Dr. Scott Winterstein, for your flexibility, willingness to provide feedback, and sincere interest in this research. Thank you for your expertise and helpful remarks on project design and analysis, particularly in your assistance with finalizing Bayesian modeling processes. I would like to express my deepest appreciation to Dr. Steven Gray for field support when I was in a tight spot, and your essential role in developing final analyses and prompt, thorough feedback whenever asked.

Words cannot express my gratitude to lead technician Brianna Bassett and field technicians Kalin Bayes, Kotaro Seguchi, Lauren Emerick, Maggie Haite, Michelle Roy, and Wiley Welsh for their hard work and friendship. Every scorching, prickly, sweaty day along the roadside was filled with laughter and fueled by an authentic interest in ecology. Even with the soggiest of boots, in densest mazes of wetland vegetation, each of you showed amazing determination and perseverance. Thank you from the bottom of my heart. I'd like to recognize Cade Shauer for your willingness to assist with canopy cover image processing, your efforts are greatly appreciated and were essential for carrying out final analyses. I was fortunate to work with my amazing lab mates within the Applied Forest and Wildlife Ecology Laboratory (AFWEL). Thank you for the valuable feedback, unwavering support, sage advice, and serving as an ongoing inspiration to challenge my perspective.

I'd like to give my sincerest thanks to my family for keeping me grounded, for their interest in my research, and for their support in all of my endeavors. Thank you to my dearest friends for keeping me sane, always lending an ear, and encouraging me to believe in myself. Melissa Winchester, thank you for the endless supply of house plants and for always having my back. Saving the dearest to my heart for last, I'd like to thank my partner Nicholas Rajewski, for every ounce of love and rock-solid support that you've given me during my journey as a graduate student (and beyond). I truly couldn't have made it through this experience without your sincere and unwavering kindness, your unbelievable patience, and insanely delicious cooking. Your encouragement makes me brave enough to accomplish all of the things I set out to do.

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DISCLAIMER

This material is based upon work supported by the Federal Highway Administration under SPR-1726. Any opinions, findings and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the Federal Highway Administration.

GENERAL INTRODUCTION

Federal regulators of the Endangered Species Act listed eastern massasauga rattlesnakes (*Sistrurus catenatus*; EMR) as threatened in 2016 (USFWS 2017a). A threatened designation indicates that a species is likely to become endangered in all or a significant portion of its range in the future (Endangered Species Act, Section 3(20)). This listing decision identifies several prominent threats to EMR including habitat loss and fragmentation, road mortality, hydrological alteration, persecution and collection, and mortality from habitat management (USFWS 2016). The current core range of EMR in Michigan is centered on the Lower Peninsula (USFWS 2016, Szymanski et al. 2016), where an estimated 187 (of 263 range wide) subpopulations currently occur (Lee and Enander 2015). With 71% of known extant populations, conservation of EMR in Michigan is critical to species recovery. Of the prominent threats to EMR identified in the listing decision (USFWS 2016), habitat loss (through human development and vegetation succession), habitat fragmentation, and road mortality potentially impact 82% of the sub-populations in Michigan (Szymanski et al. 2016). Local, state, and federal roads intersect a substantial amount of potential EMR habitat in Michigan but understanding of EMR – road interactions is generally limited to mortality from known vehicle strikes (Weatherhead and Prior 1992, Bailey et al. 2011, Rouse et al. 2011, Martin et al. 2023).

During the active season (typically April to October in southern Michigan), EMR occupy various plant communities, with habitat structure being more important than plant community composition or soil type (Bailey et al. 2012, USFWS 2016: 67194). EMR require relatively sparse overstory tree canopy cover resulting in sun-dominated areas with occasional canopy shading that results in a mosaic of ground vegetation that provides microsites for thermoregulation, abundant prey, and cover to escape predators (Bailey et al. 2011, USFWS 2016: 67194). These habitat conditions often occur in road right-of-ways (ROWs). Paterson et al. (2019) found that while EMR generally avoid crossing roads, they do not avoid habitats adjacent to roads. Though roads are known to directly and indirectly (e.g., habitat fragmentation) affect EMR (e.g., Colley et al. 2017, Baker et al. 2018, Shepard et al. 2008a,b), the spatiotemporal interactions among individual snakes and ROW maintenance activities are poorly understood (Rouse et al. 2011, USFWS 2016: 67194). Given the spatial extent of potential EMR - road interactions in Michigan, a better understanding of how to mitigate potential negative impacts of roads and road maintenance is essential for EMR conservation (Colley et al. 2017).

In 2017, the Federal Highway Administration (FHWA), Michigan Department of Transportation (MDOT), and the U.S. Fish and Wildlife Service (FWS) developed a statewide Programmatic Agreement (Programmatic) on conservation and management of EMR in relation to road construction and maintenance projects (USFWS 2018:17). The Programmatic describes best management practices to minimize potential effects of ROW activities to EMR (USFWS 2018). EMR best management practices include seasonal restrictions and exclusion fencing; knowing when and where to allocate these practices is critical (USFWS 2017b). Although guidance occurs in the Programmatic for implementation of these activities (e.g., active mowing guidelines; USFWS 2018), the Programmatic also recognizes the importance of applied research to better understand the effects of transportation projects on EMR (USFWS 2018). An effective and efficient EMR survey strategy is relevant given the Programmatic indicates that no avoidance or minimization measures are needed for proposed ROW projects "where sufficient surveys are conducted" (USFWS 2018). Additionally, the Programmatic encourages transportation and conservation agencies to conduct visual EMR surveys to aid in establishment of Conservation Focus Areas (CFA), where these entities may be required to take conservation or mitigation action (USFWS 2018:16). Visual encounter surveys are effective and cost efficient for determining site occupancy as no equipment is required and trained surveyors can effectively locate EMR regardless of experience (Casper et al. 2001, Shaffer et al. 2019). Shaffer et al. (2019) identified that the most important determinants of EMR detection during visual encounter surveys was the time spent surveying and minimum temperature during the survey (detection probability highest between 12 - 15° C). Further investigation of optimal survey conditions would aid in survey efficiency, and therefore make it easier to determine site occupancy across the species range.

Presently, MDOT and County Road Commissions maintain road ROWs throughout approximately 2,226 ha of potential EMR habitat (modeled as Tier I and Tier II; https://ecos.fws.gov/ipac/) in Michigan. These highways and associated ROWs receive numerous development and maintenance activities that have potential to affect EMR (USFWS

2018). Roads are widely perceived as barriers to snake movements and dispersal (Shepard et al. 2008a, Rouse et al. 2011, DiLeo et al. 2013, Szymanski et al 2016), though few studies have directly quantified this effect (but see Shepard et al. 2008b, Colley et al. 2017). Additionally, information on effects of maintenance activities like mowing, herbicide application, and water management projects (e.g., culvert maintenance) on EMR mortality, movements, and fitness is lacking (USFWS 2018). Research addressing these knowledge gaps will ensure that conservation measures in the Programmatic are avoiding regulatory take of EMR.

OBJECTIVES AND HYPOTHESES

Objective 1 (Chapter 1). Use the EMR survey protocol developed by Shaffer et al. (2019) to quantify likelihood of EMR site occupancy based on survey effort, ambient air temperature, and number of surveys conducted in areas adjacent to MDOT ROWs.

Hypotheses:

a) I hypothesized that EMR detection depends on their ability to maintain body temperatures within the optimal range (30-33.6° C, Harvey and Weatherhead 2011), and EMR visibility will be greatest when ambient temperatures fall within this range.
b) Ambient temperature, cloud cover, and humidity will positively correlate with EMR body visibility as these variables have been shown to influence EMR thermoregulation (Casper et al. 2001, Shaffer et al. 2019, Thacker et al. 2023).
c) EMR will be more visible in early Spring (April-May) when vegetation is short and EMR are moving from overwintering locations to active season habitats.

Objective 2 (Chapter 2). Quantify spatial and temporal patterns of EMR space use in and around MDOT ROWs. This information will be used to identify when (daily, seasonally) and where (i.e., habitat conditions) EMR are most vulnerable to ROW activities.

Hypotheses:

a) EMR use of ROWs will be greater in areas with low amounts of overstory canopy cover, and in areas that consist primarily of grass, sedge, and/or low growth vegetation (e.g., forb species).

b) EMR use of ROWs will vary seasonally, with greatest probability of ROW use during early spring when EMR emerge from hibernacula and seek areas that allow them to adequately thermoregulate, and late summer and early autumn when males increase their activity for breeding (Rouse et al. 2011).

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CHAPTER 1: DETECTION AND VISIBILITY OF EASTERN MASSASAUGA (*SISTRURUS CATENATUS*) DURING VISUAL SURVEYS IN MICHIGAN

INTRODUCTION

Eastern massasauga rattlesnakes (*Sistrurus catenatus*; EMR) have been federally listed as threatened under the Endangered Species Act since 2016 (USFWS 2016). Protection under the ESA requires effective population monitoring techniques, but identifying areas occupied by EMR can be difficult due to cryptic coloring and tendency to thermoregulate in underground burrows or under low, dense vegetation during the active season (Foster et al. 2009, Harvey and Weatherhead 2010, Shoemaker and Gibbs 2010, Szymanski et al. 2016). This behavior varies by life stage and sex (Foster et al. 2009, Harvey and Weatherhead 2010). A range of survey techniques for EMR have been developed including visual detection surveys (Casper et al. 2001, Shaffer et al. 2019, Thacker et al. 2023), drift fence camera trap systems (AHDriFT; Amber et al. 2020, Amber 2021, Amber et al. 2021), artificial cover objects (ACOs; Glowacki and Grundel 2005, Bartman et al. 2016), and funnel traps (Casper et al. 2001, Durbian and Lenhoff 2004, Glowacki and Grundel 2005, Bartman et al. 2016). Ultimately, surveys need to be reliable in discerning EMR occupancy to adequately implement ESA protections (USFWS 2017).

Federal and state agencies, businesses, and private stakeholders require survey methods that are cost effective, easy to implement, and reliable when proposed projects overlap potential habitat within EMR range. Funnel traps, ACOs, and camera trap systems are effective, but require setup and maintenance, and rapidly surveying large areas can be logistically challenging. Additionally, for some techniques (e.g., ACOs) it often takes time for EMR to acclimate to and use these objects, if they use them at all (Glowacki and Grundel 2005, Bartman et al. 2016). Visual encounter surveys offer a technique readily implemented with minimal training and costs (Casper et al. 2001, Shaffer et al. 2019, Thacker et al. 2023), but failing to detect EMR when they are present during relatively short duration (i.e., hours) visual encounter surveys can reduce effectiveness. A standardized EMR visual survey protocol for EMR by Casper et al. (2001) provides recommended survey conditions and data collection methods for cross-study comparison. Additional research on conditions needed to efficiently sample EMR with visual encounter surveys identified search effort and ambient temperature as important considerations (Shaffer et al. 2019, Thacker et al. 2023). Oftentimes, multiple surveys of the same area are required to reliably estimate site occupancy (Mackenzie 2006, Kellner and Swihart 2014), which can be costly and time consuming.

When estimating detection probability estimates can broadly vary given species rarity, distribution, behavior, environmental conditions, and site characteristics (Mackenzie 2006, Kellner and Swihart 2014). Surveys for EMR are challenging, as this species is uncommon, patchily distributed (USFWS 2016), and regulates activity based on temperature and other local environmental conditions (Weatherhead and Prior 1992, Harvey 2000, Foster et al. 2009, Harvey and Weatherhead 2010, Szymanski et al. 2016). EMR detection probability can be influenced by time of day (especially when temperatures are changing; see Casper et al. 2001, Shoemaker and Gibbs 2010), microsite composition (Shoemaker and Gibbs 2010), and time of year (i.e., Harvey and Weatherhead 2010, Shaffer et al. 2019). Therefore, it is important to understand when EMR are aboveground and visible for detection, as this will maximize visual survey accuracy, effectiveness, and efficiency.

My goal was to supplement the EMR survey model developed by Shaffer et al. (2019) by predicting percent body exposure and likelihood of EMR burrow use from radio telemetered EMR. My objectives were to identify conditions (i.e., environmental and temporal) that maximize EMR visibility during surveys. I also applied the Shaffer et al. (2019) survey method to previously unsurveyed areas within modeled EMR habitat and demonstrated how multiple surveys can be used to increase overall detection probability. Results from this research will allow natural resource managers and agencies to conduct visual surveys more effectively by identifying optimal conditions for EMR visibility.

METHODS

Initial Site Selection

Three sites across the Lower Peninsula of Michigan were selected based on anecdotal reports of EMR, modeled Tier 1 and Tier 2 habitats, and adjacent areas that had favorable habitat characteristics. The model (USFWS Unpublished; Figure 1.1) defines Tier 1 habitat as areas known to be occupied by EMR or highly likely to be occupied, and Tier 2 as areas with potential EMR habitat that may be occupied (USFWS 2018). Across EMR range, potential habitat varies widely by vegetation type (e.g., jack pine [*Pinus banksiana*] plains near Grayling to emergent marshes in southern Michigan; Szymanski et al. 2016), land use patterns, and human density (Kingsbury et al. 2003, Bailey et al. 2012, DiLeo et al. 2013, McCluskey et al. 2017, Szymanski et al. 2016). During the active season, EMR occupy several plant community types, with research suggesting that vegetation structure is more important than plant community composition or soil type (Bailey et al. 2012, USFWS 2016:67194). Generally, EMR require early successional ecotones (e.g., meadows with minimal shrub encroachment) with low overstory tree canopy cover and access to the water table through crayfish burrows, hummocks, tree roots, or other suitable structures for successful overwintering (Harvey and Weatherhead 2006, Smith 2009, Szymanski et al. 2016, Smolarz et al. 2018). During the active season, EMR often move between emergent wetlands and adjacent upland vegetation with dispersed, low canopy cover to seek refuge from predators, thermoregulate, and forage (Weatherhead and Prior 1992, Moore and Gillingham 2006, Bailey et al. 2012, Szymanski et al. 2016). Sites identified for this study consisted of wet prairie, marsh, early successional forest edges, and upland grassland with open tree canopy interspersed with shrubby, woody vegetation. Selected sites were environmentally heterogeneous, and initially within 150 m of state trunkline, corresponding to the average radius of EMR home range size in Michigan (Lee and Enander 2015). Identification and selection of study sites was completed in collaboration with the Michigan Department of Transportation (MDOT) and U.S. Fish and Wildlife Service (USFWS). Public land access was granted by the Michigan Department of Natural Resources (MDNR; permit ID: TE 225), and private landowners gave verbal permission prior to conducting research on their property, and site-specific authorizations were issued (USFWS; permit ID: #TE34563C-3).

Sub-site Selection

Within each study site (n=3), survey sub-sites were selected based on their potential for EMR occupancy. The patch size needed to support EMR depends on habitat quality, distance between microhabitats, and home range size, which can vary by life stage and reproductive status (Szymanski et al. 2016). Within a site, the search area for visual encounter surveys was constrained to 1-5 ha of potential habitat, which includes EMR average home range size in

Michigan (2.0 ha; Bissell 2006, Lee and Enander 2015, Shaffer et al. 2019). The number of sub-sites and their arrangement within a site were determined by amount of potential EMR habitat and barriers that potentially limited search access (e.g., property boundaries, lakes, ponds).

Detection Surveys

Between May and October of 2020-2022, EMR detection surveys were conducted using protocols from Shaffer et al. (2019). Within subsite boundaries, visual encounter surveys were conducted along transects oriented north-south and spaced 20 m apart (Shaffer et al. 2019). Surveyors walked a weave pattern along transects within 10 m of the transect line on each side and used a snake hook or handling tongs to separate vegetation and look under cover objects for EMR. Surveyors navigated transects with compasses and georeferenced maps produced in ArcGIS (ESRI 2020. ArcGIS Desktop: Release 10.8 Redlands, CA: Environmental Systems Research Institute) accessed remotely through Avenza Maps ® (Avenza Systems Inc.,Toronto, ON, Canada) on android tablets (SAMSUNG Glaxy Tab 8 inch Android Tablet 64GB). During surveys, researchers recorded time spent searching and ambient air temperature (at the beginning and end of each survey) with a handheld temperature gague (HoldPeak 866B digital handheld anemometer and thermometer), as both are influential to EMR detection (Shaffer et al. 2019, Thacker et al. 2023). All surveys were conducted between 0700 and 2000 hours, with >24 hours between surveys if conducted at the same site. In each survey, researchers aimed to search for ≥90 minutes of cumulative survey effort per every 2 ha (Shaffer et al. 2019). Surveys were conducted under variable weather conditions, excluding thunderstorms and heat advisories. For replicate surveys at the same subsite, I varied starting position where pairs of surveyors would either start at opposite sides of the survey boundary and meet in the middle or survey the entire search area together. If an EMR was located, surveyors stopped, made observations (e.g., local climate conditions, behavior), and recorded the time. Snakes were processed and released at the capture location and the survey was restarted to completion. If EMR were detected during surveys conducted immediately prior to roadside maintenance activities, the snake was moved to an area outside of the maintenance area. The length of my field seasons during the first two years of this research project was shortened by the COVID-19 pandemic. While adhering to federal, state, and university safety policies, I was unable to survey during early Spring in 2020 and 2021, reducing the temporal breadth of my research results.

Capture and Marking

EMR detected during formal surveys and opportunistically were picked up using snake tongs and secured in a snake bag (snake bags from Midwest tongs/handmade breathable cotton [dimensions: ~0.30 x 0.51 m]). Surveys were paused to process EMR, therefore the time lapsed while capturing and processing EMR was not included in total survey time. I transported captured EMR to a nearby area clear of understory growth, or a low traffic gravel parking lot for processing if the capture area was close to the road, difficult to access (e.g. uneven terrain, crowded vegetation), or had dense vegetation EMR could escape into. EMR were weighed in a bag using a spring scale, and I subtracted weight of the bag to determine body weight (g). After weighing, snakes were coaxed into a snake handling tube (Midwest Tongs Restraining Tubes standard size). A snake probe was used to determine sex for adults and subadults (Schaefer 1934). Females were palpated to determine reproductive status (gravid or not gravid). A handheld passive integrated transponder (PIT) tag reader was used to check for a pre-existing PIT tag (AVID: 12 mm in length, Norco, CA ; Biomark model: 8 mm in length, MiniHPT8 Pre-load Sterile Syringe). If an individual was not carrying a PIT tag, an injection site was swabbed with alcohol along the lower dorsal region of the body above the cloaca and below the widest point of the body and a PIT tag injected between the skin and body cavity. Injection sites were sealed with a cyanoacrylate-based adhesive (e.g., Loctite waterproof super glue; Cobb et al. 2005, Howze et al. 2012, Shaffer et al. 2019).

Following PIT tagging, a very high frequency (VHF) transmitter (Holohil; PD-2 3.8 g; Appendix A) was attached to all adults that were large enough (i.e., transmitters < 5% of total body weight). Before attachment, the bottom of transmitters were roughened to increase surface area for attachment and transmitters were glued to the dorsal side of the body, off center of the spine, and just below the widest portion of the body. All transmitters were secured using Loctite waterproof super glue and a loose strip (not to restrict movement or breathing) of electrical tape to provide further security until glue had fully set. The length of the transmitter antennae never exceeded the body length of snakes. After processing, EMR were placed in the handling bag and transported to capture location for release. All equipment was sanitized with an approximately 10% bleach water solution or disinfecting wipes following protocols from USFWS (outlined in permit; Allender et al. 2018). All capture and handling protocols adhered to guidelines approved by the USFWS (Native Threatened Species-Recovery, Threatened Wildlife Permit #ES34563C-3), Michigan Department of Natural Resources (MDNR; Scientific Collectors Permit #TE225), and Michigan State University's Institutional Animal Care and Use Committee (IACUC; PROO201900452).

Telemetry and Relocation

Telemetered snakes were relocated 1-5 times per week at variable times of day and during variable weather conditions to discern potential influences on body exposure and burrow use from April-October. Telemetry receivers (model R-1000, Communications Specialists, Inc., Orange, CA) were paired with Yagi and H antennas to home on locations of individuals. Once an individual was relocated, I estimated percent body exposure (i.e., unobstructed by vegetation or cover) and whether an individual was inside a burrow. Use of a burrow was confirmed if a strong signal was emanating only from a burrow entrance, but no visual or auditory presence could be confirmed. Though searcher presence on EMR behavior and movements has been documented in gravid EMR (Parent and Weatherhead 2000), I exclusively used the homing method as it was necessary to observe individual body exposure and record burrow use. However, I took care to observe individuals from a distance and minimize observer disturbance to snakes. When an EMR was relocated, I recorded location in Universal Transverse Mercator (UTM) coordinates, weather conditions, and behavior.

ANALYSIS

Detection Modeling

Shaffer et al. (2019) produced a model that predicts EMR detection probability from visual encounter surveys based on minimum daily temperature and time spent surveying within 2 ha. Minimum daily temperature in my study was collected from the nearest weather station (data obtained from the Climate Data Online Data Tools from the National Oceanic and Atmospheric Administration National Centers for Environmental Information [NOAA], and Michigan State University Enviroweather database), with a mean distance of 26.5 km from study sites. Search effort was represented by total time spent surveying multiplied by the number of surveyors and scaled to a 2 ha area to align with the Shaffer et al. (2019) model. Minimum daily temperature and scaled search time were input into the Shaffer et al. (2019) model to provide an estimate of EMR detection probability for each survey. If I surveyed a site more than once, I calculated the cumulative detection probability. To do this I determined the probability of not detecting a snake during each survey by subtracting the survey detection probability from one (Equation 1). As additional surveys were conducted, I multiplied the probabilities of not detecting EMR for each survey to portray a cumulative detection probability (Equation 1).

$$p_{Cum} = (1 - p_1) * (1 - p_2) * (1 - p_n) \qquad \textit{Equation 1}$$

Where p_{Cum} is the cumulative detection probability for an area surveyed multiple times, p_1 is the estimated detection probability from Shaffer et al. (2019) for the first survey, with subsequent p_n for each additional survey conducted.

Body Exposure and Burrow Use Modeling

I hypothesized that cloud cover would be important for EMR detection (Casper et al. 2001, Bartman et al. 2016, Thacker 2020, Kudla et al. 2021, Thacker et al. 2023), and noted from personal observations and communications with other EMR researchers greater body exposure during overcast conditions when temperatures tend to be relatively cooler. Activity of EMR also varies depending on time of day (Szymanski et al. 2016), meriting inclusion of time as a predictor variable in modeling. Julian day was also included as a predictor given that EMR movements and behavior change seasonally (Marshall et al. 2006, Harvey and Weatherhead 2010, DeGregorio et al. 2011, Harvey and Weatherhead 2011, Szymanski et al. 2016) and to also serve as a proxy for changing vegetation structure during the growing season. As EMR populations in this study were observed within 300m of state trunkline, distance from road for each EMR location was included in the model. I included this variable to determine if roads and associated activity (e.g., ground vibrations from passing traffic) may influence EMR body exposure. Temperature was included in the model given it was deemed significant in other EMR detection probability models (Shaffer et al. 2019). Though humidity was not found to influence EMR detection probability in previous studies (Shaffer et al. 2019), I included humidity to further evaluate if it influenced EMR visibility and burrow use. I found that the digital anemometer used to measure temperature and humidity at the location of the snake occasionally yielded some unrealistically hot temperature values. Therefore, anemometer temperature data that exceeded 1.5 times the 3rd quartile of all temperature measurements were removed from the dataset. Given EMR movements vary depending on sex and reproductive status (Foster et al. 2009, Harvey and Weatherhead 2010), sex was also included to determine if body exposure varied between males and females. Individual snake identification was included as a random effect for each model.

I developed two Bayesian hierarchical models using the "brms" package (Bürkner 2021) in R (R Core Team 2022). Models took the following general form:

$$y_n \sim D(\psi_{1n}, \psi_{2n}, \dots, \psi_{kn})$$

Where y_n represents the predicted response based on *n* locations from 35 observed EMR, ψ_k represents the variables (1, 2, k) measured for each n location, and *D* the estimated response distribution (Bürkner 2021). For body exposure, I used individual percent body exposure recorded during each relocation event as the response variable. Given that my response variable was a percent, I specified a beta response distribution that took the form:

$$y \sim \mathrm{Beta}(\psi_1 = \mu, \psi_2 = \phi) = \frac{y^{\mu\phi-1}(1-y)^{(1-\mu)\phi-1}}{\mathrm{B}(\mu\phi, (1-\mu)\phi)}$$

where B is the Beta function, y is the predicted response, μ is the mean, and ϕ the precision (i.e., standard error, Bürkner 2021). Predictor variables for the percent body exposure model included cloud cover, hour of the day, humidity, sex, and temperature, with a random effect for EMR identifier.

For burrow use, I used a binary response variable and specified a Bernoulli response distribution that took the following form:

$$y \sim Bernoulli(\psi) = \psi^y(1-\psi)^{1-y}$$

where *y* is the response (1 = observed using a burrow, 0 = not observed using a burrow), ψ $[0,1]$ is the success probability modeled using a Bernoulli distribution (Bürkner 2021). Predictor variables for the burrow use model included cloud cover, hour of the day, humidity, sex, and Julian date, with a random effect for EMR identifier.

I ran all models for 4000 Monte Carlo Markov Chain (MCMC) sampling iterations across four chains. Multicollinearity was evaluated by calculating variance inflation factors (VIFs) for all covariates in the model where any covariate with a value >3.0 was removed (Zuur et al. 2009). Anticipating a polynomial relationship for temperature and Julian day with body exposure and burrow use, I developed separate models where temperature and Julian day were modelled as linear and a second-degree polynomial. To compare the linear and polynomial models, I used loo function of leave-one-out cross validation (LOOIC; Vehtari et al. 2017) and retained the top-ranking model. Trace and density plots were generated to investigate model diagnostics, and I evaluated R-hat values to ascertain if variance of model parameters between- and within-chain estimates agreed between model iterations. Variables were deemed significant when 95% credible intervals did not overlap 0.

RESULTS

Detection Surveys

From May through October in 2020 to 2023, I conducted 54 visual encounter surveys within 35 sub-sites ($\bar{x}$ = 1.54 surveys/subsite, SE = 0.18, range = 1-7 surveys) at three sites in the southern Lower Peninsula of Michigan (Table 1.1). I surveyed 28 sub-sites a single time, one sub-site two times, and six sub-sites more than two times (Table 1.1). EMR occupancy was confirmed at four sub-sites, one in Oakland and three in Lenawee County (Table 1.1). I calculated cumulative probability of detection for sub-sites that were surveyed more than one time (n=7). Cumulative detection probabilities varied from 0.01 – 1.00 for each sub-site ($\bar{x}$ = 0.16, SE = 0.26; Table 1.1). During surveys, five EMR were detected (male = 1, female = 4), all of which were subsequently captured and telemetered for monitoring. In addition to formal EMR surveys, field crews conducted approximately 630 hours of informal searches (to find more EMR for radio-tagging) from 2020 to 2022. Twenty-four additional EMR were located during informal surveys. Biological data collected on captured EMR are summarized in Appendix B and includes PIT-tag ID number, weight, lengths, sex, reproductive status, number of rattles, and encounter type (i.e., visual or auditory detection). EMR detection surveys were conducted prior to and after completion of ROW mowing on two occasions, and once after an herbicide application along a road safety barrier (survey data included in the detection survey analysis).

No EMR were detected prior to or post mowing maintenance. Prior to herbicide application, one adult EMR was located while surveying. Lastly, field crews found multiple neonates and young of the year EMR and the information on those encounters is included in Appendix C.

Modeling

The majority (n=15) of EMR captured were females (compared to 9 males), of which 12 were gravid based on field palpations of the body. I used 173 locations from 23 radio-tagged adults (14 females (12 gravid), 9 males), and three locations from unmarked EMR encountered during searches for body exposure and burrow use modeling (176 total locations). Field crews purposefully located telemetered EMR across a wide variety of environmental conditions and times of day (Table 1.2). I did not identify multicollinearity among variables and R-hat values were <1.05. However, a scatter plot of temperature and Julian day suggested some correlation, therefore, I did not model these variables together. Temperature was included in the body exposure model due to significance of the variable in predicting EMR detection probabilities (Shaffer et al. 2019). Body exposure models were built with temperature as a quadratic term (LOOIC = -455.9), and a linear term (LOOIC = -456.1), where the linear model ranked slightly higher. As the quadratic model competed with the linear model, I included a graph of the quadratic model in Appendix D, noting that estimated body exposure asymptotes in the thermoneutral range for EMR. The quadratic model for Julian day (LOOIC = 103.9) ranked higher than the linear Julian date model (LOOIC = 107.6), so I retained the quadratic relationship for Julian date. I also compared quadratic terms for Julian day and temperature in the burrow use model and found that including Julian day as a quadratic term (LOOIC = 105.0) slightly outperformed the quadratic temperature model (104.8), therefore I retained quadratic Julian day as a variable for modeling EMR burrow use.

Body Exposure Model

On average, I obtained 8 locations (SE = 0.51) on each telemetered adult EMR. Average body exposure for observed snakes was 42% (SE = 3%) and ranged from 0-100%. Percent body exposure during the active season was positively influenced by ambient temperature ($\beta = 0.28$, SE = 0.11, 95% CI = 0.06 – 0.51; Table 1.3), where body exposure increased from ~25% at ~15° C to ~55% at ~40° C (Figure 1.2). I failed to find an effect of cloud cover, hour of day, humidity, or sex on EMR body exposure (Table 1.3).

Burrow Use Model

I observed EMR using small mammal burrows in upland grassland prairie with sandy soil, hummocks, and in prairie fens throughout the active season, presumably to seek refuge from predators or to meet thermoregulatory needs (Moore and Gillingham 2006). During the overwintering season (November-March), EMR were observed using crayfish and small mammal burrows, and hummocks along ecotones where forested or dense shrub cover met the edge of a wetland. Of the 176 observations included in the model, I found EMR using burrows 20 times (~11% of observations). For models of burrow use, Julian day predicted burrow use, particularly during the last half of the active season (Table 1.4). The relationship for Julian day generally portrayed EMR shifting from overwintering to active seasons and estimated that likelihood of EMR burrow use is <0.05 during most of the active season (Figure 1.3). I also found that probability of using a burrow varied between male and female EMR ($\beta = 2.35$, SE = 1.27, 95% CI = 0.18 – 5.24; Table 1.4). Average burrow use for females (~2% of locations) was lower compared to males (~9% of locations), but males showed substantially more variation in burrow use (Figure 1.4). I failed to find an effect of cloud cover, time of day, or humidity on likelihood of EMR burrow use (Table 1.4). I documented most burrow use in May and September when greater than one-third of EMR locations in those months were in burrows. I note that my sample sizes were small for October as field work concluded.

DISCUSSION

An effective detection survey for EMR is predicated on individuals being visible and available (e.g., aboveground) to detect, which may be influenced by animal behavior and environmental and temporal factors (Casper et al. 2001, Shaffer et al. 2019, Thacker et al. 2023). I found that EMR were most exposed when ambient temperatures approximated the thermal-neutral zone (30-33.6° C, Harvey and Weatherhead 2011). My results indicated that although EMR are visible and available for visual detection at cooler temperatures (~25% body exposed at ~15° C), body exposure doubled at 35° C. Others suggested that EMR detection probability decreases later in the day (Casper et al. 2001, Shoemaker and Gibbs 2010), but I did not observe evidence that EMR body exposure changed as the day progressed. Similarly, Shaffer et al. (2019) found that survey start time was not influential to EMR detection, suggesting that surveys may be conducted at any time of day if temperatures are appropriate. I also found that EMR use of burrows was low (<5% of locations) during the peak of the active season, indicating that EMR are above ground and available for detection at this time. I observed a difference in burrow use between males and females, with males more prone to use burrows during the active season (but this behavior was highly variable).

Previous models for EMR detection (e.g., Shaffer et al. 2019, Thacker et al. 2023) did not include EMR behavior (i.e., burrow use and amount of body exposed while basking). Consistent with other research, detection probability for individual EMR surveys was generally low (i.e., <0.10) with few exceptions, stressing the importance of repeated surveys in the same areas to confirm occupancy. I did not collect ambient daily temperature during formal surveys and instead used data from the closest weather station (26.5 km away). Hence, a limitation of this study was the large mean distance from survey sub-sites to the nearest weather station, which potentially influenced estimated detection probabilities from the Shaffer et al. (2019) model. The Shaffer et al. (2019) model penalizes surveys ($\beta = -1.13$) as temperatures increase, thus estimated detection probabilities from individual surveys in my study may be higher than portrayed by the Shaffer et al. (2019) model because I observed a positive effect of temperature on body exposure. Additionally, surveys were paused to capture and process EMR, which likely impacted the detection probability results due to changes in weather conditions (e.g., temperature, cloud cover) that could have occurred while processing the snake. I recommend avoiding the snake once detected, recording the approximate location, and returning to capture the individual after the survey is completed. With repeated surveys, detection probabilities can approach 1.00, however field crews in this study detected EMR during relatively poor survey conditions (e.g., high daily temperatures) according to the Shaffer et al. (2019) model (detection probability = 0.01). A component of the animal detection process is abundance (Kellner and Swihart 2014), and high abundance can help offset poor survey conditions. Although I did not estimate abundance for my study sites, the sub-site in Oakland County where an EMR was detected during poor survey conditions was embedded in an area where field crews found and telemetered multiple EMR, suggesting that higher abundance potentially played a role in survey results. Surveys conducted earlier in the active season (April-May) when vegetation is relatively low and less likely to obscure snakes may also improve detection, but my results suggest that burrow use is high at this time. I found that the EMR detection survey model proposed by Shaffer et al. (2019) was a useful way to document reliability of sub-site occupancy status but note that the model may be penalizing surveys conducted during warmer temperatures when snakes were most exposed during my study.

Casper et al. (2001) concluded that EMR detection would be highest when surveying between 20-30° C, but research conducted by Shaffer et al. (2019) indicated that detection probability approached 0.00 at 30° C. Additionally, Shaffer et al. (2019) suggested that detection probability was maximized when minimum daily temperatures were 12.8° C (but this estimate was highly uncertain), with detection probability non-linearly declining to 24° C. The temperature variable in the Shaffer et al. (2019) model received minimal support (AIC_{weight} = 0.04) compared to survey search time (AIC_{weight} = 0.96), but temperature was a significant variable in the final model ($p = 0.003$). My body exposure results contradict Shaffer et al. (2019). In this study, EMR body exposure (and presumably likelihood of visually detecting an EMR) linearly increased with increasing temperatures, with body exposure approaching 50% near the thermoneutral range for EMR (30-33.6° C; Harvey and Weatherhead 2011). I note that the Shaffer et al. (2019) model was based on few adult females (i.e., three; federal restrictions limited the number subjected to surgical transmitter implants), whereas much of my data came from gravid females which may partially explain the difference. Furthermore, although Shaffer et al. (2019) explored change in temperature during the survey as a detection covariate it was not significant. Shaffer et al. (2019) recorded temperature at the start and end of surveys, whereas I recorded temperatures when EMR were located. Perhaps cooler mornings (i.e., Shaffer et al. 2019) that rapidly warm to the thermoneutral zone are conducive to detecting EMR.

In my study, cloud cover did not influence EMR body exposure. Ambient temperature and cloud cover were not correlated in my study, but these variables may interact to influence EMR visibility. Lack of direct sunlight to the ground on cloudy days may cause EMR to use locations that are more visually exposed to meet thermoregulatory needs. Similarly, hour of the day was not significant in my study (consistent with Shaffer et al. 2019), but I noted a moderate negative trend on body exposure suggesting that EMR may become less visible from morning to afternoon. I recommend conducting visual detection surveys for EMR during the morning when there is full daylight, and on days featuring warmer ambient temperatures (as temperatures approach 30° C) to maximize potential visibility of EMR for surveyors. Although I found a linear relationship between temperature and EMR body exposure, I suspect that EMR body exposure eventually plateaus as internal body temperatures reach the thermoneutral range (30-33.6° C; Harvey and Weatherhead 2011). Indeed, I found evidence in the form of a competing model that suggested the temperature relationship was quadratic, peaking at about (~33° C; Appendix D).

Field technicians in our study were unfamiliar with EMR detection surveys and were trained at the beginning of each season. Training included identifying EMR by sight and sound, habitat characteristics, seasonal space use patterns, and basic EMR biology. In this study, EMR were detected by individuals without prior experience when estimated probability of detection was low. This finding indicates that with proper training, visual encounter surveys can be implemented by workers with a variety of experience (Appendix H). My results align with findings from Shaffer et al. (2019) who documented that prior survey field experience did not significantly influence detection during EMR visual detection surveys (Shaffer et al. 2019).

EMR were observed using burrows throughout the active season presumably for shelter or to meet thermoregulatory needs. Burrow use was highest (based on proportion of EMR locations in burrows) during spring emergence from, and fall return to hibernacula. EMR in my study tended to remain active around the hibernacula during spring and fall, frequently accessing burrows as surface environmental conditions became unsuitable. I found lowest burrow use during July, suggesting that EMR are aboveground and available for detection. However, body visibility during July may be reduced due to temperatures above 33° C and well-developed vegetation structure. Probability of burrow use increased beginning in late July, generally corresponding to the time of year when gravid females give birth, followed by movement to overwintering sites (Szymanski et al. 2016). By late September and early October, I found that burrow use started to exponentially increase, corresponding to previously documented EMR overwintering behavior (Szymanski et al. 2016). Based on these results, I suggest surveying in the early season (April-early June) on optimal temperature days when EMR may be more exposed and above ground and when vegetation is less developed. If surveys must be conducted mid-summer, I recommend searching in July when temperatures are warmer but on days <33° C. I recommend improving the burrow use model by observing tagged EMR across a range of life stages (e.g., age, reproductive status) in addition to sex.

Regional differences in EMR behavior have been noted among subpopulations (Weatherhead and Prior 1992, Jones et al. 2012, Hileman 2016, Szymanski et al. 2016, Hileman et al. 2017). As results and models of this research are regionally restricted to southern Michigan, USA, I caution on applying these models beyond similar vegetation types and environmental conditions. I recommended that further testing of body exposure and detection probabilities be conducted at other locations across EMR range with varying environmental and habitat conditions, like research that has been conducted on EMR population characteristics (e.g., Jones et al. 2012).

MANAGEMENT IMPLICATIONS

Surveys for federally listed species are often implemented to meet regulatory compliance requirements. Increasing efficiency and effectiveness of surveys can save time and money while providing valuable information for EMR conservation planning, management, and assessment. Surveys should be conducted when EMR are most visible, i.e., they are above ground and exposed. I found that EMR visibility is greatest on days when temperatures approach 30° C, when EMR body visibility is ~50%. Additionally, EMRs are most likely to be above ground during July in southern Michigan, yet vegetation at that time is tall and dense. As a compromise, I recommend conducting EMR surveys during optimal temperatures during later May and June when use of burrows is relatively low, and vegetation is not fully developed. In areas in modeled Tier 1 or Tier 2 EMR habitat subjected to MDOT maintenance or construction activities, I recommend repeated surveys until cumulative detection probability approaches 1.00. My results are specific to the southern Lower Peninsula of Michigan, USA, or other portions of the EMR home range with similar environmental conditions.

ACKNOWLEDGEMENTS

Thank you to Brianna Bassett, Kalin Bayes, Kotaro Seguchi, Lauren Emerick, Maggie Haite, Michelle Roy, and Wiley Welsh for assistance with EMR surveying and monitoring. I also thank my co-advisors, Drs. Gary Roloff and Henry (Rique) Campa III, and committee member Dr. Scott Winterstein for their knowledge, advice, and support. I'm especially grateful to Dr. Steven Gray for analytical assistance. A special thank you to the Michigan Department of Transportation (MDOT) for funding this project (OR#19-137). I coordinated with MDOT to finalize survey site locations and met annually to review research progress and results (the MDOT was not directly involved in data collection, analysis, or writing of this publication). Last but not least, thank you to Michigan State University, the Michigan Ecological Services Field Office (U.S. Fish and Wildlife Service), Michigan Department of Natural Resources, and U.S. Forest Service for their collaboration and support during this project.

TABLES

TABLE 1.1. Number of visual encounter surveys conducted for eastern massasauga rattlesnakes (EMR), cumulative detection probability, and number of EMR detected for 35 study sub-sites (1-5 ha in size) in southern Michigan, USA, 2020-2022.

Sub-site	County	Surveys	Cumulative Detection Probability	EMR Detected
1	Lenawee	1	0.02	0
2	Eaton	1	0.21	0
3	Eaton	1	0.05	0
4	Calhoun	3	0.24	0
5	Lenawee	1	0.03	0
6	Oakland	7	1.00	0
7	Eaton	1	0.05	0
8	Eaton	1	0.05	0
9	Eaton	1	0.14	0
10	Eaton	1	0.06	0
11	Eaton	1	0.05	0
12	Eaton	1	0.78	0
13	Eaton	1	0.05	0
14	Eaton	1	0.10	0
15	Oakland	5	0.57	0
16	Oakland	1	0.01	1
17	Oscoda	1	0.06	0
18	Oscoda	1	0.02	0
19	Oscoda	1	0.07	0
20	Oscoda	2	0.08	0
21	Oscoda	1	0.06	0
22	Oscoda	1	0.08	0
23	Calhoun	3	0.31	0
24	Washtenaw	1	0.04	0
25	Lenawee	3	1.00	1
26	Lenawee	3	0.18	1
27	Lenawee	1	0.07	2
28	Eaton	1	0.02	0
29	Eaton	1	0.01	0
30	Eaton	1	0.02	0
31	Eaton	1	0.04	0
32	Eaton	1	0.02	0
33	Eaton	1	0.01	0
34	Washtenaw	1	0.03	0
35	Washtenaw	1	0.03	0

TABLE 1.2. Variables, definitions, mean, standard errors (SE), and range of field-recorded values for Bayesian regression models of eastern massasauga percent body exposure and burrow use during the active season in southern Michigan, USA, 2020 – 2022.

Variables	**Definition**	**Mean (SE)**	**Range**
Humidity (%)	Relative humidity measured at the snake location.	55.7 (0.7)	14.2 - 100
Temperature (° C)	Ambient temperature at the snake location.	26.4 (0.3)	9.4 - 39.6
Hour of Day	Hour of day the snake was located.	-	7:34:00 - 18:28:00
Julian Day	Date the snake was located. Represented as a count of days since January 1st of each year.	-	96 - 295
Cloud Cover (%)	Ocular estimate of cloud cover at snake location.	48.3 (2.2)	0 - 100

TABLE 1.3. Model estimates, standard errors (SE), upper and lower 95% credible intervals (CI) for predicting eastern massasauga percent body exposure during the active season in southern Michigan, USA, 2020-2022.

	Estimate	**SE**	**Lower 95% CI**	**Upper 95% CI**
Cloud Cover	0.21	0.12	-0.02	0.45
Hour	-0.18	0.11	-0.40	0.03
Humidity	0.01	0.01	-0.02	0.03
Temperature	0.28	0.11	0.06	0.51
Sex (Male)	-0.60	0.41	-1.40	0.24

TABLE 1.4. Model estimates, standard errors (SE), upper and lower 95% credible intervals (CI) for predicting likelihood of eastern massasauga burrow use during the active season in southern Michigan, USA, 2020-2022.

	Estimate	**SE**	**Lower 95% CI**	**Upper 95% CI**
Cloud Cover	-0.18	0.38	-0.93	0.56
Hour	-0.11	0.41	-0.92	0.71
Humidity	0.00	0.03	-0.06	0.07
Julian Day 1	-4.37	4.79	-15.06	4.23
Julian Day 2	14.50	4.93	5.26	24.75
Sex (Male)	2.35	1.27	0.18	5.24

FIGURES

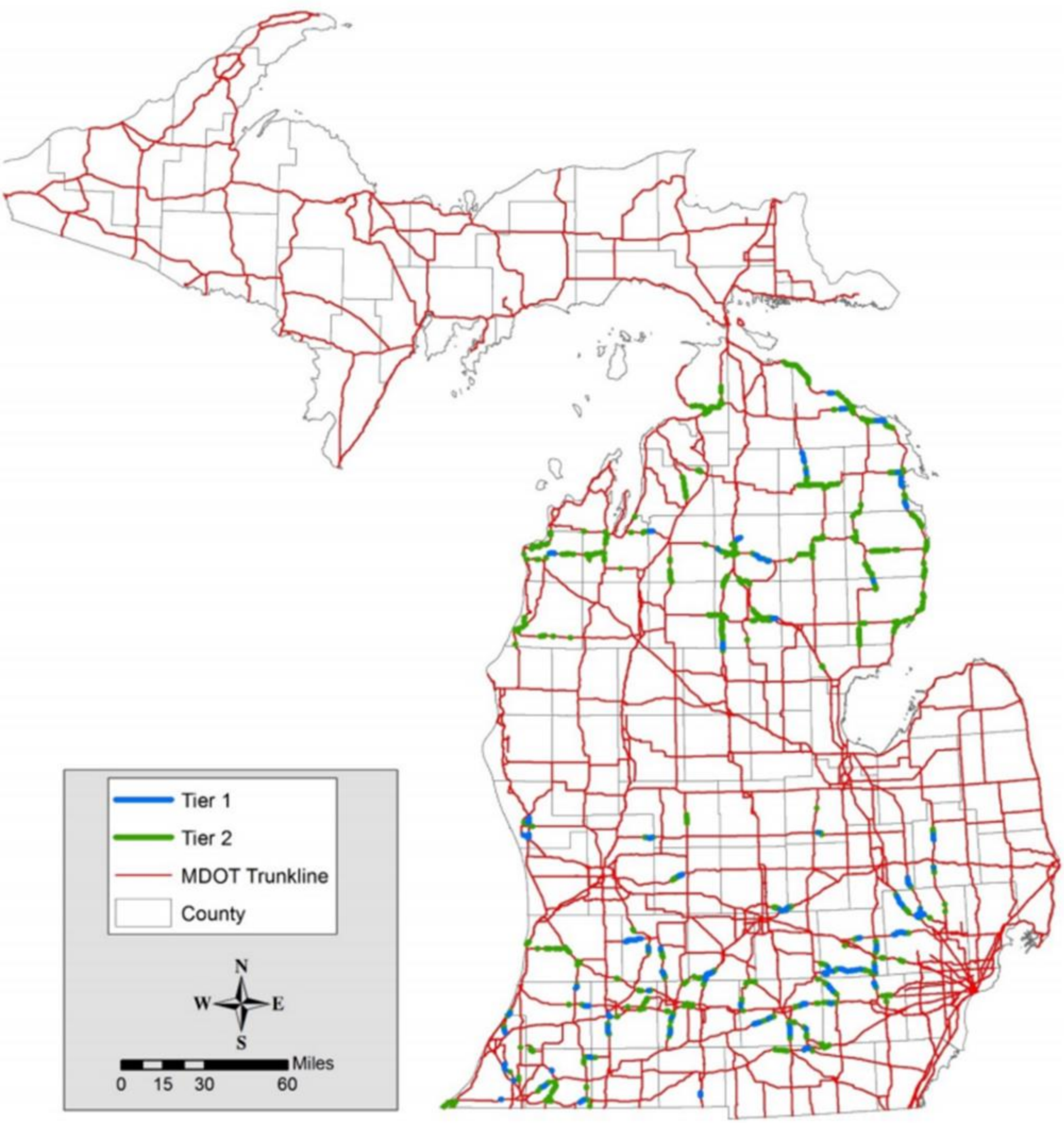

FIGURE 1.1. Regulated state trunkline that intersect with modeled Tier 1 and Tier 2 eastern massasauga habitat in Michigan, USA (USFWS 2018).

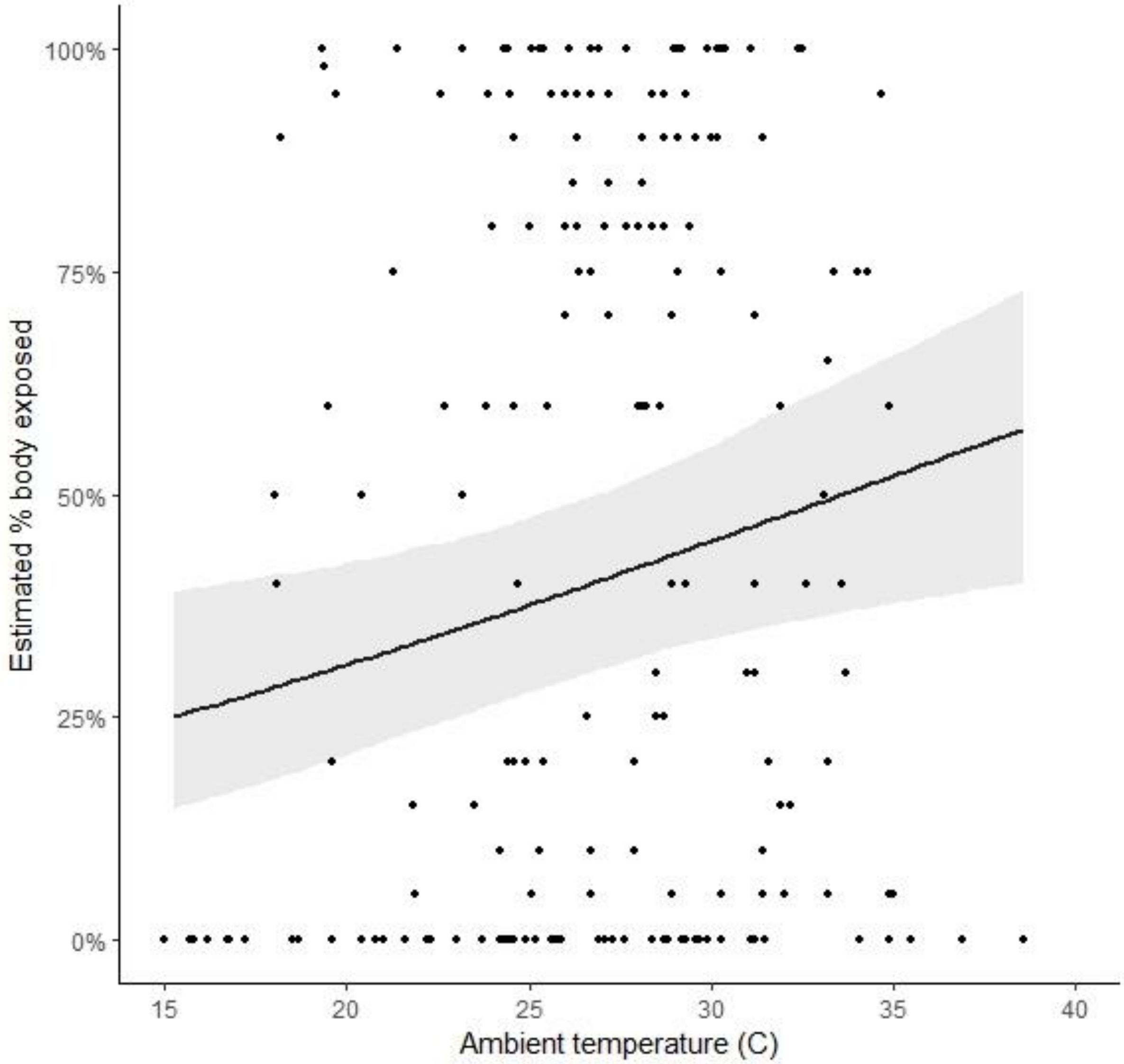

FIGURE 1.2. Estimated percent body exposure of eastern massasauga rattlesnakes and ambient air temperature (Celsius) measured at snake locations during the active season (April-October) in southern Michigan, USA, 2020-2022. Estimated percent body exposure is indicated by the solid line, 95% credible intervals are indicated by the gray ribbon, and observed data by black dots.

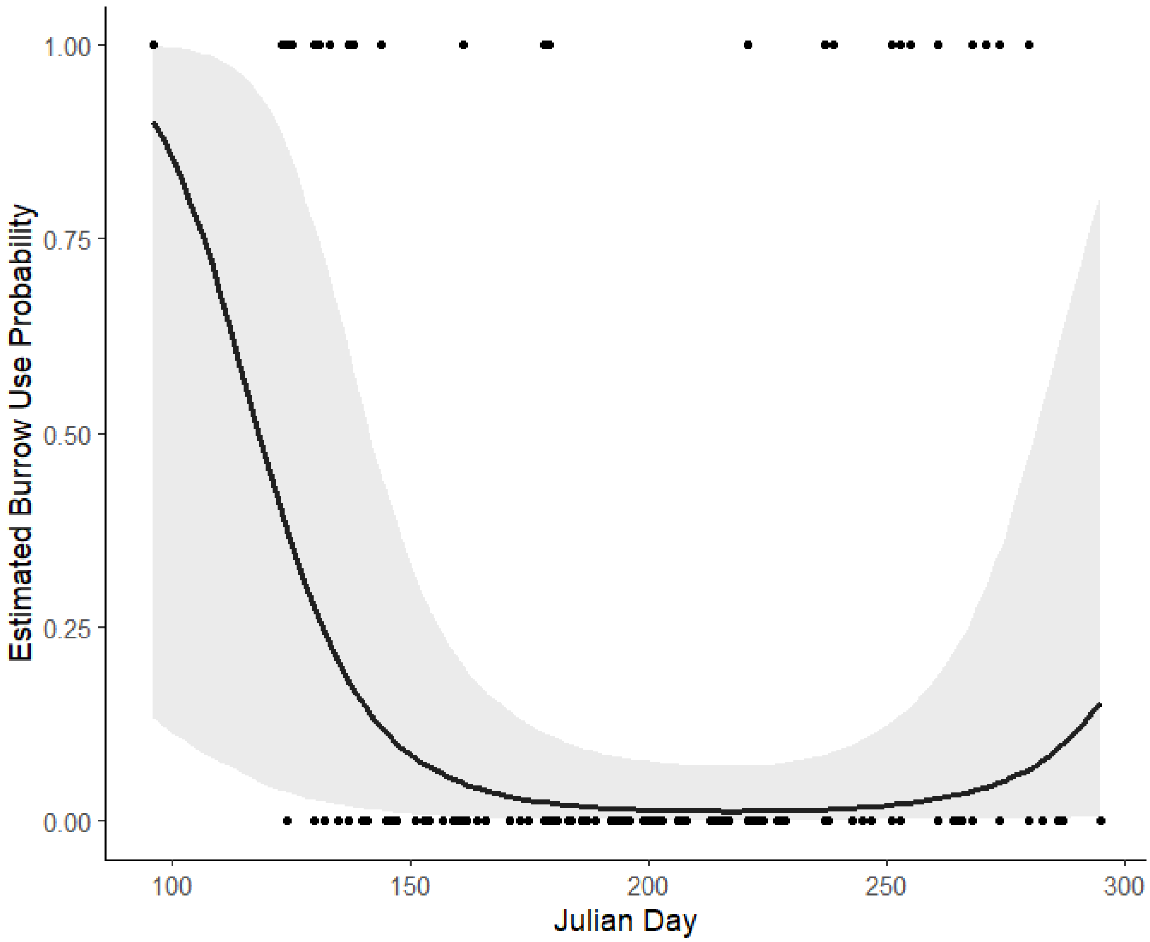

FIGURE 1.3. Estimated probability of burrow use by eastern massasauga rattlesnakes during the active season (April-October) in southern Michigan, USA, 2020-2022. Estimated percent body exposure is indicated by the solid line and 95% credible intervals are indicated by the gray ribbon. Julian Day 200 = July 19.

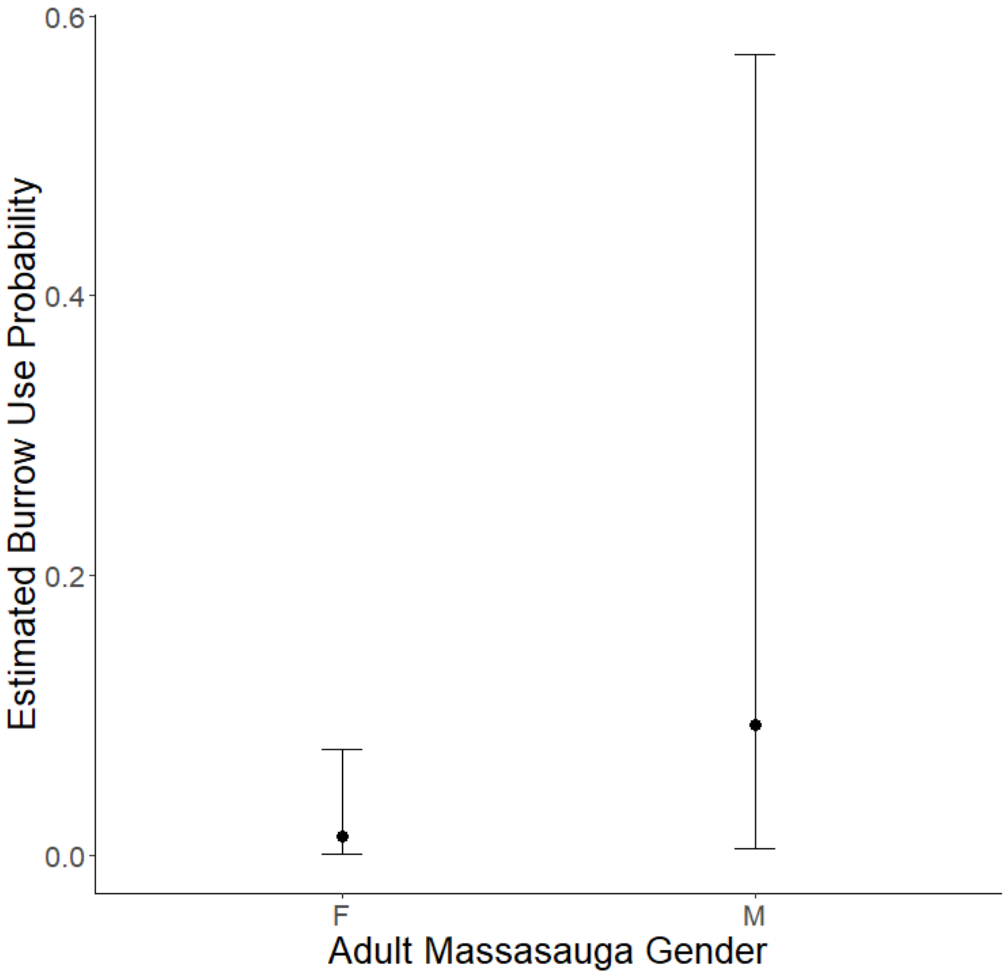

FIGURE 1.4. Estimated probability of burrow use by eastern massasauga rattlesnakes by sex during the active season (April-October) in southern Michigan, USA, 2020-2022. Mean probability of use indicated by solid dot and whiskers represent 95% standard error. The maximum probability of use is equal to 1.

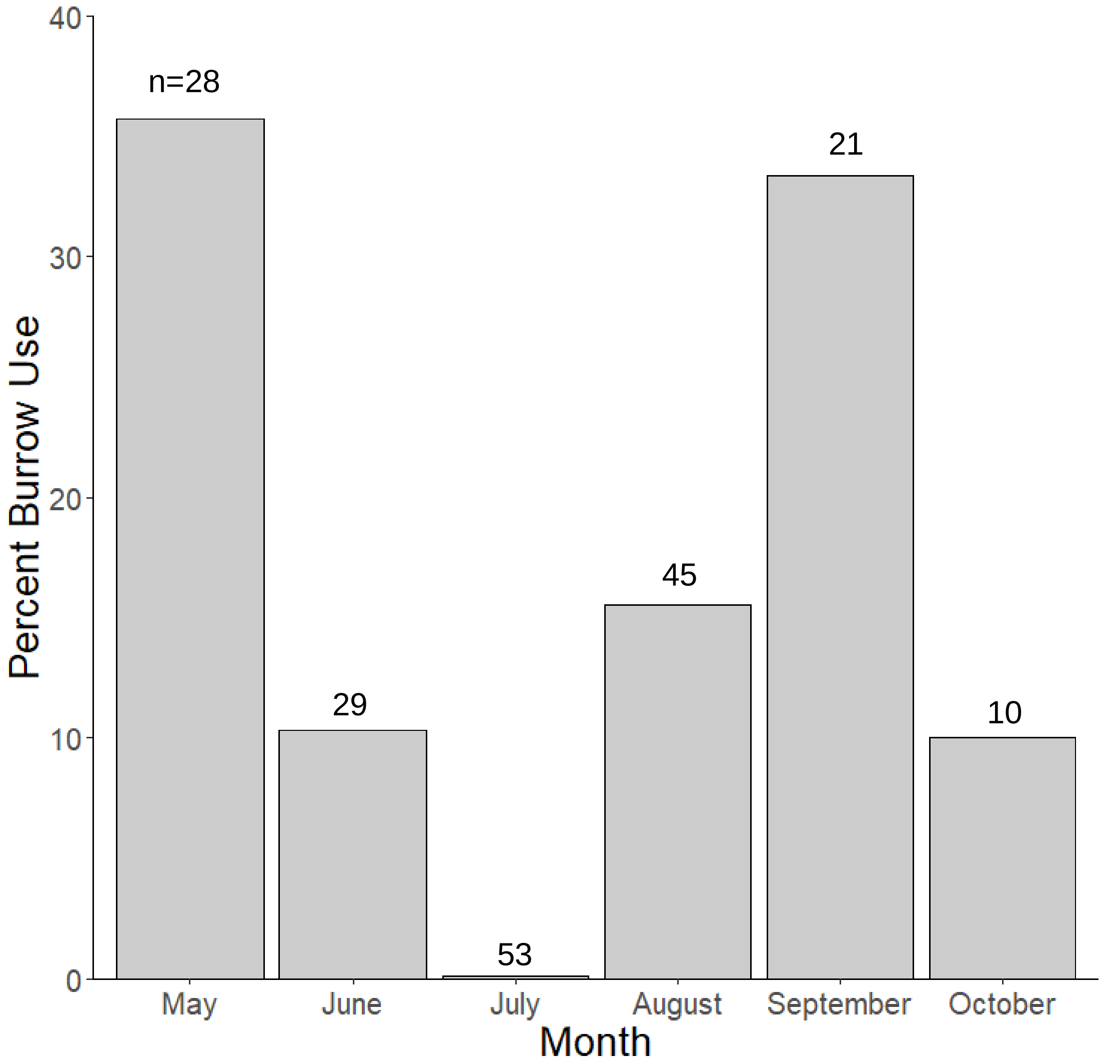

FIGURE 1.5. Percentage of total observations (n = the total number of observations per month) where eastern massasauga rattlesnakes were found using a burrow during the active season from May to October, based on data from two subpopulations in southern Michigan, USA, 2020-2022.

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CHAPTER 2: EASTERN MASSASAUGA RATTLESNAKE (*SISTRURUS CATENATUS*) SPACE USE NEAR MAINTAINED ROADSIDES IN MICHIGAN

INTRODUCTION

Rattlesnakes traverse roads to reach seasonal habitats (Seigel 1986, Seigel and Pilgrim 2002, Jochimsen 2005, Brehme et al. 2013), and may use roads to meet thermoregulatory needs (Colley et al. 2017). However, road-related mortality can threaten snake population persistence (Brehme et al. 2018). Eastern massasauga rattlesnakes (EMR; *Sistrurus catenatus*) are federally listed as threatened under the Endangered Species Act (USFWS 2016), due in large part to habitat fragmentation and loss from agricultural and urban development that includes roads and associated infrastructures (Paterson et al. 2021, Martin et al. 2023). Our understanding of EMR space use near roads is limited to road avoidance (Andrews and Gibbons 2005, McGregor et al. 2008, Paterson et al. 2019), declining occupancy attributed to habitat loss and isolation from road building and expansion (Paterson et al. 2021), and road related mortality (Seigel and Pilgrim 2002, Rouse et al. 2011, Paterson et al. 2019).

Snake use of roadsides varies by geographic location, vegetation, climate, age and structure of the road (e.g., width, number of lanes), traffic patterns, road density (i.e., number of roads in an area; DiLeo et al. 2013), time of day, and road surface substrate (Andrews and Gibbons 2005, Rouse et al. 2011). Despite general aversion to crossing roads, EMR use habitat around roads (Paterson et al. 2019). Published research describing EMR space use patterns around roads and what environmental characteristics might be attracting EMR to roadsides are lacking. Understanding where and how EMR use roadsides will better inform management to reduce impacts of roads, road maintenance, and potential mortality of EMR.

Based on current understanding of EMR habitat requirements, habitat in southern Michigan includes early to mid-successional wetland and upland areas, with patchy herbaceous vegetation and dense, low-growing woody cover (Bissell 2006, Shoemaker and Gibbs 2010, Bailey et al. 2011, Thacker et al. 2023). Live herbaceous cover (e.g., grass, sedge, forbs) typically contains gaps that aid in thermoregulation (Bissell 2006, Bailey et al. 2011). Ecotones along these vegetation types, particularly proximal to dense forest, may also be important for EMR habitat suitability (Bissell 2006) and overwintering (Weatherhead and Prior 1992, Harvey and

Weatherhead 2006, Smith 2009). Maintained road right-of-ways (ROWs) offer habitat conditions suitable for EMR (e.g., open canopy, early successional upland areas with grass and low vegetation cover, and sparsely distributed low woody vegetation). Previous research indicates that the presence and density of woody vegetation and amount of overstory canopy cover tend to negatively relate to EMR space use in southern lower Michigan (Bissell 2006, Bailey et al. 2011, Shaffer 2018, Thacker et al. 2023). However, based on variation in reported woody stem densities used by EMR (Shaffer 2018) and canopy cover (Harvey and Weatherhead 2006, Moore and Gillingham 2006, Johnson et al. 2016, Thacker et al. 2023), further research to determine if woody vegetation is a strong indicator of local site use is needed (Shaffer 2018).

Documented EMR use of roadsides is concerning given areas along roads are often intensively managed (e.g., mowing, herbicides). Natural resource managers require more information about EMR space use near roads to better inform the timing and approaches that could be used for managing roadsides to reduce risks to EMR. My research sought to evaluate seasonal (April – October) temperature patterns and land cover characteristics of roadsides used by EMR, and how patterns of EMR space use may be influenced by climate, environmental conditions, and season. I hypothesized that EMR would show greater use in ROWs consisting of low herbaceous cover (e.g., grasses/sedges, forbs), particularly if ROWs were connected to larger areas of occupied habitat. Furthermore, I hypothesized that EMR space use of roadsides would vary seasonally, with EMR using ROW more in early spring after they emerge from overwintering sites and seek adequate thermoregulatory conditions (Rouse et al. 2011). I also predicted that males would increase their activity near ROWs in late summer and fall for breeding (Rouse et al. 2011).

STUDY AREA

Site Selection

I located two sites occupied by EMR adjacent to Michigan Department of Transportation (MDOT) maintained roadsides in the Lower Peninsula of Michigan. For my study, maintained roadsides included areas along state or federal trunkline that were periodically managed via mowing or herbicide application. Site selection was initially based on occurrence of Tier 1 or Tier 2 modeled EMR habitat (Figure 1.1, USFWS 2018), and final study site selection was approved by MDOT. I initially surveyed for EMR within 300 m of the road edge, which exceeds the radius of the average home range for EMR in Michigan (Lee and Enander 2015). Field visits to potential study sites broadly confirmed suitable habitat conditions (e.g., wet prairie, emergent marsh, early successional forest edges, and upland grassland interspersed with woody vegetation), and I used EMR visual encounter surveys to determine occupancy status (Shaffer et al. 2019). Study site size depended largely on boundaries created by inaccessible private property and natural transitions to less suitable land cover types for EMR. Smaller sub-sites (1-5 ha) were generated within study sites for visual detection and ground cover surveys. Public land access was granted by the Michigan Department of Natural Resources (MDNR; permit ID: TE 225) and site-specific authorizations were issued (USFWS; permit ID: #TE34563C-3). Habitat conditions and apparent road access for EMR varied between the two sites and thus I present each independently.

Site 1 - Lenawee

A US Route bordered the north side of Site 1 in Lenawee County, MI, and the ROW was ~5 m wide with no fence. The ROW was maintained (~2 times per year) via herbicide application and shrub removal during this study, which varied in timing and frequency of occurrence from 2020-2022. The ROW and adjacent upland vegetation were on a south facing slope, consisting of upland grass-prairie and sparse shrub cover (Figure 2.1). The bottom of the slope converged with wet prairie and marsh. Mixed hardwood forests bordered the east and west, and the southern border met a river. Surveyed sub-sites (ranging 1-5 ha) were part of a larger interconnected wetland system, with no apparent barriers to EMR movement between the surveyed areas and adjacent suitable cover types.

Site 2 - Oakland

At Site 2 in Oakland County, MI, a US Interstate Highway bordered the northeastern side, and the ROW was ~25 m wide from pavement to a chain-link fence. Beyond the fence, a south facing slope of upland grass and forb vegetation met a wet-prairie fen. From 2020-2022 mowing was conducted within 5m of the road and was observed to occur two times each year during the study months. A strip (~30 m wide) of dense forest lined the ROW fence between the fen and the roadside (Figure 2.2). A culvert bisected the ROW fence where lower density early successional woody vegetation was mixed with dense herbaceous wetland plants. The culvert and other small streams and tributaries led to a small lake (0.95 ha) at the center of the study site. Beyond the ROW fence (i.e., away from the road), dense forest encircled the lake and an open canopy wet-prairie fen that was ~375 m across. This site was interconnected to a larger, patchy wetland network that was segregated by roads, urban development, and dense woody growth.

METHODS

Vegetation Surveys and Canopy Cover

I collected vegetation data along a systematic grid that overlapped areas where EMR visual detection surveys were conducted (referred to as sub-sites that ranged in size from 1-5 ha). I spaced north-south transects 20 m apart and collected vegetation data every 20 m along transects. Surveyors navigated to vegetation survey locations with compasses and georeferenced maps produced in ArcGIS (ESRI 2020. ArcGIS Desktop: Release 10.8 Redlands, CA: Environmental Systems Research Institute) accessed remotely through Avenza Maps ® (Avenza Systems Inc., Toronto, ON, Canada) on android tablets (SAMSUNG Galaxy Tab 8 inch Android Tablet 64GB). Researchers recorded presence (1) or absence (0) of ground cover that included woody vegetation, low vegetation cover (non-grass/sedge herbaceous cover and woody vegetation under 0.5 m), grasses and sedges, standing water (water present visibly on the surface), and bare soil (>~100 cm^2) in 4-1x1 m sections within a 2x2 m quadrat (Appendix E). I selected these characteristics based on prior EMR literature (Bissell 2006, Shoemaker and Gibbs 2010, Bailey et al. 2011, Thacker et al. 2023) and personal observations. I summed each ground cover type present for the four quadrats at each plot, resulting in values ranging from 0-4 that represented cover classes 0%, 25%, 50%, 75%, and 100%. Grasses and sedges (grouped) were differentiated from other low vegetation cover as they potentially provide less EMR cover than forbs or low-growing woody vegetation (based on observations of EMR in this study consistently using patches of giant mullein (*Verbascum thapsus*) near roadsides in a grass dominated area). At the center of each vegetation survey plot, I took a digital image of overstory cover >30 cm above the ground (Thacker et al. 2023) using a fish-eye lens on a cell-phone camera. I used Gap Light Analyzer (GLA; Frazer et al. 1999) to determine percent canopy openness in each photograph and then inverted these values to represent percent closed canopy.

I also measured vegetation height in the ROW and adjacent areas every ~2 weeks in 2022 at each site to characterize changes in vegetation height over the active season. Vegetation height data were collected independent of EMR survey sub-sites, and I surveyed 20 locations per site along a "T" shaped design (Appendix F). The first 7 samples were located 10 m parallel to the road pavement (Appendix F). Sample locations were 2 m apart (Appendix F). Another 7 samples were located 15 m away and parallel to the road pavement at the same spacing (Appendix F). The final 6 samples extended 20 to 45 m perpendicular from the other locations and away from the road pavement (Appendix F).

Temperature Loggers

I used iButton temperature loggers (iButtonLink Technology model DS1921G-F5# Thermochron 4k; n=14) to passively collect temperature readings above and belowground every 90 minutes at each site from May-October 2021-2022. Aboveground loggers rested on the ground surface in a plastic bag (to protect from moisture), whereas belowground loggers were buried in a plastic bag ~0.15 m under the soil surface (Appendix G). I placed 7 pairs (i.e., above and belowground) of temperature loggers (loggers hereafter) along a transect perpendicular from the road and into adjacent EMR habitat. I placed the first logger pair 10 m from the road pavement. I then spaced subsequent logger pairs depending on EMR locations collected during the active season and known overwintering locations, with the goal of portraying temperature changes in areas used by EMR while active and inactive (i.e., in burrows). After placing the initial logger at Site 1, I placed the six subsequent logger pairs 35 m apart (beginning 35 m from the road; 25 m from logger 1) along a 210 m transect. At Site 2 I placed loggers 50 m apart (beginning 50 m from the road; 40 m from logger pair 1) along a 300 m transect. I used scent removal spray (Wildlife Research Center Scent Killer Spray Odorless Formula) to reduce wildlife tampering with the devices. Data were downloaded from the temperature loggers every ~2 weeks and any damaged equipment replaced.

Site 1 - Lenawee

I placed paired temperature loggers in numerous vegetation cover types. The logger nearest the road (10 m away) was in low vegetation cover under a low growing shrub, which was removed during the overwintering season before April 2022. I observed that mowing ROW maintenance overlapped the location of this logger. Loggers 35 m and 70 m from the road were placed in an open canopy, tall grass upland on a south facing slope. The next logger (105 m away) was near a known hibernacula under low, early successional woody cover. Cover types 140 m away included mid-successional forest with closed canopy. The next logger (175 m away) was under a small shrub in an open canopy wet prairie. I placed the final logger 210 m away in a closed canopy wet prairie consisting of early successional woody vegetation.

Site 2 - Oakland

Logger 1 (10 m from the road) was within the ROW at the edge of a closed canopy hardwood forest in early successional grass and forb cover. Mowing was not observed to overlap the location of the logger. The second logger (50 m from road) was in dense, closed canopy forest. Loggers 3 (100 m from road) and 4 (150 m from road) were in a wet prairie/fen within tall patches of cattails (*Typha* spp.) and phragmites (*Phragmites* spp.). Logger 5 (200 m from road) was under a small patch of trees under a hummock. Logger 6 (200 m from road) was in an open canopy wet-prairie/fen, and logger 7 was in closed canopy wet-prairie/fen.

Capture, Marking, and Locating EMR

I captured, marked (using very high frequency (VHF) transmitters and PIT tags), and monitored individual EMR found during visual encounter surveys and opportunistic (i.e., wandering visual encounter surveys) searching. Researchers picked up EMR using snake tongs and secured the individual in a snake bag (snake bags from Midwest tongs/handmade breathable cotton [dimensions: ~0.30x0.51 m]). EMR were weighed in the bag using a spring scale, and I subtracted weight of the bag to determine body weight (g). After weighing, snakes were coaxed into a snake handling tube (Midwest Tongs Restraining Tubes standard size). A snake probe (standard sexing probe set, 8 piece set with ball tips) was used to determine sex for adults and subadults (Schaefer 1934). I palpated females to determine reproductive status (gravid or not gravid) and used a handheld passive integrated transponder (PIT) tag reader to check for a pre-existing PIT tag (AVID: 12 mm in length, Norco, CA ; Biomark model: 8 mm in length, MiniHPT8 Pre-load Sterile Syringe). If an individual was not PIT tagged, I swabbed an injection site with alcohol along the lower dorsal region of the body above the cloaca and below the widest point of the body and injected a PIT tag between the skin and body cavity. Injection sites were sealed with a cyanoacrylate-based adhesive (e.g., Loctite waterproof super glue; Cobb et al. 2005, Howze et al. 2012, Shaffer et al. 2019).

Following PIT tagging, I attached VHF transmitters (Holohil; PD-2 3.8 g; Appendix A) to adults weighing over ~80 grams (i.e., transmitters < 5% of total body weight) on the posterior dorsal side below the widest part of the body and offset of the spine, on the opposite side of the PIT tag. I used Loctite waterproof super glue to adhere the transmitter to the body, and a loose strip (not to restrict movement or breathing) of electrical tape to allow the glue to fully set. After handling, all equipment was sanitized with an approximately 10% bleach water solution or disinfecting wipes following protocols from USFWS (outlined in permit; Allender et al. 2018). I adhered to all capture and handling protocols approved by the USFWS (Native Threatened Species-Recovery, Threatened Wildlife Permit #ES34563C-3), Michigan Department of Natural Resources (MDNR; Scientific Collectors Permit #TE225), and Michigan State University's Institutional Animal Care and Use Committee (IACUC; PROO201900452).

Telemetered snakes were relocated 1-5 times per week at variable times of day and weather conditions from April to October (2020-2022). Receivers (model R-1000, Communications Specialists, Inc., Orange, CA) were paired with Yagi and H antennas to locate individuals. Though prior studies have documented an impact of searcher presence on behavior and movements for gravid EMR (Parent and Weatherhead 2000), field crews used the homing method (Mech 1983, Bauder and Barnhart 2014) to collect most EMR locations; some locations were calculated via triangulation. During homing, field crews observed individuals from a distance to minimize observer disturbance to snakes. When an EMR was relocated, researchers recorded location in Universal Transverse Mercator (UTM) coordinates.

ANALYSIS

Vegetation Characterization

I generated boxplots to portray vegetation heights near roadsides in 2-week intervals from mid-June to the end of August. Vegetation heights were grouped into four distance (i.e., away from the road) categories, values (n=7) 10 m from the road pavement (or ROW height), 15 m from the road pavement (n=7, near ROW), 20-30 m from the road pavement (n=3, Medium Far), and 35-45 m from the road (n=3, Far).

Seasonal Temperature Change

Some temperatures collected by loggers were unrealistic hot likely attributed to logger malfunction, therefore, I excluded temperature data that exceeded 1.5 times the 3rd quartile of all temperature measurements. Average temperatures were summarized over 2-week intervals from May to October for each logger for 2021 to 2022. Box plots were generated separately for aboveground and belowground loggers, representing temperature changes for 2-week intervals.

Home Range Estimation and Visualization

I generated Brownian-Bridge kernel home ranges using the "adehabitatHR" package (Horne et al. 2007) in R (R Core Team 2022). This type of home range analysis minimizes individual variation and has lower reported error for reptiles, even for individuals with relatively few relocations (Silva et al. 2020). First, I generated movement trajectories between successive locations for each telemetered individual. I specified a 10 m resolution for all resultant kernels and a 5 m smoothing parameter (to align with average accuracy of GPS used in this study; Silva et al. 2020). I inverted volumetric values so that higher values correspond with higher probability of use and low values associated with the periphery of the home range.

I described average probability of use near roads for each study site by combining probability of use surfaces from individual EMR for individuals with >20 locations. This process resulted in a composite site-level probability of use surface for all telemetered EMR. Probability of use values were portrayed in a 10x10 m resolution grid, and calculated by overlapping individual home range grids, summing the use probability within each grid space, then dividing the summed value by the sum of the probability density values from the entire home range surface. This gives the proportionate density of use by all snakes within the used area. This value was then multiplied by 100 to convert to percent probability of use. I then mapped the composite probability of use surface and displayed seasonal shifts in space use by color-coding (by 2-week periods) individual locations at each site.

To elucidate relationships between vegetation cover and EMR probability of use, I overlaid the composite probability of use surface with the vegetation survey grid, and each overlapping vegetation sample location was assigned a corresponding EMR probability of use value (for those use values >0). The EMR use value at these locations represented a systematic sample of EMR use to serve as a response variable in modeling. Additionally, distance from vegetation survey locations to the road pavement was modeled as a predictor of EMR use intensity.

Space Use Modeling

I developed a Bayesian regression model using the "brms" package (Bürkner 2021) in R (R Core Team 2022) to predict intensity of EMR space use. Cover of grasses and sedges, other low vegetation cover, woody vegetation, bare soil, and percent closed canopy were used as model covariates. Models took the following general form:

$$y_n \sim D(\psi_{1n}, \psi_{2n}, \ldots, \psi_{kn})$$

Where y represents the EMR use probability at location *n*, ψ_k represents parameter estimates for variables *1-k* at location *n*, and *D* the estimated response distribution (Bürkner 2021). Given the structure of the response variable (i.e., 1-100), I specified a beta response distribution that took the form:

$$y \sim \mathrm{Beta}(\psi_1 = \mu, \psi_2 = \phi) = \frac{y^{\mu\phi-1}(1-y)^{(1-\mu)\phi-1}}{\mathrm{B}(\mu\phi,(1-\mu)\phi)},$$

where B is the Beta function, y is the response, μ is the mean, and ϕ the precision (Bürkner 2021). I ran models for 4000 Monte Carlo Markov Chain (MCMC) sampling iterations across four chains and evaluated multicollinearity by calculating variance inflation factors (VIFs) for all covariates in the model where any covariate with VIF >3.0 was removed (Zuur et al. 2009). Based on exploratory linear regression plots of EMR use probability and environmental variables, I anticipated a potential polynomial relationship between distance to road and EMR use at Site 2. Therefore, I developed separate models where distance to road was modeled as linear and a second-degree polynomial. These models were then compared using LOOIC and I retained the top-ranking model. Trace and density plots were generated to investigate model diagnostics. Variables were deemed significant when 95% credible intervals did not overlap 0.

RESULTS

Vegetation Structure and Composition

I collected composition data at 88 plots for Site 1 and 71 plots for Site 2 (Table 2.1); these plots overlapped apparent suitable EMR habitat (later verified with telemetry data). Grass/sedge cover was high (≥94%) at both sites, with low (<1 m tall) vegetation cover also relatively high (>72%; Table 2.1). Site 1 had greater cover (~60%) of taller (≥0.5 m) woody vegetation compared to Site 2 (~40%), and bare soil and water cover were low (≤13%) at both sites (Table 2.1). Vegetatively, Sites 1 and 2 in my study were generally similar, except that Site 1 had more tall woody cover. Vegetation cover estimates were precise (i.e., low standard deviations), suggesting homogeneity among plots at a site (Table 2.1).

Seasonal Temperature Patterns

Site 1 - Lenawee

Median aboveground surface temperatures ranged between 15.0° C and 21.3° C during the active season (i.e., April to October) and no consistent pattern with distance from road was observed (Figure 2.4). I found substantial variation in aboveground temperatures within two-week periods during the active season, with some measures exceeding 40° C (Figure 2.4). Conversely, I found a quadratic pattern in belowground temperatures, with a temperature peak from July 1-14th to August 15-31st (median temperatures ranging from 17.8° C to 20.8° C), and that loggers closer to roads (Logger IDs 1-3) tended to record warmer median temperatures (Figure 2.5). Belowground temperatures were consistently higher closest to the road (10 m away), with less variation in median belowground temperature across the site in September and October (Figure 2.5. On average, belowground temperatures closest to the road (10 m away) were 1.5° C higher compared to the farthest temperature logger at the site (210 m away).

Site 2 - Oakland

I observed similar patterns in median above- (range ~15.0° C to 21.3° C) and below- (~13.5° C to ~20.5° C) ground temperature readings at Site 2, with no discernable pattern in aboveground temperatures throughout the summer and no consistent pattern with distance from the road; Figure 2.6). However, belowground loggers indicated that average temperatures followed a quadratic pattern over the active season and were greatest from June 15-30th to August 15-31st (Figure 2.7). At Site 2 distance from road was less influential on belowground temperatures compared to Site 1 (Figure 2.7), potentially because of the closed canopy forest adjacent to the ROW (Figure 2.2). Belowground temperatures in the spring (May 1-14) were 2-4° C cooler than above ground temperatures, particularly in the lower-lying areas farthest from the road (Figure 2.7). On average, belowground temperatures closest to the road (10 m away) were 1.0° C higher compared to the farthest temperature logger at the site (300 m away).

Telemetry and Observations

I observed 54 unique EMR in this study; 23 – neonates, 1 – juvenile, 1 – sub-adult, 5 – non-captured adults, and 24 – captured and telemetered adults; Appendices B, C). Monitoring for 24 telemetered EMR varied due to drop off and/or loss of glue-on transmitters or transmitter battery failure, with the average transmitter remaining attached for 12 days (n=61 captures, SE = 1.96 days, range = 1-64 days). If a transmitter was shed, I sought to recapture that individual to continue monitoring. I recaptured five individuals at least once from 2020-2022; two individuals were re-captured across more than one season. Of the 381 total EMR locations gathered across both study sites, 26% were collected using triangulation (average triangulation error for three data collectors was 10.1 m (SE=1.43), the remaining locations were from observations of untagged EMR or telemetry homing. The homing method was used exclusively in 2022.

Site 1 - Lenawee

Of the 24 EMR observed from 2020-2022, I captured and tagged 14 at Site 1. From May – August, EMR (particularly gravid females) were regularly (1-5x per week) observed within 10 m of the roadside (Figure 2.8). I observed five EMR litters at this site, and birthing locations ranged from 1-10 m off the road pavement. Post birthing, I observed adults and neonates using vegetation beneath staghorn sumac (*Rhus typhina*), discarded rubber tiles (e.g., roofing tiles), or small mammal burrows as cover and refuge for ~1 week before dispersing. In general, EMR located near the roadside were found in upland vegetation dominated by grasses and forbs, with sparse, low woody cover. Some EMR were also observed using wet prairie and marshes within 300 m of the road, predominately early and late during the active season (Figure 2.8). I observed three overwintering sites (ranging ~60 m to ~240 m from the road; Figure 2.8) based on locations of telemetered snakes in the late season (late September-October). Hibernacula consisted of crayfish burrows within early successional low woody cover, or underneath hummocks and root knots in a fen densely covered with staghorn sumac.

I created movement trajectories for 13 individuals with ≥2 locations (12 adults, 1 sub-adult). Based on these trajectories, average total movement of EMR individuals over 2-week periods from May 1st to October 31st (n=197 location pairs) averaged = 36.0 m (SE = 2.6, range = 4.0 - 112.3 m) and spanned ~0.03 ha (~312 m east to west). EMR had the lowest average movements from May 1^{st}-14^{th} (n=4 location pairs), with average movement distance of 4.0 m (SE = 1.3). EMR moved most between September 1^{st}-14^{th} (n=13 location pairs), with average movement distance of 112.3 m (SE = 2.6).

Median vegetation heights from June 15 to August 31 (measured in 2021) were generally >40 cm; the exception was in the ROW which was mowed in August (Figure 2.9). On average, I found EMR within 25 m of the road until mowing, when average distance increased to >75 m away. It is not clear if EMR moved away from the road in response to mowing, changes in temperature near the roadside, or if the movement corresponded to an overall shift in space use as gravid females (n=6) moved back to the wet prairie after giving birth (Foster et al. 2009).

Site 2 – Oakland

I captured and radio-tagged 10 EMR at Site 2 from 2020-2022 and observed 14 EMR overall. Overwintering sites (two confirmed hibernacula; ~40 m and ~300 m from the road; Figure 2.10) had similar conditions as Site 1, with adult EMR using crayfish burrows in early successional woody vegetation along forest-wetland ecotones. Areas south of the ROW fence (i.e., away from the road) were used by all observed EMR life stages during the active season and consisted of wet prairie-fen, surrounded by closed-canopy forest with a small lake in the center and several small outflowing streams. Large, dense patches of invasive narrowleaf cattails (*T. angustifolia*) and phragmites (*P. australis*) occurred around the lake. During the study, researchers found two EMR within the interstate ROW; one was deceased and the other was injured and died within 24 hours of initial encounter. A necropsy was unable to be performed and cause of death was undetermined.

Average total movement at Site 2 for 9 EMR over 2-week periods from May to October (n = 125 location pairs) averaged 77.4 m (SE = 10.0; range = 5.0 - 194.8 m) and spanned ~0.05 ha (~458 m along the longest axis). EMR showed lowest average movements from May 1st-14th (n = 6 location pairs) with average movements of 5.0 m (SE = 1.0 m). EMR moved the most between October 15th - 31st (n = 2 location pairs) averaging = 194.8 m (SE = 185.3 m) and from September 15th - 31st (n = 12 location pairs) averaging = 162.0 m (SE = 55.4 m). Whereas observed neonates at Site 1 all occurred within ~40 m of the road, neonates at Site 2 were observed >~200 m from the road in the prairie fen.

Median vegetation heights in the ROW and in near ROW zones were consistently < 60 cm (Figure 2.11). Vegetation heights in the ROW were consistently shorter compared to other distances away from the road, but radio-tagged EMR generally stayed >130 m from the road throughout the active season (Figure 2.10).

Home Range

Site 1 - Lenawee

I used four (3 – female, 1 – male) individuals with ≥20 locations for home range analysis (average = 30 locations/EMR (SE = 3; range = 24-37). Composite (i.e., for multiple EMR with potentially overlapping home ranges) EMR use ranged from 1-85% (Figure 2.8). I found concentrated EMR use along the road, with most use occurring from May – August (Figure 2.8). Observations of non-telemetered individuals (n = 6) and individuals with <20 locations (n = 12) overlapped modeled areas of use 100% of the time (Figure 2.8).

Site 2 - Oakland

I retained three EMR (2 – female, 1 – male) with ≥20 locations (locations, average = 31 locations/EMR (SE = 3; range = 27-36) to create individual home ranges at Site 2 (Figure 2.10). Composite use ranged from (1-76%; Figure 2.10). Core use areas tended to occur >25 m from the road, with a highly concentrated use zone in fen habitat ~160 m from the road (Figure 2.10). Observations of non-telemetered individuals (n = 4) and individuals with <20 locations (n = 7) overlapped modeled areas of use 100% of the time at site 2 (Figure 2.10).

Use Intensity and Vegetation Cover

Visual inspection of biplots revealed that ground cover (grass and sedge, low vegetation cover, woody vegetation, bare soil, and standing water) did not influence EMR probability of use at either site. Thus, I excluded these variables from modeling, and final site-level models included only distance to road and canopy cover as predictor variables.

Site 1 - Lenawee

I assigned an EMR use probability to 88 vegetation sampling locations at Site 1. The Site 1 model included canopy cover and distance to road as linear terms. Proximity to road did not influence EMR space use (β = -0.002, 95% CI = -0.005 – 0.001; Table 2.2), and canopy cover negatively correlated with EMR use (β= -0.010, 95% CI = -0.014 – -0.006; Table 2.2), where increasing canopy cover (0% to 90%) reduced EMR use from 45% to 20%; Figure 2.12). All predictors had R-hat values <1.05.

Site 2 - Oakland

I assigned 71 vegetation sampling locations an EMR use probability at Site 2. I found that modeling distance to road as linear (LOOIC = -116.9) was less supported than a model that included distance to road as a second-degree polynomial term (LOOIC = -126.2). Canopy cover had no effect on EMR space use (β = -0.001, 95% CI = -0.004 – 0.002; Table 2.3). Distance to road affected EMR probability of use at Site 2, with use increasing from 0 to ~100m away (β = -1.128, 95% CI = -1.957 – -0.299), and then declining beyond 100m (β = -1.421, 95% CI = -2.219 – -0.610; Table 2.3). The effect size was greater beyond 100 m (Figure 2.13). Estimated EMR use probability was ~40% near the road and increased until ~100m from the road when use began declining by 1.42% for every meter of distance (Figure 2.13). All predictors had R-hat values <1.05.

DISCUSSION

In general, information on EMR space use around roads and how EMR potentially respond to regular ROW maintenance is lacking; I only found 12 peer-refereed articles focused on addressing EMR-road interactions (Andrews and Gibbons 2005, Miller 2006, McGregor et al. 2008, Shepard et al. 2008a, b, Rouse et al. 2011, DiLeo et al. 2013, Colley et al. 2017, Matthews 2018, Paterson et al. 2019, Choquette et al. 2020, Paterson et al. 2021). I found EMR using

ROWs at two sites studied in central Michigan, however, intensity of space use differed between the sites. This difference is likely attributed to differences in spatial configuration of habitat, vegetation composition and structure, and corresponding soil temperatures. I documented core use areas for EMR (and successful reproduction) within 25 m of a heavily traveled state highway at a study site that had a continuous transition of suitable habitat (i.e., low tree cover, moderate cover of herbaceous vegetation; Bissell 2006, Bailey et al. 2011) from wet, lower areas to the drier, higher elevation locations along the road (~300 m total distance). Conversely, EMR core use areas were >25 m from a road at another study site where suitable habitat was bisected by a narrow (~30 m) strip of closed canopy forest. Based on my observations of EMR space use at this site, unsuitable cover types separating the road from adjacent suitable cover types may limit EMR use of roadsides. Although conditions in the ROW may mimic suitable habitat for EMR, observations at Site 2 suggest that EMR were more likely to occupy the road, potentially because the area of suitable habitat was relatively narrow with cooler belowground temperatures compared to temperatures in the strip of forest separating the occupied fen from the roadside. Further investigation across the range of EMR is warranted to investigate whether this pattern holds across more sites, but my results suggest that maintaining unsuitable vegetation types along ROWs may help reduce EMR-road interactions. Additionally, ROW that are part of unbroken lowland to wetland EMR habitat may be high use areas warranting EMR surveys. My below ground temperature data indicate that roadsides can provide temperatures (i.e., closer to the thermoneutral range for EMR) that are apparently attractive to EMR (especially gravid females). Furthermore, my results indicate that using localized measures of vegetation structure (e.g., cover of grasses or forbs), which are often used to estimate site occupancy for EMR (Marshall et al. 2006, Harvey and Weatherhead 2006, Moore and Gillingham 2006, Bailey et al. 2011, DeGregorio et al. 2011), are not effective for predicting core use areas within home ranges.

My results indicated that sites with higher average belowground temperatures near roads influence early season space use of EMR. Belowground temperatures may vary depending on presence of urban structures, vegetation cover, anthropogenic vegetation disturbance, elevation, and slope (Shreve 1924, Tang et al. 2011, Song et al. 2013, Roundy and

Chambers 2021). Thermoregulation is an important factor affecting habitat selecting for EMR (Harvey and Weatherhead 2006, 2010, 2011, Foster et al. 2009, Shoemaker and Gibbs 2010, Johnson et al. 2016), and the effect is often more pronounced for gravid females especially early in the active season (Foster et al. 2009, Harvey and Weatherhead 2010, 2011). Although researchers often measure aboveground temperatures to understand EMR detection probability (Shaffer et al. 2019, Thacker 2020, Thacker et al. 2023), average aboveground temperatures were relatively consistent throughout the active season for my study sites. Hence, use of average aboveground temperatures to identify core use areas for EMR appears limited (within the range of temperature conditions I measured). However, I documented considerable variation in aboveground temperatures within two-week periods and EMR likely respond to this variation in temperature more than a mean value. In areas of continuous habitat from wetlands to upland roadsides, the timing of EMR concentrating use along roadsides seemed to correspond to warmer belowground temperatures. The belowground temperature pattern was less pronounced at the site with discontinuous habitat from the wetland to upland roadside, with warmer temperatures occurring an open, south-westerly aspect 100-150 m away from the road. Prior research has indicated that changes in belowground temperatures can affect EMR spring emergence (Mauger and Wilson 1999) so EMR response during the active season to belowground temperatures is not surprising, but this relationship to EMR use intensity and roads warrants further study.

Studies on occupancy requirements for EMR indicate that vegetation structure is more influential than plant community composition or soil type (Bailey et al. 2012, USFWS 2016: 67194). A variety of land cover types and microhabitat characteristics are used by EMR (Harvey and Weatherhead 2006, Moore and Gillingham 2006, DeGregorio et al. 2011, Bailey et al. 2012, Szymanski et al. 2016), but current EMR sub-populations may occur in less than optimal areas due to habitat loss and fragmentation, declining habitat quality (e.g., from pollution and runoff, forest encroachment), and reduced connectivity (e.g., closed canopy forest, urban structures, roads). In this study, connectivity between ROW and adjacent habitats played an important role in EMR space use, and ultimately use intensity along roadsides. Interestingly, I failed to detect road-related mortalities at the site with continuous habitat from the wetland to the upland roadside, yet the roadside at this site was regularly used by gravid EMR for birthing sites. One telemetered EMR birthed at the same roadside site for two (non-consecutive) years. Anecdotally, I observed an EMR along the roadside in this study recoiling in response to large passing vehicles, but not fleeing, despite findings that EMR may avoid roadsides as a result traffic noise and vibration (Rouse et al. 2011, Paterson et al. 2019).

Core use areas for EMR during the active season in my study tended to center on warmer upland areas dominated by herbaceous vegetation <75 cm tall (Site 1) and warmer, open canopy prairie-fen (Site 2). Areas overlapping closed canopy forest and areas dominated by dense invasive forbs (e.g., *Phragmites* spp.) are deemed low quality for EMR (Reinert and Buskar 1992, Sage 2005, Bissell 2006, Harvey and Weatherhead 2006, Bailey et al. 2012) and were available but not used by EMR in this study. My results indicated that given suitable habitat, microhabitat conditions play less of a role in defining EMR space use than overall structure of land cover and habitat contiguity around roads (Andrews and Gibbons 2005). In areas with continuous habitat to roadsides and where concerns over EMR vulnerability to vehicle strikes exist, bisecting that habitat with closed canopy forest (~30 m wide in this study) may help reduce EMR roadside use. However, I found that EMR at one of my sites appeared to select roadside habitats for birthing and, in the absence of documented mortality from road strikes, these areas may serve as important population source habitats and should be managed as such.

I found some evidence that vegetation height near roads influences EMR space use at one of our sites, where snakes on average were farther from the road when vegetation height decreased in the ROW after mowing maintenance had occurred. However, movement coinciding with shorter vegetation from a mowing event was only observed once, and therefore this relationship should be explored further. The timing of mowing I observed coincided with seasonal movements away from birthing sites, which makes it difficult to ascertain if an increase in average EMR distance to the road was a result of seasonal movement or mowing. Differences in implementation of scheduled mowing maintenance between the two study sites limited my ability to monitor EMR movements prior to, during, and post maintenance. Following USFWS best management practices, mowing was to be conducted in similar ways at similar times of the active season at both sites (USFWS 2018). It would be valuable for management agencies to know if intensity of roadside maintenance could affect EMR use near roads or how EMR behavior and movement patterns may change in response to maintenance intensity. I recommend that future research projects systematically monitor ROWs managed with variable maintenance intensity (e.g., mowing) that potentially degrades habitat quality for EMR more than once during the active season, maintenance that degrades habitat only in the early season (prior to August) before litters are being born, and at a site where no maintenance occurs in the ROW.

Regularly occupying maintained ROWs puts EMR at increased risk of mortality (Rouse et al. 2011, DiLeo et al. 2013, Paterson et al. 2019), which is particularly concerning given the abundance of reproductively mature EMR and neonates observed <10 m of the road in this study. Road related mortality decreases when suitable habitat is available and accessible without crossing a road (Seigel and Pilgrim 2002). I observed this pattern at my study site, where EMR used habitat absent of dense forest between the wetland to roadside uplands. I documented low EMR roadside use and two road-related mortalities at the study site with discontinuous habitat (Site 2), suggesting that habitat in the ROW was not suitable to support EMR (Siegel and Pilgrim 2002). It is likely EMR are traveling through the ROW but not consistently occupying the space. Alternatively, roadside conditions (e.g., higher ambient temperatures; Bogren et al. 2002) could draw EMR to the roadside where they experience mortality while trying to meet their life requisites (e.g., thermoregulation during gestation).

Previous research failed to identify negative impacts of road density and road-related habitat loss on reptile occupancy (Paterson et al. 2021), whereas other studies documented negative impacts of roads on reptiles (Jochimsen 2005) and EMR specifically (Andrews and Gibbons 2005, Miller 2006, McGregor et al. 2008, Shepard et al. 2008a, b, Rouse et al. 2011, DiLeo et al. 2013, Colley et al. 2017, Matthews 2018, Paterson et al. 2019, 2021, Choquette et al. 2020). My study found that landscape context (i.e., continuous vs discontinuous habitats, or habitat quality) is an important consideration when documenting road effects on EMR. The most consistently used roadside in my study was along a US Route with two lane traffic (~89 kmh (55 mph)) speed limit, annual average daily traffic (AADT) = ~5300; MDOT 2023) located near a recreational venue that regularly holds large events. My least used roadside where I documented EMR mortality was a 6-lane Interstate Route (~113 km/hr (70 mph)) speed limit, AADT = ~60,700; MDOT 2023). My study suggests that roadside habitats can contribute to EMR conservation, but a deeper understanding of how road type, structure, age of the road and potential isolation of EMR sub-populations, and traffic, influences EMR space use is needed (Dileo et al. 2013, Paterson et al. 2021).

Given previous literature suggesting that EMR space use is driven by fine-scale habitat characteristics (Thacker et al. 2023) and that landscape context and road use varied between my two sites, I took a site-level approach in this study. I caution that my analysis represents a small sample of roadside habitats used by EMR in southern Michigan. As such, there is still a need to explore EMR space use and interactions with roads across a broader extent. I recommend future studies explore impacts of habitat connectivity, habitat quality and structure, and characteristics of the road to discern where EMR rely on roadside habitats. My study was also limited by frequent dropping of radio-transmitters. Use of implanted transmitters is recommended for long-term monitoring, as acceptable implant methods are available (Bissell 2006, Bailey et al. 2011, Shaffer 2018) that do not require repeated handling of EMR. Moreover, implanted transmitters are conducive to long-term monitoring efforts as the transmitter is retained when individuals shed their skin. Based on personal observations while surveying potential study sites along roadsides across eight counties at the start of this project, size and quality of habitat adjacent to roads (and associated soil temperatures), connectivity between hibernacula and birthing sites, and distance between ROW and adjacent habitat appear to play the largest role in EMR space use along roadsides. In this study, EMR did not avoid using roadsides, which aligns with prior literature (Paterson et al. 2019). Movement patterns of EMR may not be consistent over time and 1-2 years of data may not sufficiently describe space use for EMR in a changing landscape (Fitch 1999, Seigel and Pilgrim 2002). This is particularly true for areas that are actively managed, such as roadsides.

MANAGEMENT IMPLICATIONS

Managers responsible for EMR conservation and roads are faced with a dilemma. In some instances (i.e., continuous habitat in this study), roadsides can serve as important population sources for EMR. In other instances (i.e., areas with discontinuous EMR habitat between core use areas and roadsides), use of ROW habitat by EMR is lower but those habitats may not hold EMR, causing movements potentially into the road. The dilemma is whether to discourage roadside use (and potentially disrupt important source habitat) by disconnecting core use areas from roadsides, or potentially increasing roadside use (and perhaps providing source habitat) by making discontinuous habitat more continuous. I found closed canopy forest can serve as a limitation to EMR movements between areas used by EMR in the active season and managed roadsides and thus, management of closed canopies can be used to affect ROW use by EMR. I recommend further research into the potential of maintaining strips of dense (90-100% canopy cover) native trees and shrubs between active season habitat and roadsides to reduce EMR ROW use. This strip (~30 m wide in this study) potentially resulted in cooler belowground soil temperatures which are less appealing to gravid EMR. If managers want to increase habitat quality within roadsides, I recommend placing artificial cover (e.g., coverboards, down and dead woody debris from other vegetation management practices) near the ROW as refuge sites. I regularly observed EMR using roadside litter and a fallen traffic sign as cover in a ROW.

ACKNOWLEDGEMENTS

Thank you to Brianna Bassett, Kalin Bayes, Kotaro Seguchi, Lauren Emerick, Maggie Haite, Michelle Roy, and Wiley Welsh for assistance with EMR surveying and monitoring. I also thank my co-advisors, Drs. Gary Roloff and Henry (Rique) Campa III, and committee member Dr. Scott Winterstein for their knowledge, advice, and support. I'm especially grateful to Dr. Steven Gray for analytical assistance. A special thank you to the Michigan Department of Transportation (MDOT) for funding this project (OR#19-137). I coordinated with MDOT to finalize survey site locations and met annually to review research progress and results (the MDOT was not directly involved in data collection, analysis, or writing of this publication). Last but not least, thank you to Michigan State University, the Michigan Ecological Services Field Office (U.S. Fish and Wildlife Service), Michigan Department of Natural Resources, and U.S. Forest Service for their collaboration and support during this project.

TABLES

TABLE 2.1. Variables and mean (standard deviation; SD) and range of measured values by site used to model probability of eastern massasauga space use in southern Michigan, USA, 2020-2022 (Site 1 = Lenawee County, Site 2 = Oakland County).

	Site 1 (88 plots)		Site 2 (71 plots)	
Variable	Mean (SD)	Range	Mean (SD)	Range
Ground Frequency (%) [a]				
Grass/sedge	94.0 (4.8)	0.0-100.0	97.3 (4.8)	0.0-100.0
Low (<1m tall) vegetation	72.8 (4.3)	0.0-100.0	93.0 (4.8)	0.0-100.0
Woody vegetation (≥0.5m tall)	60.3 (3.8)	0.0-100.0	39.8 (3.3)	0.0-100.0
Bare soil	8.3 (1.5)	0.0-100.0	6.8 (1.3)	0.0-100.0
Water	6.5 (1.3)	0.0-100.0	13.0 (1.8)	0.0-100.0
Canopy cover (%)[b]	53.9 (2.4)	0.0 – 89.2	44.4 (3.3)	0.0 – 93.7
Distance to road (m)[c]	80.3 (5.3)	3.3 – 155.6	114.2 (10.6)	0.1 – 260.2

[a] Based on presence/absence in 4 – 1 x 1 m sub-plots in a 2 x 2 m area.

[b] Open sky >30 cm above the sample location as estimated from hemispherical photography (Thacker et al. 2023).

[c] Euclidean distance from vegetation sample point to road pavement.

TABLE 2.2. Estimates of eastern massasauga probability of space use from a Bayesian regression model (Beta distribution) for Site 1 (Lenawee County) in southern Michigan, USA, 2020-2022. SE = standard error; 95% CI = 95% credible interval.

Variable	**Estimate (SE)**	**95% CI**
Closed Canopy	-0.010 (0.000)	-0.014, -0.006
Distance to Road	-0.005 (0.000)	-0.005, 0.001

TABLE 2.3. Estimates of eastern massasauga probability of space use from a Bayesian regression model (Beta distribution) for Site 2 (Oakland County) in southern Michigan, USA, 2020-2022. SE = standard error; 95% CI = 95% credible interval.

	Estimate (SE)	**95% CI**
Closed Canopy (Linear)	-0.001 (0.00)	-0.004, 0.002
Distance to Road (poly 1)	-1.128 (0.42)	-1.957, -0.299
Distance to Road (poly 2)	-1.421 (0.41)	-2.219, -0.610

FIGURES

FIGURE 2.1. Road right-of-way at a site occupied by eastern massasauga rattlesnakes (Site 1) with continuous wetland to upland habitat in Lenawee County, MI, USA. August 4, 2021 (Photo: J. Rajewski).

FIGURE 2.2. Road right-of-way at a site occupied by eastern massasauga rattlensakes (Site 2) with discontinuous wetland to upland habitat in Oakland County, MI, USA. August 5, 2021 (Photo: J. Rajewski).

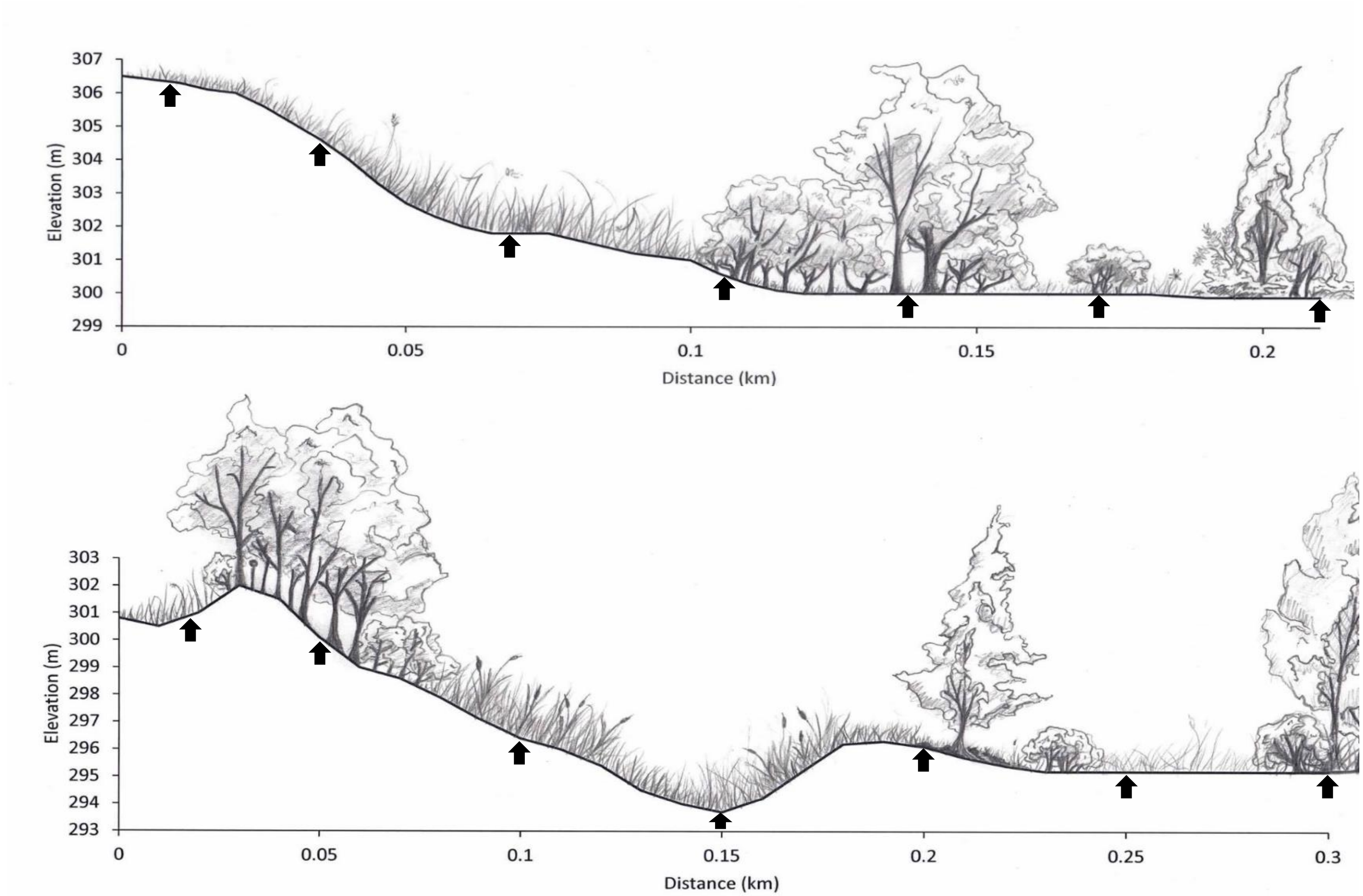

FIGURE 2.3. Cross section figures show vegetation composition and elevation change across transects, along which temperature data was collected from May-October (2020-2022). Arrows show locations of 7 pairs (n=14 total) of temperature loggers (iButtonLink Technology model DS1921G-F5# Thermochron 4k), placed above- and belowground (0.15 m subsurface). Temperatures were collected at 2 distinct sites occupied by eastern massasauga rattlesnakes (*Sistrurus catenatus*) in Lenawee County (top) and Oakland County (bottom). The edge of the road coincides with a distance of 0 m.

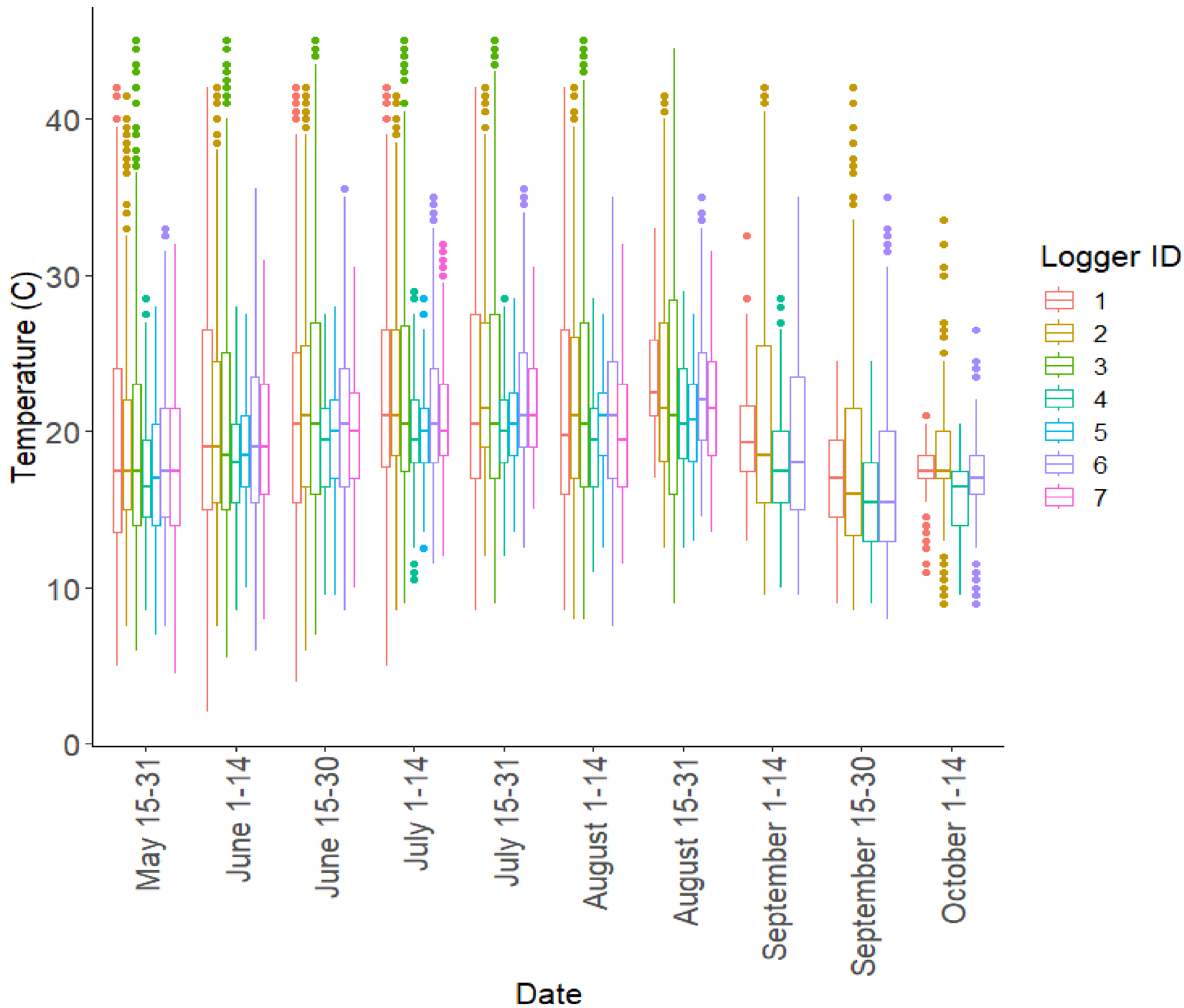

FIGURE 2.4. Bi-weekly mean surface temperatures arranged by increasing distance to road (left to right) at Site 1 from May-October (2021-2022) in Lenawee County, Michigan, USA. Temperature logger 1 was placed 10 m from the road, and 2-7 were placed every 35 m from the road respectively up to 210 m away. Loggers 1-7 are displayed sequentially left to right within each bi-weekly period. From September to October data were only available for loggers 1, 2, 4, and 6, arranged sequentially.

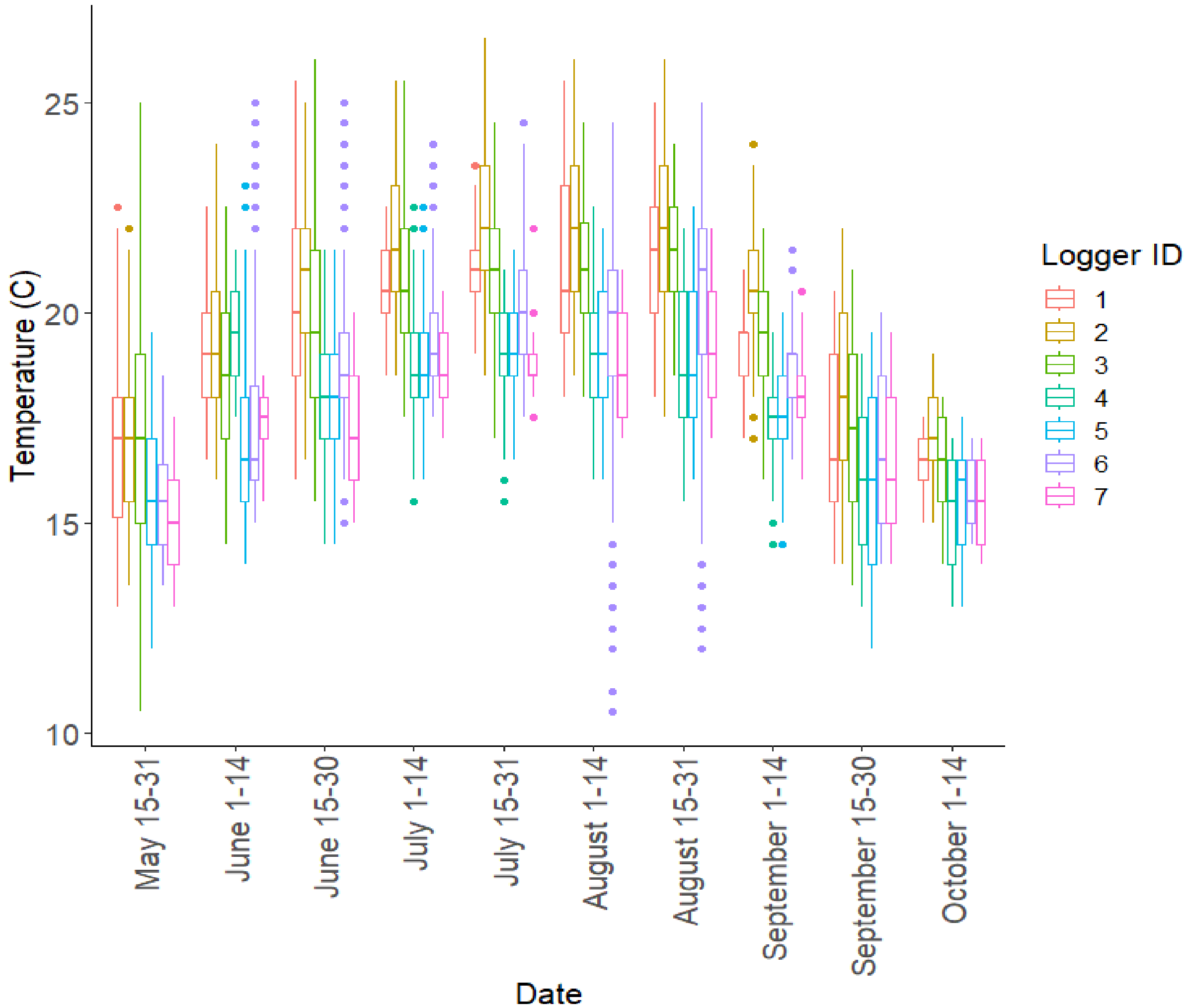

FIGURE 2.5. Bi-weekly mean sub-surface temperatures arranged by increasing distance to road (left to right) at Site 1 from May-October (2021-2022) in Lenawee County, Michigan, USA. Temperature logger 1 was placed 10 m from the road, and 2-7 were placed every 35 m from the road respectively up to 210 m away. Loggers 1-7 are displayed sequentially left to right within each bi-weekly period. Temperature data for logger 4 were unavailable in May.

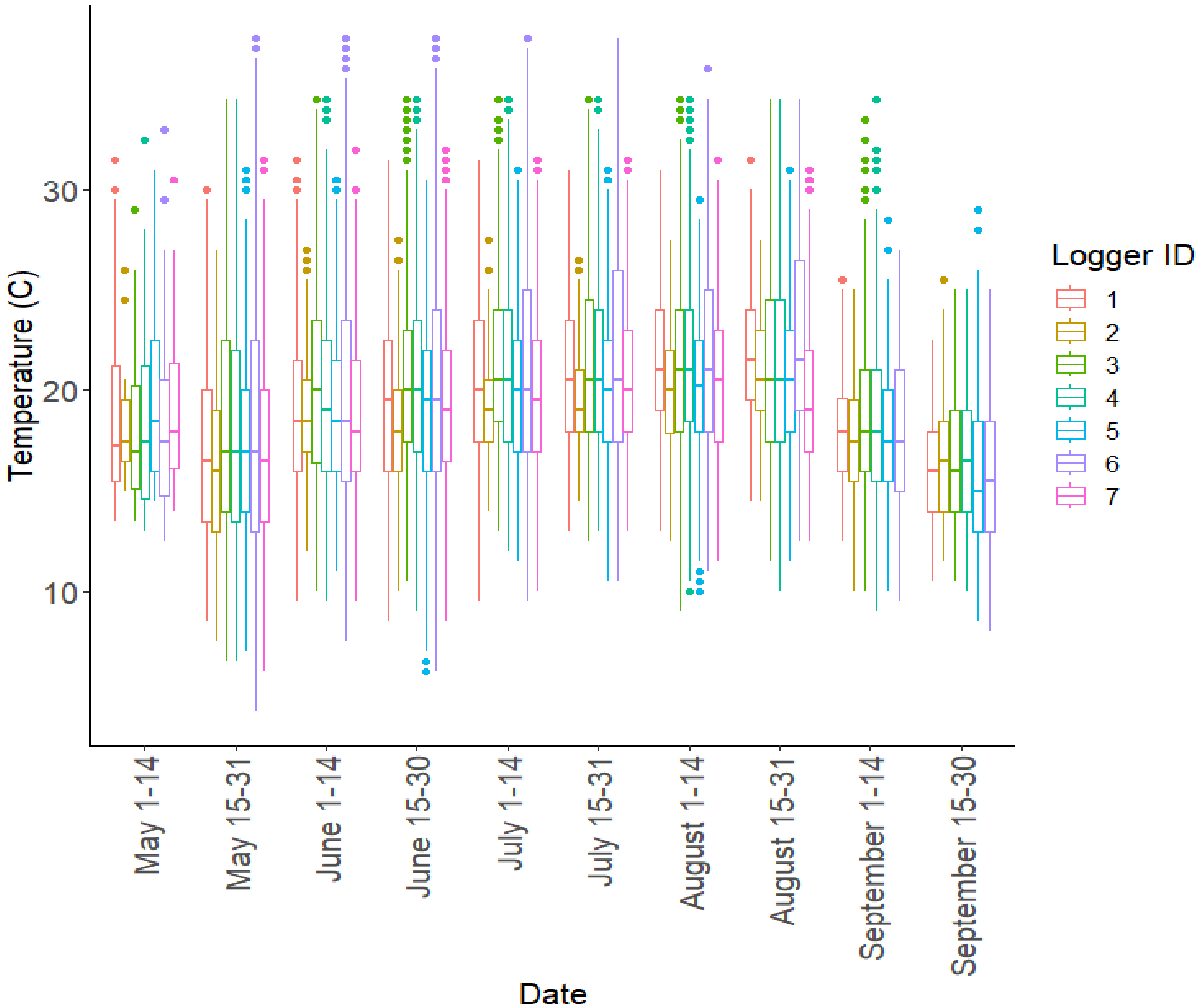

FIGURE 2.6. Bi-weekly mean surface temperatures arranged by increasing distance to road (left to right) at Site 2 from May-October (2021-2022) in Oakland County, Michigan, USA. Temperature logger 1 was placed 10 m from the road, and 2-7 were placed every 50 m from the road respectively up to 300 m away. Logger 1-7 are placed sequentially left to right within each bi-weekly period. From September to October data were not available for logger 7.

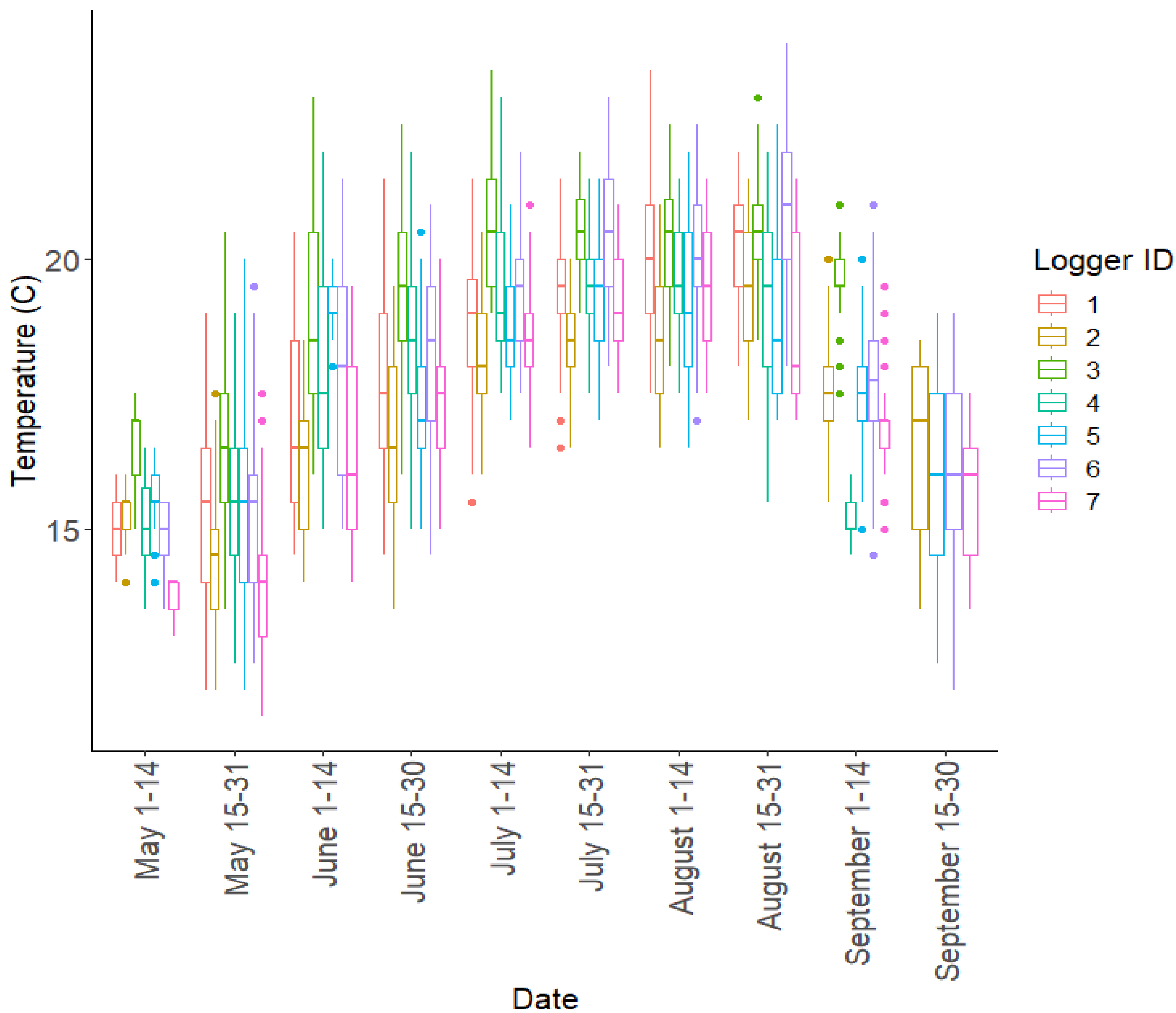

FIGURE 2.7. Bi-weekly mean sub-surface temperatures arranged by increasing distance to road (left to right) at Site 2 from May-October (2021-2022) in Oakland County, Michigan, USA. Temperature logger 1 was placed 10 m from the road, and 2-7 were placed every 50 m from the road respectively up to 300 m away. Logger 1-7 are placed sequentially left to right within each bi-weekly period. Temperature data were not available at logger 1 throughout September, or for loggers 3 and 4 from September 15-31st.

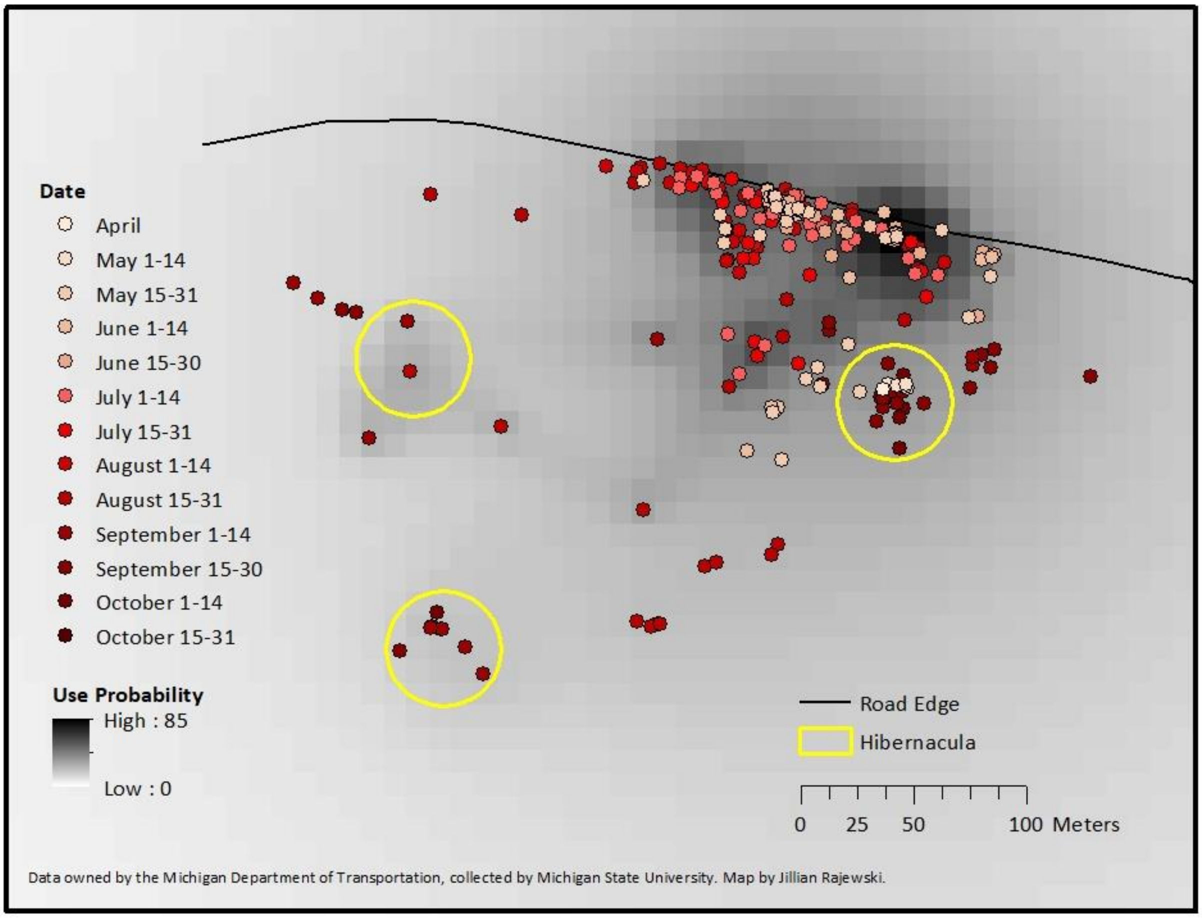

FIGURE 2.8. Predicted eastern massasauga rattlesnake probability of use (scaled 0 – 100) and associated locations for 2-week intervals during the active season in Lenawee County (Site 1), Michigan, USA (2020-2022). The edge of the road pavement (solid black line) is included for reference.

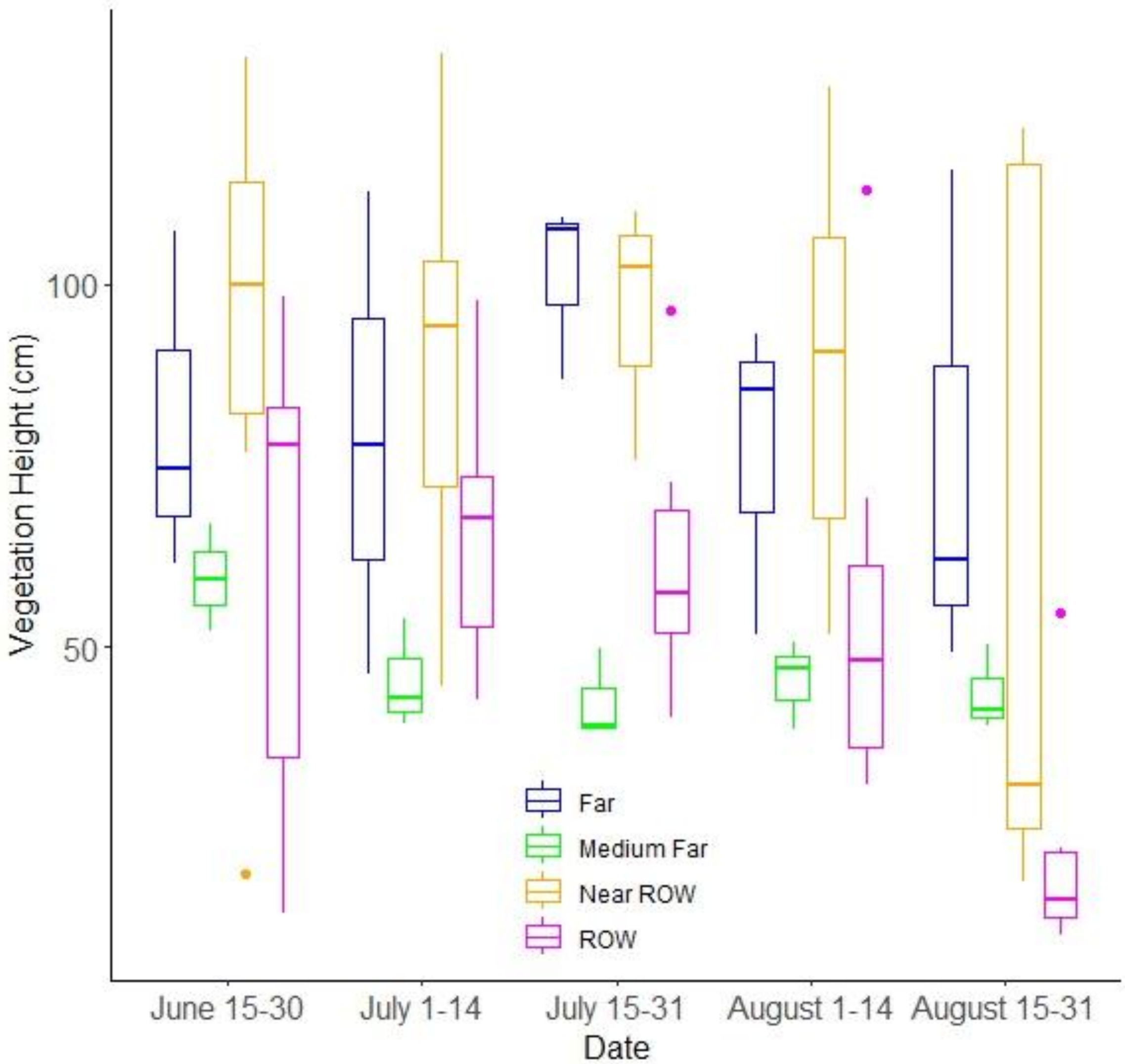

FIGURE 2.9. Median, upper (25%) and lower (75%) quartiles (boxes), 95% confidence intervals (whiskers), and outliers (points) for vegetation heights measured 10 m (right-of-way; ROW), 15 m (Near ROW), 20 and 30 m (Medium Far), and 35 and 45 m away from the road pavement. Vegetation height measurements were summarized over 2-week intervals from mid-June through August in Lenawee County (Site 1), Michigan, USA (2020-2022).

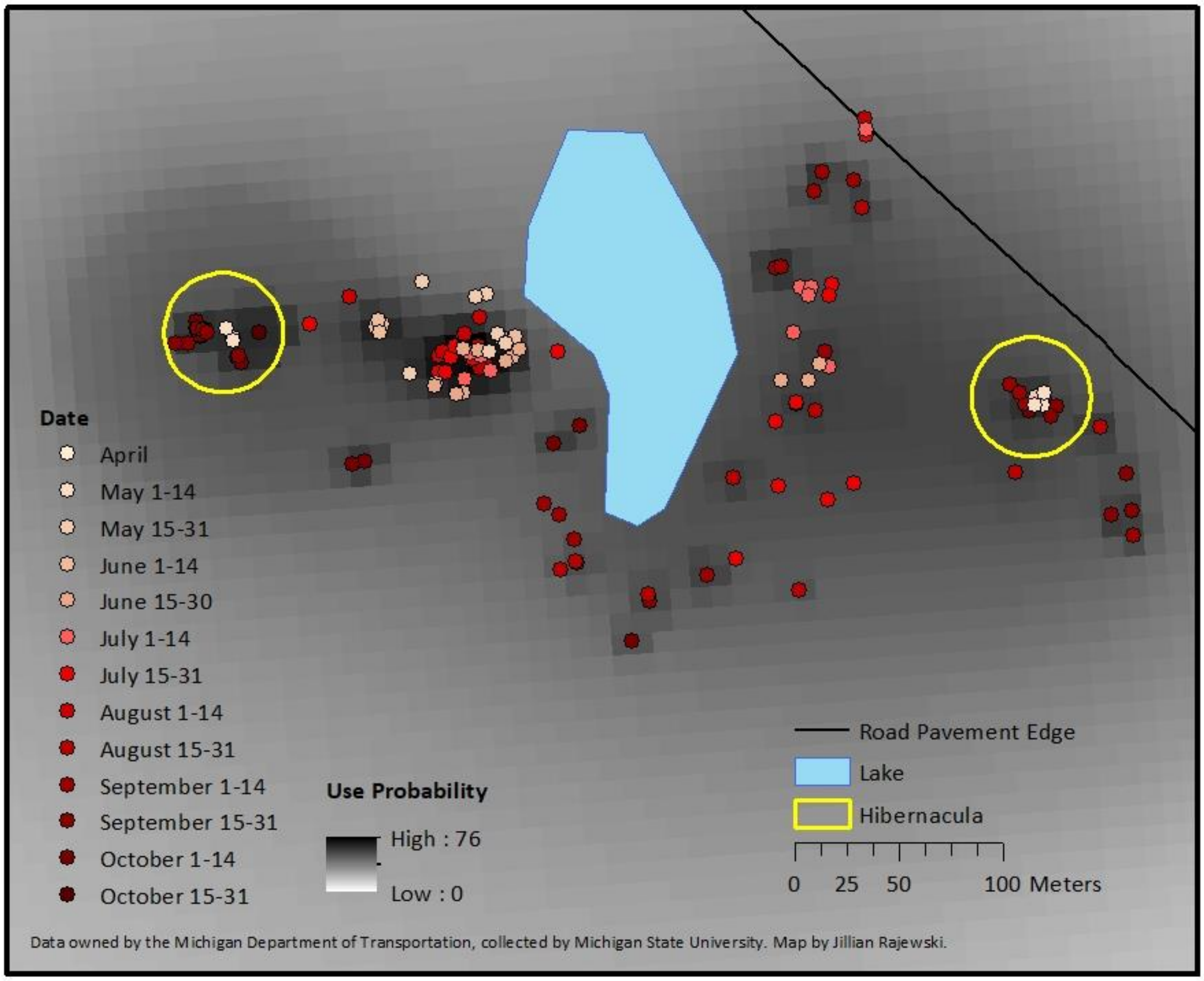

FIGURE 2.10. Predicted eastern massasauga rattlesnake probability of use (scaled 0 – 100) and associated locations for 2-week intervals during the active season in Oakland County (Site 2), Michigan, USA (2020-2022). The edge of the road pavement (solid black line) is included for reference.

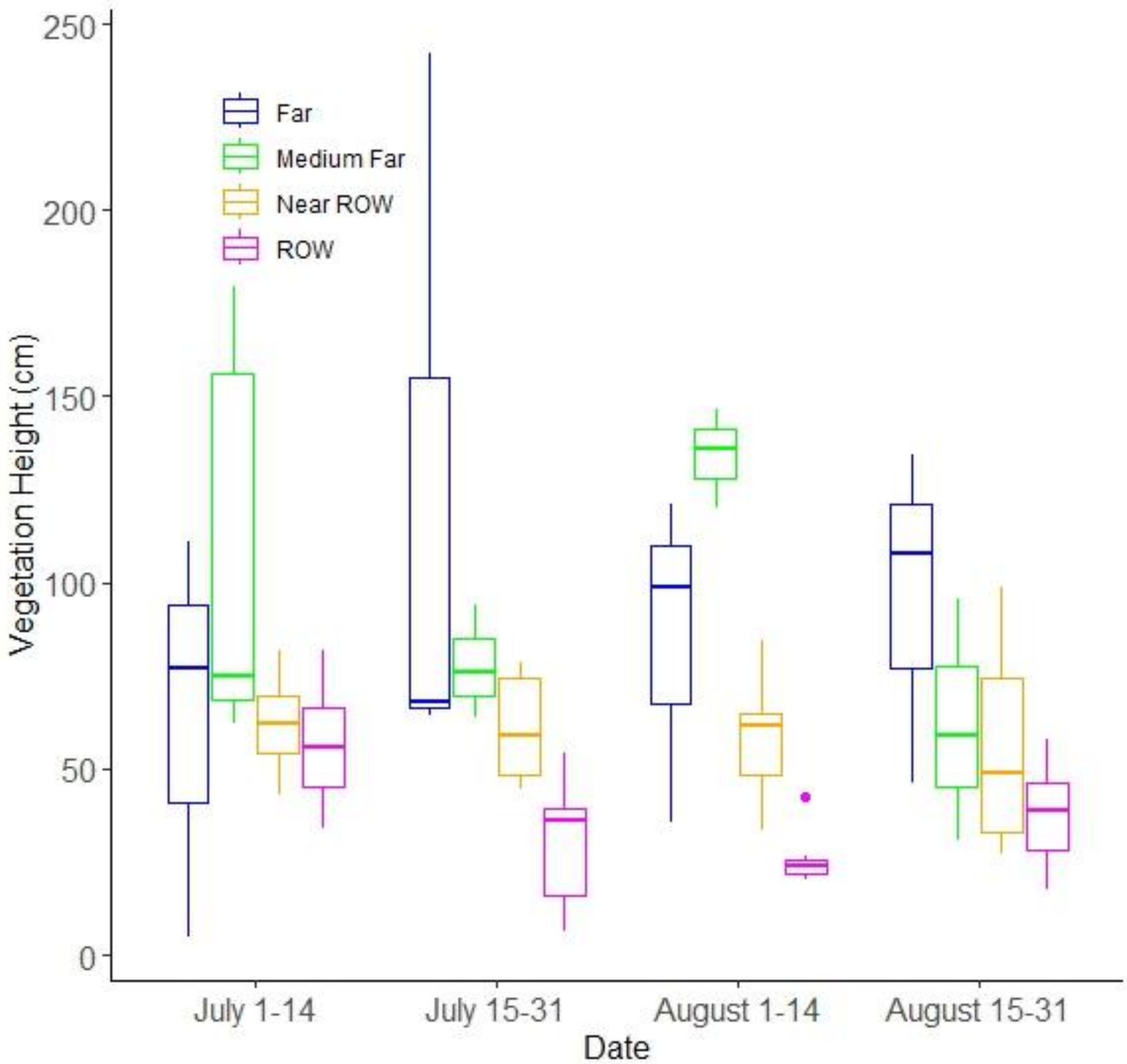

FIGURE 2.11. Median, upper (25%) and lower (75%) quartiles (boxes), 95% confidence intervals (whiskers), and outliers (points) for vegetation heights measured at 10 m (right-of-way; ROW), 15 m (Near ROW), 20 and 30 m (Medium Far), and 35 and 45 m from the road pavement. Vegetation height measurements were summarized over 2-week intervals from early July through August in Oakland County (Site 2), Michigan, USA (2020-2022).

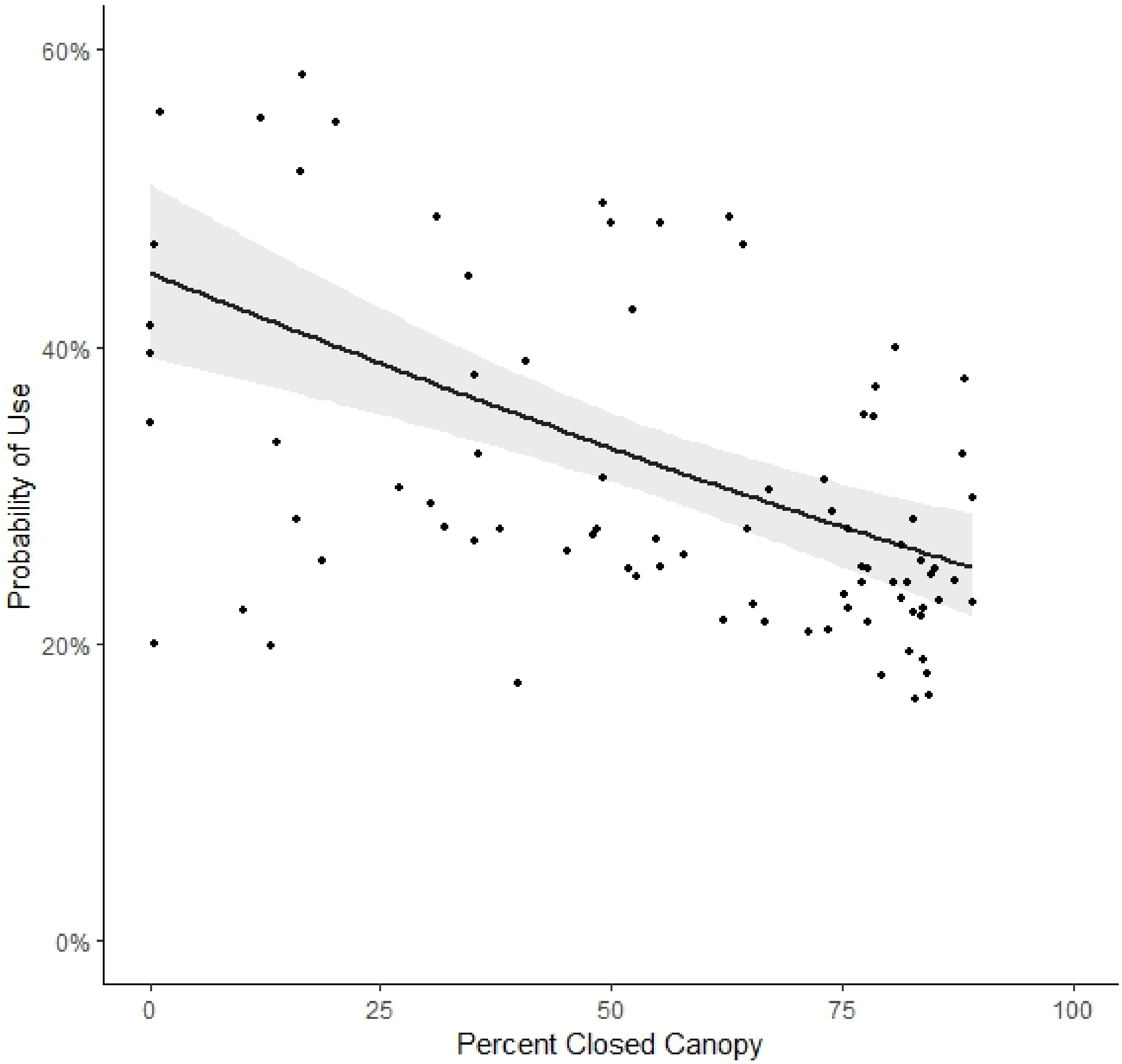

FIGURE 2.12. Predicted eastern massasauga probability of use (and 95% credible intervals) and percent canopy cover during the active season (April to October) in Lenawee County (Site 1), Michigan, USA (2020-2022).

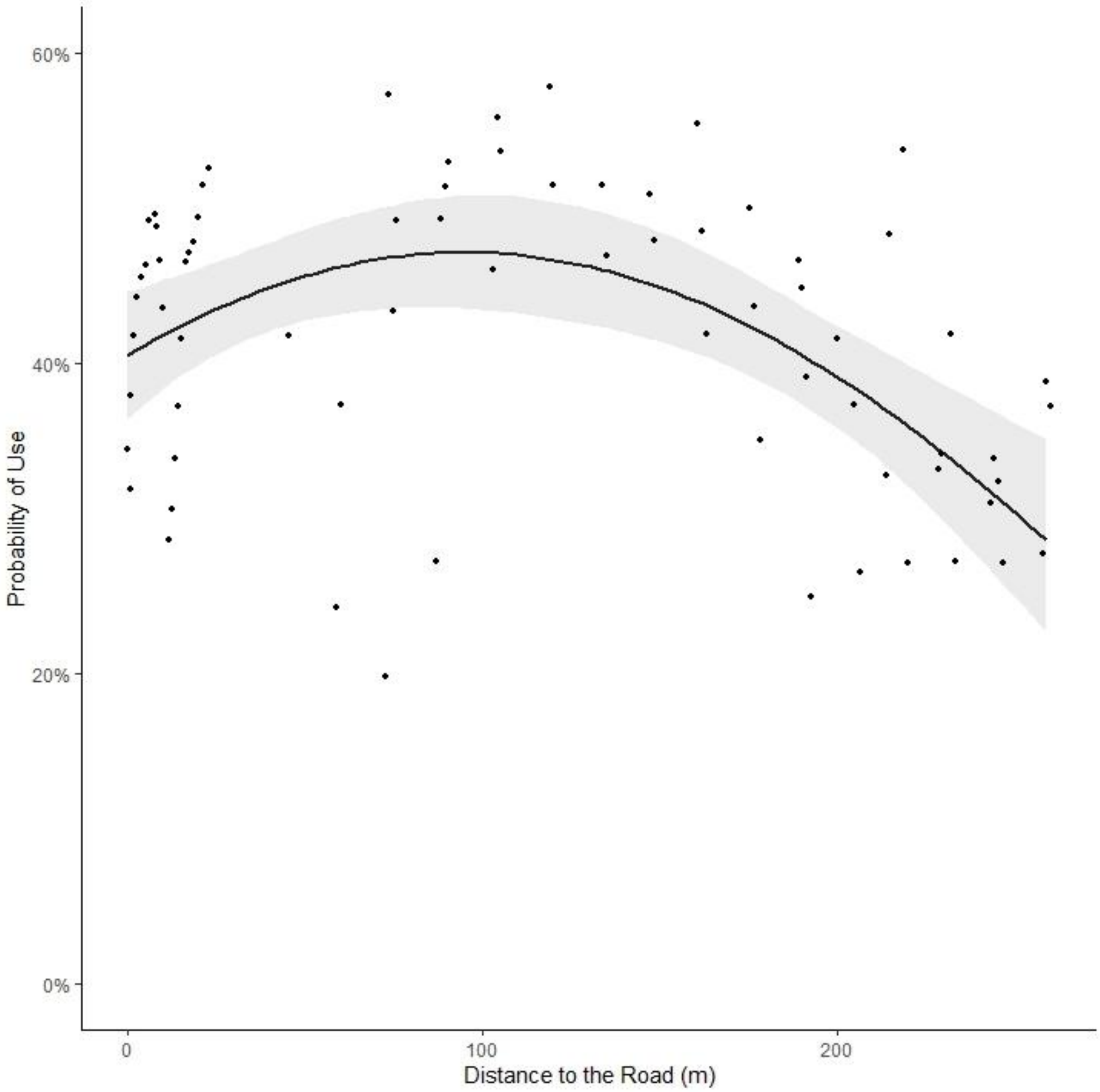

FIGURE 2.13. Predicted eastern massasauga probability of use (and 95% credible intervals) and distance to paved road during the active season (April to October) in Oakland County (Site 2), Michigan, USA (2020-2022).

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CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Prior to ROW project implementation, visual surveys may be required to confirm EMR site occupancy, or to ensure EMR individuals do not occupy the project area at the time of implementation. To increase effectiveness and efficiency of visual survey methods prior to ROW project implementation, this research identified conditions during which EMR were most likely available for visual detection. Time of day did not significantly impact visibility, indicating visual encounter surveys during the active season can be conducted at any time of day (consistent with Shaffer et al. 2019). I found that temperature positively influenced body visibility, and that percent of EMR body exposure was maximized (~50% of body visible) as temperature approached 30° C. For maximum visibility of EMR during visual encounter surveys, surveys should be conducted on days when minimum daily temperature are around 13° C (~25% body exposure), with temperatures projected to warm to 30° C (~50% body exposure) from early morning to early afternoon. The beginning of research in this study coincided with the onset of COVID-19 pandemic safety restrictions. Therefore, I was unable to survey until late Spring (mid-late May) in 2020 and 2021, after EMR moved from hibernacula and vegetation was more developed, which likely impacted survey and monitoring results. However, I documented seasonal shifts in space use (i.e., EMR moved closer to roads as the active season progressed), so surveying too early in the active season along ROW may not be effective for determining EMR occupancy in ROW. Though seasonality (e.g., month of the active season) in my study had no significant effect on EMR visibility (i.e., how much of the body was exposed), EMR are likely aboveground and therefore most available for detection in early spring (April-May) when vegetation cover is low and EMR are slow and lethargic post emergence from hibernacula (Kingsbury et al. 2003, Harvey and Weatherhead 2006, Markle et al. 2020), and tend to move towards roadsides later in May. Additionally, EMR in my study were mostly aboveground in July.

This research confirmed EMR space use of maintained roadsides (Weatherhead and Prior 1992, Shepard et al. 2008, Bailey et al. 2012, Colley 2015). In my study, active season home ranges for radio tagged EMR based on ≥20 relocations averaged 0.03 (Site 1) to 0.05 (Site 2) ha, and EMR exhibited relatively localized movements. These home range estimates are lower than observed in other EMR studies (Table 3.1), suggesting that space use for EMR populations occupying roadside habitats may be limited by habitat fragmentation (Kudla et al. 2021). However, I documented reproduction at both of my study sites, indicating that the necessary habitat components (i.e., basking, breeding, feeding, overwintering habitat; Bissel 2006, Durbian et al. 2006, DeGregorio et al. 2011, Bailey et al. 2012, Shaffer 2018) occur within a smaller area. My home ranges are based on a limited sample during the active season from mostly gravid females (male EMR are known to move more; Weatherhead and Prior 1992, Jellen et al. 2007, DeGregorio et al. 2011), therefore future research should seek to further elucidate EMR home range size and space use near maintained roads.

Observed land cover types used by EMR in this study reflects vegetation and cover type selection reported in prior literature (Kingsbury et al. 2003, Bissel 2006, Bailey et al. 2012, Shaffer 2018). Existing habitat suitability, macro- and micro-habitat selection models are likely relevant when attempting to identify occupied EMR habitat occurring along roadsides (Harvey and Weatherhead 2006, Marshall et al. 2006, Moore and Gillingham 2006, DeGregorio et al. 2011). Structure of ground cover was not found to influence intensity of EMR space use in road ROWs or adjacent areas in this study, however prior research (e.g., Harvey and Weatherhead 2006, Marshall et al. 2006, Moore and Gillingham 2006, DeGregorio et al. 2011) indicated microhabitat structure influences space use. My microsite measurements were conducted in areas only used by EMR (i.e., intensity of use >0%), suggesting that within home ranges the variables I measured did not differentiate core use areas. I suspect that fine scale vegetation heterogeneity (e.g., individual shrubs or tall forbs interspersed in lower grower grass and forbs) and soil temperatures influence localized space. Further investigation of micro, and macro-habitat influencing space use near roads is warranted.

During the active season, EMR individuals primarily selected early successional grassland (which was similar in appearance and structure to ROW land cover), and wet prairie-fen. Overwintering habitat at both sites occurred along ecotones where forest met a wetland edge, with presence of low, dense woody vegetation. Hibernacula structures consisted of crayfish burrows and hummocks. Closed overstory canopy was found to negatively affect EMR probability of use near roads and in adjacent areas (probability of use approaching 0.2 when closed canopy cover approaches 80%). These findings support conclusions made in prior literature that dense forest canopy cover negatively impacts EMR occupancy (Thacker et al. 2023) and amount of available habitat during the active season (Reinert and Buskar 1992, Shoemaker and Gibbs 2010, Bailey et al. 2012, Thacker et al. 2023).

Overall, the presence of neonates, yearlings, and juveniles within roadside habitats indicates that sufficient resources exist to meet energy requirements for successful reproduction (Bonnet et al. 1999, Bissel 2006). I confirmed multi-year survival for EMR of reproductive age females (Site 1 = 2, Site 2 = 1), suggesting site fidelity during the active season. Observations from Site 1 indicated that EMR regularly use areas adjacent to roads and meet life requisites in these areas. There were apparent differences in structure of land cover, road characteristics, and maintenance (e.g., mowing, herbicide application) between Sites 1 and 2 that may have influenced space use patterns and observed mortality around roads. Future researchers are encouraged to further quantify demographics for roadside populations to assess the impact of roads on survival rates, reproductive success, and overall population structure.

A surprising result from my study was the regularity with which gravid females were observed using road ROWs and adjacent upland areas at Site 1 during gestation and using these areas to have their litters. Space use near roads was disproportionate between the two occupied sites in my study, where core areas of high use (approaching 89% use intensity) at Site 1 occurred within 20 m of the road, where core areas of use (approaching 94%) at Site 2 were more dispersed but occurred beyond 20 m from the road. I found that areas with high intensity of use by EMR coincided with higher belowground temperature readings relative to the surrounding area. Warmer belowground temperatures corresponded to temperature loggers placed in areas with open canopy, low presence of woody vegetation, and in fen and grassland cover types. For researchers and managers seeking to predict core areas of use by EMR near roadways, I recommend determining areas with higher average belowground temperatures than surrounding areas, and where suitable habitat conditions for EMR occur. Use of temperature loggers with regular (bi-weekly) maintenance was simple, effective, inexpensive, and causes minimal disturbance to EMR habitat. Due to personally observed device failures, if using this method, I recommend monitoring loggers on days where average local daily temperatures may exceed the temperature range limit provided by the manufacturer, particularly for loggers near roadsides or in moist soil conditions.

TABLES

TABLE 3.1. Summary of home range and movement data for eastern massasauga rattlesnakes range wide including publication reference, location, age class(es), estimated home range size, average total and daily distances moved.

Author(s)	Location	Age Class(es)[a]	Estimated Range Size (ha)	Average Total Movement (m)	Average Daily Movement (m)
Reinert and Kodrich (1982)	Pennsylvania, USA	A	1.00	.[b]	9.1
Weatherhead and Prior (1992)	Ontario, CA	A	25.00	.	56 (Summer), 132 (Fall)
Johnson (2000)	New York, USA	A	26.20	2,751.3	19.5
Kingsbury et al. (2003)	Michigan, USA	A	4.03	.	.
Moore and Gillingham (2006)	Michigan, USA	A	NA	.	6.87
Jellen et al. (2007)	Pennsylvania, USA	N	NA	257.3	5.27
Durbian et al. (2008)	Missouri, Wisconsin, USA	A, N	NA	.	.
DeGregorio et al. (2011)	Michigan, USA	A	NA	.	25.5
Bailey et al. (2012)	Michigan, USA	A	NA	.	.
Present study	Michigan, USA	A, SA	0.04	757	.

[a] A = Adult, N = Neonate, SA = Sub-adult, J = Juvenile as described in corresponding reference.
[b] Not calculated.

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APPENDIX A:

EASTERN MASSASAUGA RATTLESNAKE WITH VHF TRANSMITTER ATTACHED

FIGURE A.1. A gravid female eastern massasauga rattlesnake (*Sistrurus catenatus*) with a very high frequency (VHF) transmitter (Holohil; PD-2 3.8g) attached via Loctite waterproof super glue. A loose strip of electrical tape was used to secure the transmitter in place.

APPENDIX B:

DOCUMENTATION OF ADULT EASTERN MASSASAUGA RATTLESNAKES

TABLE B.1. Biological data collected for eastern massasauga rattlesnakes (*Sistrurus catenatus*) upon capture during the active season in southern Michigan, USA, 2020-2022. Date of capture, site, abbreviated PIT tag identifier (last three digits), body weight, snout to vent length (SVL), total length (snout to last rattle), sex, gravid based on body palpation, number of tail rattles, and encounter type. Blank PIT values are recaptures.

Date	Site	PIT	Weight (g)	SVL Length (cm)	Total Length (cm)	Sex	Gravid	Rattles	Encounter Type
5/15/2022	1	973	390	57	61	F	Y	4	Visual
5/15/2022	1	287	353	60.5	69	F	Y	5	Visual
5/15/2022	1	196	290	58.5	64	F	Y	6	Visual
5/17/2021	1	849	120	46	51	F	NA	5	Auditory
5/25/2021	1		118	46.7	51.9	F	NA	5	Visual
6/2/2022	1		200	50	55	F	Y	7	Visual
8/19/2020	1		144	46	51.5	F	NA	5	Visual
5/20/2022	1	507	263	57	65	F	Y	10	Visual
5/24/2022	1	117	180	61	63	F	Y	7	Visual
5/25/2022	2	580	179	47	54.5	F	Y	7	Visual
5/27/2022	2	262	244	57	62	F	N	10	Visual
6/20/2022	2		260	55.7	61.1	F	NA	11	Visual
6/8/2021	1	792	221	51.5	65.5	F	Y	12	Visual
6/16/2021	1		224	56.5	66.3	F	Y	12	Visual
6/24/2021	1		228	58	68.5	F	Y	13	Visual
7/14/2021	1		216	56.5	63	F	Y	9	Visual
6/18/2021	1	462	NA	55	63.5	M	NA	7	Visual
6/22/2021	1		303	NA	NA	M	NA	7	Visual

TABLE B.1. (cont'd)

Date	Site	PIT_ID	Weight (g)	SVL Length (cm)	Total Length (cm)	Sex	Gravid	Rattles	Encounter Type
7/6/2021	1		390	55	61	M	NA	8	Visual
6/21/2021	2	190	116	44.5	50.5	M	NA	6	Visual
6/30/2021	1	886	190	51.5	58	M	NA	7	Visual
6/30/2021	1	468	268	55.5	61.5	F	Y	6	Visual
7/13/2021	2	034	270	51.4	56.5	M	NA	7	Auditory
7/14/2021	1	440	371	59	64	M	NA	12	Visual
7/18/2022	2	504	216	52	62	F	Y	9	Visual
8/4/2022	1	912	340	55	63	F	Y	10	Visual
8/9/2022	1	666	080	37.8	45.2	F	NA	5	Visual
8/11/2022	2	813	NA	57.5	68	M	NA	11	Visual
8/17/2022	2		294	57.5	68	M	NA	11	Visual
8/17/2020	2	104	171	44	50	F	Y	7	Visual
8/19/2020	1	278	135	43	50.5	M	NA	6	Auditory
8/24/2021	2	602	186	49	54	M	NA	6	Visual
8/25/2020	2	091	92	41.5	47.5	M	NA	4	Visual
9/30/2020	1	806	20	22	23	F	N	2	Visual

APPENDIX C:

DOCUMENTATION OF NEONATE EASTERN MASSASAUGA RATTLESNAKES

TABLE C.1. Biological data collected for neonate eastern massasauga rattlesnake (*Sistrurus catenatus*) individuals during the active season in southern Michigan, USA, 2020-2022. Date of capture, site ID (1 = Lenawee County, 2 = Oakland County), temperature and humidity at time of capture, abbreviated parent PIT-ID, observed individuals, and life stage notes are listed. In total, 23 neonates and young of the year were observed.

Date	Site	Temp (C)	Humidity (%)	Parent PIT_ID	Total Observed Individuals	Life Stage Notes
9/21/2020	1	20.9	47.8	NA	1	Neonate
8/13/2021	1	23.5	22.0	468	5	Neonate[a]
8/17/2021	1	32.8	56.1	440	2	Neonate Litter
8/17/2021	1	27.3	72.0	462	6	Neonate Litter
5/19/2022	2	24.8	76.6	NA	1	Young of the Year
5/27/2022	2	26.7	69.4	NA	1	Young of the Year
7/7/2022	2	27.5	54.7	NA	1	Young of the Year
7/18/2022	1	30.1	64.8	NA	1	Young of the Year
8/11/2022	1	33.2	41.3	973	2	Neonate Litter[b]
8/12/2022	1	24.9	52.7	973	NA	Neonate Litter
8/15/2022	1	31.1	48.0	912	3	Neonate Litter[c]
8/16/2022	1	30.0	48.7	912	NA	Neonate Litter

[a] Of the 5 living neonates in the litter, 1 was captured and measured (weight = 11 g, Total Length = 20.5 cm).

[b] An additional 4 non-viable embryos were found in this litter.

[c] An additional 3 non-viable embryos were found in this litter.

APPENDIX D:

PREDICTION OF EASTERN MASSASAUGA PERCENT BODY EXPOSURE AND AMBIENT AIR TEMPERATURE

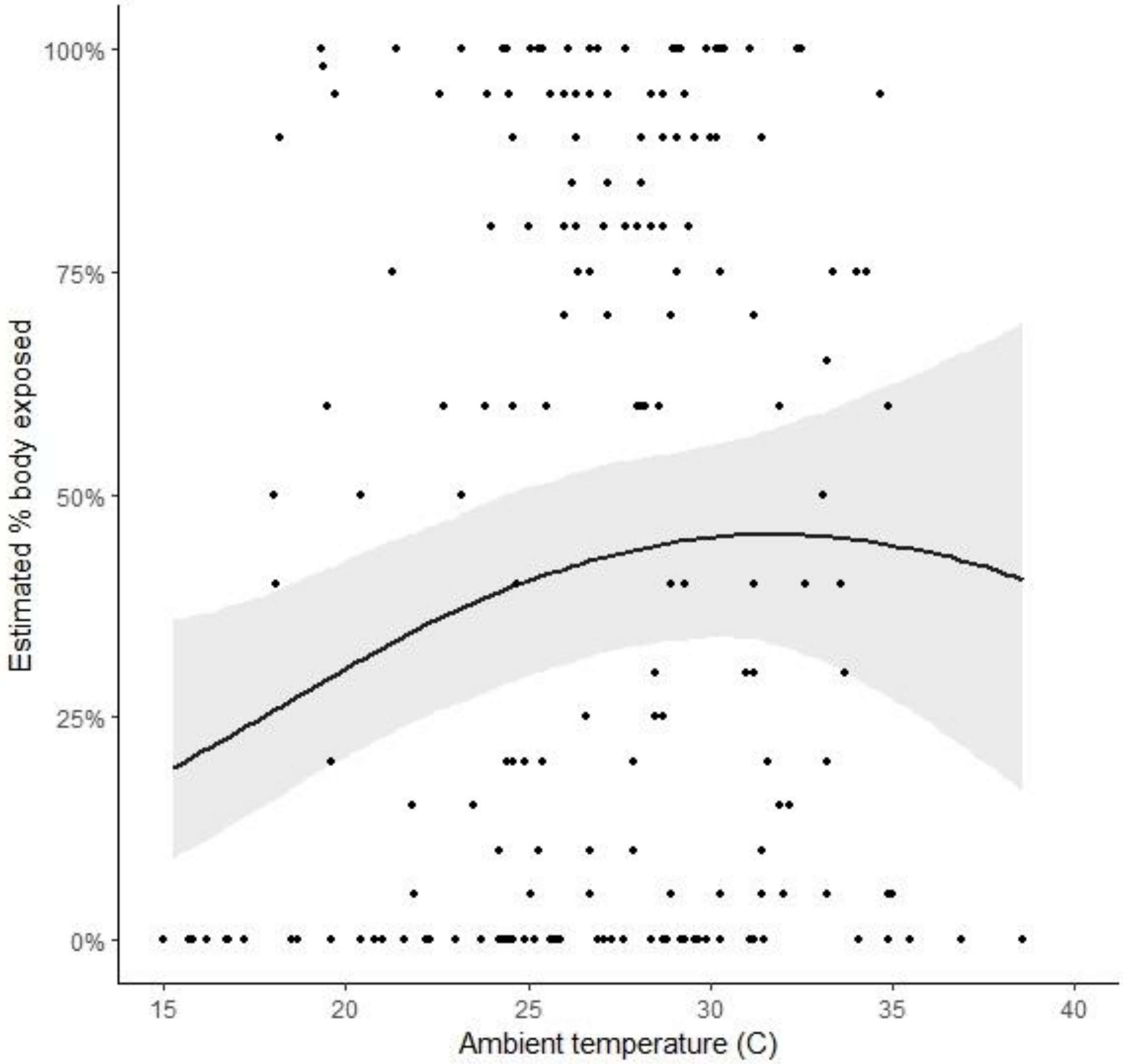

FIGURE D.1. Prediction of eastern massasauga (*Sistrurus catenatus*) percent body exposure and ambient air temperature (Celsius) during the active season (April-October) in southern Michigan, USA, 2020-2022. Percent body exposure is indicated by the solid line and 95% credible intervals are indicated by the gray band.

APPENDIX E:

VEGETATION SURVEY QUADRAT SAMPLING METHOD

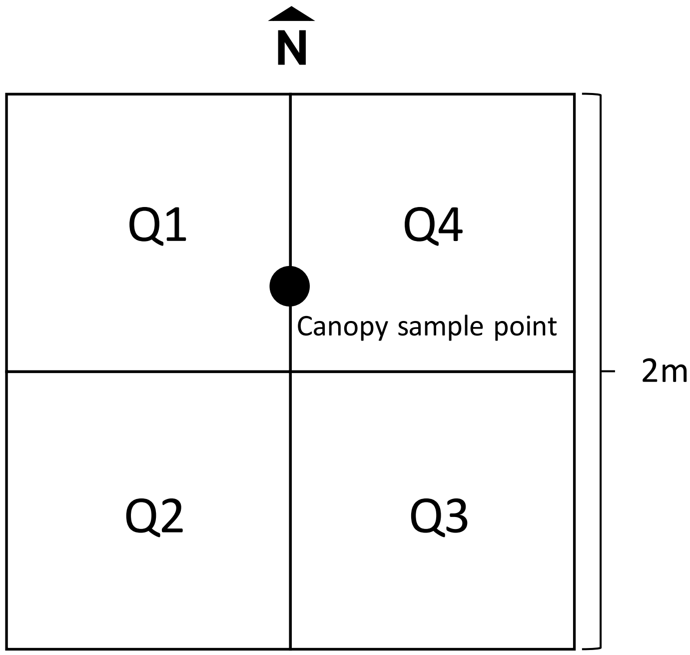

FIGURE E.1. Vegetation sampling quadrat (2x2 m) split into 4-1x1 m sample sections. Presence (0) or absence (1) of woody vegetation, low vegetation cover (non-grass/sedge herbaceous cover and woody vegetation under 0.5m), grasses and sedges, standing water (water present visibly on the surface), and bare soil (>~100 cm^2) was recorded in each quadrat (Q1, 2, 3, 4), and presence values were summed for the plot. Hemispheric canopy cover images were taken 30 cm above the ground at the center of each quadrat.

APPENDIX F:

VEGETATION HEIGHT SAMPLING METHOD

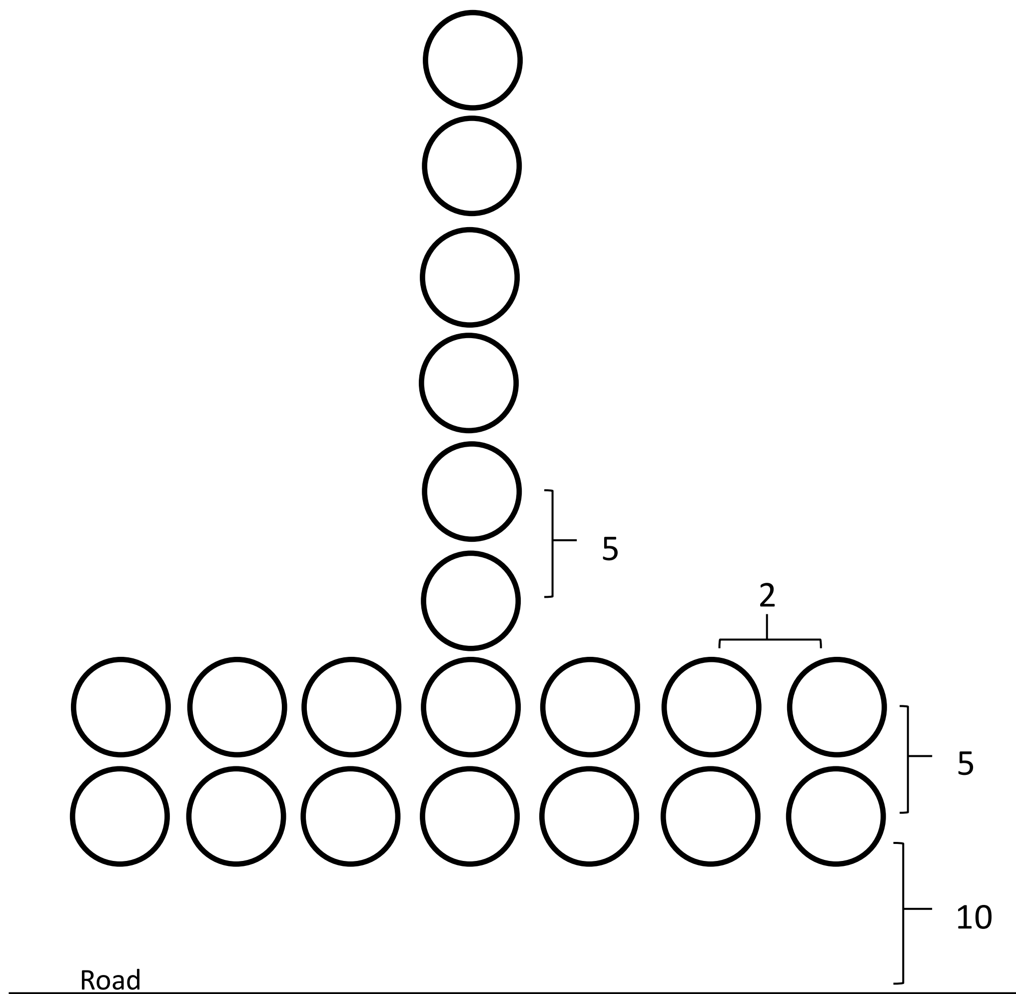

FIGURE F.1. Vegetation height samples (n = 20) were collected between 10 m and 45 m of a state-maintained road at sites confirmed to be occupied by eastern massasauga rattlesnakes (*Sistrurus catenatus*). Sampling began 10 m from the road, and subsequent samples were taken 5 m apart east to west (for the first seven, and next seven vegetation samples closest to the road), and 2 m apart north to south.

APPENDIX G:
TEMPERATURE LOGGER ORIENTATION

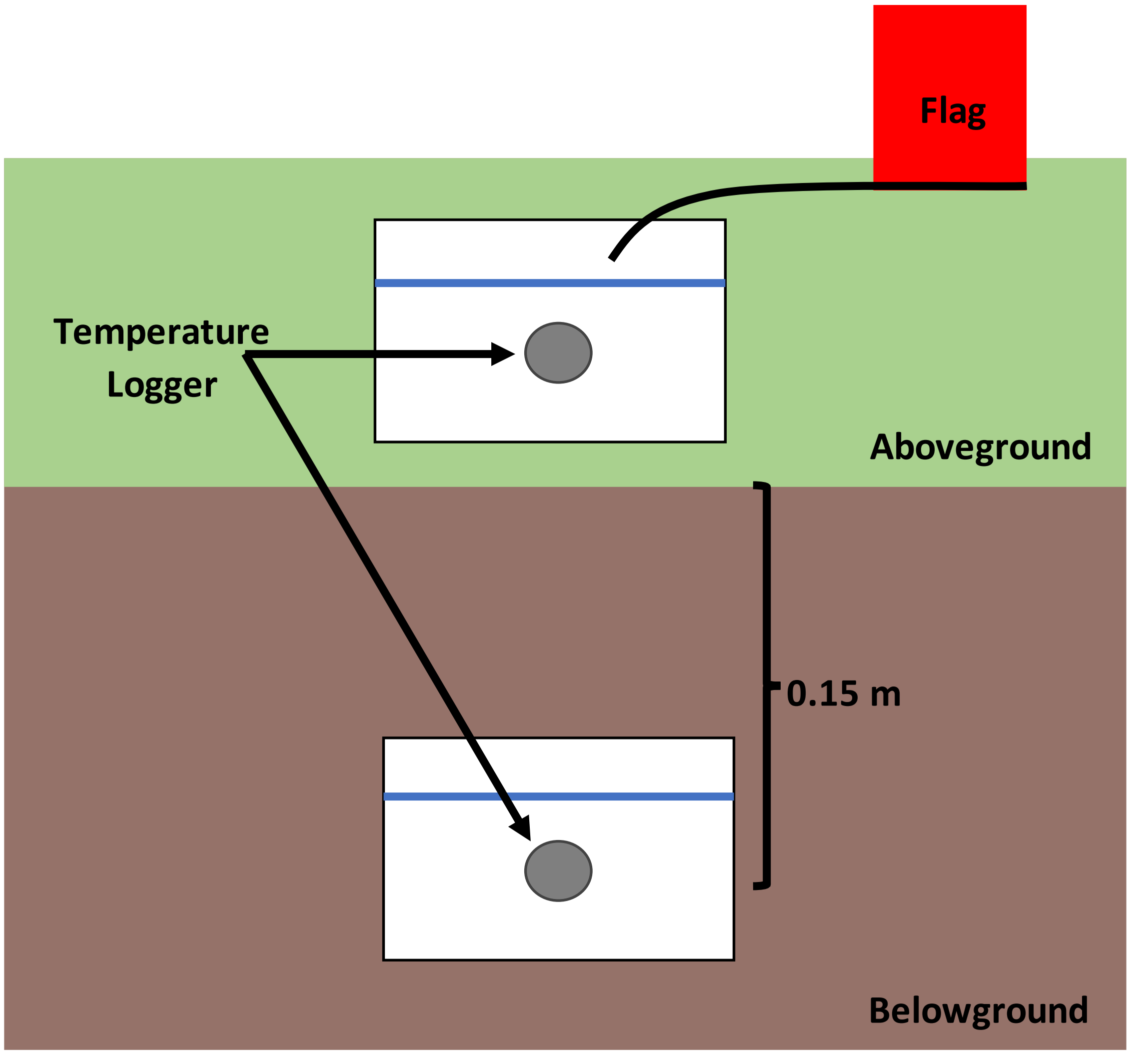

FIGURE G.1. Temperature logger (iButtonLink Technology model DS1921G-F5# Thermochron 4k) pair orientation above- (soil surface) and belowground (0.15 m subsurface). Temperature loggers were sealed in plastic zipper bags. A brightly colored flag was attached to the surface temperature logger bag for relocation. If in the road right-of-way the flag was bent parallel to the ground (pictured above) to avoid disrupting maintenance activities.

APPENDIX H:

RECOMMENDED TRAINING GUIDE

Recommended Materials:

- Snake tongs or hooks
- Calf high, thick boots
- High visibility vest
- Helmet and safety glasses (along roadsides as necessary)
- Tablet with GPS capabilities
- Compass

Survey Standard Operating Procedure:

- Record the date, survey plot ID, number of surveyors, minimum daily air temperature (from local weather station the following day), and survey start time.
- As you travel along the transect weave back and forth ~10 m to either side of the transect line to search for EMR.
- Walk at a casual, slow pace to thoroughly search under vegetation.
    - EMR can hide in place under vegetation rather than flee, therefore auditory detection can be just as important as visual detection during surveys. Lightly disturbing vegetation with a snake hook or tongs can cause EMR to rattle.
        - Rattling can sound like a bee or a fly buzzing. As bees can be found resting in similar vegetation structures that EMR use, it's recommended to confirm the source of the sound as an EMR.
- If an EMR is located, briefly record the time, date, location, behavior, and any other information relevant to the goal of the surveyors. Continue the survey after data recording is complete.
- Once surveyors have sufficiently covered the survey area, record survey end time.

Recommended Training Period: 2 days

Recommended Training Timeline:

Day 1: Review general EMR biology and habitat requirements. Introduce and review survey methods.

- Highly recommended to travel to a confirmed occupied site with high quality habitat prior to survey training so surveys can develop a visual perspective of the habitat.
- Walk through a survey and how to conduct one. Trainees should observe a trainer.
- See source materials below.
  - https://www.michigan.gov/-/media/Project/Websites/MDOT/Business/Local-Government/Local-Agency-Program/NEPA/Combined-EMR-and-BMPs-for-Eastern-Massasauga-Rattlesnake-041217.pdf?rev=70e6bdf5b7ba4198840af625a7d31342
  - https://mnfi.anr.msu.edu/species/eastern-massasauga-rattlesnake
- Have trainee and trainer conduct a survey together. Trainer should focus on trainee technique, thoroughness of searching, and pace. Decoy artificial snakes may be deployed to help refine the search image and test accuracy.
- If more than one practice survey is conducted, limit to two practice surveys in a day to avoid survey fatigue, which limits the ability of the surveyor to detect EMR.
- Ample break time between surveys should be allowed to prevent eye strain and body fatigue.

Day 2: Trainees should conduct two surveys on their own shadowed by an experienced surveyor at two distinct sites. Decoy artificial snakes maybe deployed to help refine search image and test accuracy.

- Continue training until trainer is confident that trainee can reliably detect EMR under a variety of habitat conditions.