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Great Lakes Snake: Estimating the Occupancy and Detection Probabilities of the Eastern Massasauga Rattlesnake *(Sistrurus catenatus)*

Arin June Thacker
Grand Valley State University

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Arin June Thacker

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Dedication

This thesis is dedicated to my mother, Nancy E. Thacker, whose endless support, encouragement, and love made this possible. Thank you for raising me to believe that I can accomplish anything. I would like to thank Stephen V. Wineski for comforting and encouraging me always. I love you both dearly.

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Abstract

Detailed knowledge of a species' range and distribution is important for understanding species persistence and developing species management plans. This issue is particularly pronounced in threatened species with wide-spread range and a low detectability in their natural environment, as surveying and successfully encountering this type of species is oftentimes difficult. One such species is the eastern massasauga rattlesnake (*Sistrurus catenatus*), a small-bodied pit viper with a distribution centered around the Great Lakes region. We used single-season occupancy modeling in order to reassess the status of historic massasauga occurrences. We evaluated factors affecting eastern massasauga detection probability from a long-term dataset to inform a standardized survey protocol. We surveyed 34 sites throughout Michigan's lower peninsula from May through September of 2018 and 2019. We measured site- and survey-specific covariates at each site to inform occupancy and detection probabilities, respectively. Additionally, we used data from 2013-2019 collected from a population of massasaugas located in Southwest Michigan to inform detection-specific models. We found that average canopy cover best predicted occupancy probabilities, while total search effort best explained detection probabilities. From the long-term data, additive effects of total search effort, substrate temperature, the Julian day of year, and total site area best explained differences in eastern massasauga detection probabilities in Southwest Michigan. Our results may be used to guide future surveys efforts for the eastern massasauga at sites with unknown population status. Additionally, our findings suggest that eastern massasaugas may benefit from management plans that encourage reductions in average canopy cover while maintaining adequate refugia from predators and harsh conditions.

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Table 3. Model selection results for the eastern massasauga rattlesnake in a southwest Michigan population surveyed between 2013-2019. Models are ranked using Akaike’s Information Criterion adjusted for small sample size (AIC_c). Header ‘Model’ represents the model evaluated, ‘AIC_c’ indicates the values derived from Akaike’s Information Criterion adjusted for small sample size. Delta AIC_c is represented by ΔAICc and indicates the difference between a model and the top-ranked model. Model weight is represented by w_i. (*L)* represents model likelihood. K indicates the numbers of parameters included in each model. Relative model fit is represented by -2*Log(*L*).

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Chapter 1 – Introduction

Introduction

Detailed knowledge of a species' distribution and geographic extent are both critical for making effective conservation decisions and developing recovery plans for threatened or endangered species. Contemporary estimates of a species' distribution allow managers to effectively allocate resources to enhance or maintain habitat and can impact decisions regarding land management techniques used. Knowledge pertaining to a species' distribution is also important in elucidating how habitat fragmentation, both natural and anthropogenic, will influence the overall geographic range of a species (Guisan and Thuiller, 2005). Additionally, global climate change has shifted numerous species' geographic ranges (Parmesan and Yohe, 2003), making knowledge of historic ranges particularly important in predicting and responding to these changes.

A common source of error in delineating a species' geographic extent is through false absences. False absences occur when a species is incorrectly described as being absent from a patch of habitat due to an investigator's inability to detect it (MacKenzie et al., 2003). Not accounting for these false absences can lead to biases in parameter estimates (e.g., occupancy probabilities, detection probabilities, etc.), and can lead to incorrect inferences about the population's dynamics (Kellner and Swihart, 2014; MacKenzie et al., 2002). False absences are particularly prevalent in cryptic species with patchy distributions that occur at low densities. Species such as these typically have low detection probabilities, which can lead to knowledge gaps in our understanding of their life history and population demographics (MacKenzie et al., 2017).

Occupancy modeling is a technique that predicts the proportion of area a focal species occupies, while accounting for site- and survey-specific detectability (MacKenzie et al., 2002). Using occupancy modeling is a cost-effective and efficient method to evaluate extant population distribution and landscape features associated with occupancy (Mazerolle et al., 2005). This method is particularly advantageous for species with low detection probabilities that occur at relatively low densities throughout a large geographic range (Durso et al., 2011; McGrath et al., 2015), since abundance estimates for these species are typically resource and time intensive (MacKenzie and Nichols, 2004). Consequently, occupancy modeling has been used in studies focused on rare or cryptic species such as Sumatran tigers (Hines et al., 2010), Eastern box turtles (Erb et al., 2015), and an endemic species of Brazilian bromeliad tree frog (Barata et al., 2017).

One such cryptic species is the eastern massasauga (*Sistrurus catenatus*), an imperiled North American rattlesnake with a distribution centered around the Great Lakes region. The eastern massasauga is endangered or threatened in every state or province in which it occurs and is listed as threatened under the United States Endangered Species Act as of September 2016 (Federal Register, 2016). Massasaugas are a wetland-dependent species, and as such have severely declined in numbers and extent due to habitat degradation, destruction, and fragmentation (Syzmanski et al. 2015). Additional threats to massasauga population viability include disease (Allender et al., 2011, 2016), road kills (Shepard et al., 2008), and direct human persecution (Baker et al., 2016; Parent and Weatherhead, 2000). Although there have been a myriad of studies in regard to home range sizes and habitat associations (Bailey et al., 2012; Degregorio et al., 2011; Harvey and Weatherhead, 2010; Moore and Gillingham, 2006), there are still knowledge gaps pertaining to massasauga life history traits, population demographics, and distribution throughout their range (Hileman et al., 2017; Szymanski et al., 2015).

Purpose

The primary purpose of this project was to re-assess the population statuses of several sites throughout the lower peninsula of Michigan. Using occupancy modeling and presence/absence data at sites with historic massasauga occurrence data our goal in this research was to determine what site- and survey-specific covariates would influence massasauga occupancy and detection probabilities, respectively. We also sought to develop a new survey protocol using long-term data collected from a population in Southwest Michigan.

Scope

Results of this project will inform research and management of massasauga populations throughout their range in Michigan's lower peninsula. Although results will largely inform future conservation and management plans in Michigan, results could potentially be used to inform sites outside of the state with similar habitat characteristics. Results of this study could also be used in comparisons throughout the massasaugas range to lend insights into regional differences in factors that support population persistence. Additionally, the methodologies of this study could be used in future studies of range-wide analyses of cryptic species.

Assumptions

In Chapter 2.1 and 2.2 when using single-season single-species occupancy models we assumed that populations were closed to changes in occupancy between surveys. Two consecutive field seasons were used for these analyses; however, we believed that the changes at the population occupancy status would not occur over one inactive season thus allowing me to use a single-season model. We assumed that population status for each site was unknown prior to surveys. Additionally, we assumed that sites were independent of each other by implementing a minimum of a 2 km buffer between sites during site selection of the study design. We assumed that occupancy and detection probabilities were not constant across all units and surveys but were accounted for in our models using site- and survey-specific covariates.

Objectives

For Chapter 2.1 our objectives were to 1) use local-scale, site-specific characteristics to estimate occupancy probabilities of the eastern massasauga rattlesnake throughout Michigan's lower peninsula and 2) use survey-specific covariates to estimate the detection probabilities of the eastern massasauga rattlesnake. For chapter 2.2 our objective was to identify factors that would affect the detection probability of the eastern massasauga to inform a standardized survey protocol.

Significance

Michigan is considered an important stronghold for eastern massasauga persistence, with an estimated 232 remaining populations located throughout the lower peninsula (Syzmanski *et al.* 2015). However, this estimate includes several historic populations with unknown status, but were presumed to be still present. With their recent listing as a threatened species under the U.S. Endangered Species Act, and the historic decline of wetlands in Michigan (Fizzell et al., 2005.), state-wide massasauga conservation efforts should be increased. Yet effective conservation strategies cannot be implemented without accurate knowledge of the species' contemporary distribution within the state. This study will be the first to reassess the occupancy status of these unknown historic populations throughout the state and variables associated with local extirpations.

The protections afforded to the eastern massasauga have implications for the conservation of open-canopied wetlands, a critical habitat that has become increasingly rare. Sustaining the health and function of wetlands is not only important for maintaining wildlife habitat, but additionally for preserving the ecosystem services they provide. Wetlands are one of the most valuable ecosystems, as they provide protections against flooding, filter and transform nutrients, sequester carbon, and recharge groundwater (Zedler and Kercher, 2005). Although wetland restoration has been a priority for land managers, many wetland mitigation or restoration efforts have been ineffective in execution and continued monitoring (Kozich and Halvorsen, 2012). The results of this study will likely identify impaired wetlands with extant populations of massasaugas, thus serving as a guide for restoration planning. Identifying these areas in which resources and effort can be effectively invested will aid in preserving and maintaining these economically and biologically invaluable ecosystems. Results will additionally identify which landscape and site characteristics that foster extant populations of eastern massasaugas, furthering our knowledge of this species distribution and habitat associations.

Definitions

Detection probability (p) – the probability that a species of interest will be detected at a site during a defined survey period.

Occupancy probability (Ψ) – the probability that a species of interest is present at a site during a defined survey period.

Chapter 2.1

Factors Affecting Occupancy and Detection Probabilities of the Eastern Massasauga rattlesnake (*Sistrurus catenatus*) in Michigan.

Arin J. Thacker[1], Eric T. Hileman[2], Paul Keenlance[1], Jennifer A. Moore[1]

[1] Department of Biology, Grand Valley State University, 1 Campus Drive, Allendale, Michigan, 49401, USA

[2] Department of Wildlife, Fisheries and Aquaculture, Mississippi State University, Mississippi State, Mississippi 39762, USA

Abstract

Knowledge of a wildlife species distribution throughout a landscape is key in developing long-term conservation practices and management plans. This issue is particularly relevant for threatened or rare species with a broad geographic range and low detectability in their preferred habitats, as surveying and successfully encountering this type of species is oftentimes difficult. One such species is the eastern massasauga (*Sistrurus catenatus*), a small-bodied, federally threatened rattlesnake with a distribution centered around the Great Lakes region. We used single-season occupancy modeling in order to reassess the status of historic massasauga sites (n=34) distributed throughout Michigan's Lower Peninsula. We measured site- and survey-specific covariates at each site to inform occupancy and detection probabilities, respectively. Average canopy cover best predicted occupancy probabilities, while search effort best predicted detection probabilities. Our top model which included canopy cover (occupancy) and search effort (detection) estimated an average occupancy probability of 0.33 (CI = 0.12-0.64) and average detection probability of 0.65 (CI = 0.43-0.83). Our results may be used to guide future surveys efforts for the eastern massasauga at sites with unknown population status. Additionally, our findings suggest that persistence of eastern massasauga populations relies upon low canopy cover (<60%), so management practices aimed at reducing woody invasive species, or setting back natural succession, will benefit this species.

Introduction

Wild plant and animal populations have been dramatically impacted in the face of changes in global climate regimes, destruction and alterations of landscape mosaic and function, and significant increases of urbanization during the 20th century (Ceballos and Ehrlich, 2002; Parmesan and Yohe, 2003; Lenoir and Svenning 2015). These factors have led to significant changes in both the biotic and abiotic factors that comprise a species habitat, and have thus changed the quality, connectivity, and availability of suitable habitat. Knowledge of a species' precise current distribution within their geographic extent has therefore become critical baseline data for informing conservation efforts. Incomplete biogeographical data may lead to misguided management practices based on imprecise associations between species and habitat characteristics. The potential for biased inferences regarding population dynamics, such as local colonization or extinction rates, or factors correlated with species persistence exists when distributional data are incomplete (Bland et al., 2015; McGrath et al., 2015). Additionally, a species' current distribution should be put into context with their historical range in order to understand the drivers of population contractions or expansions (Tingley and Beissinger, 2009; Laliberte and Ripple, 2003).

Use of historic distributional data allows us to assess how wildlife populations have changed over time and predict how they may change in the face of future climatic and geographic conditions (Lütolf et al., 2009). This type of historic data may range in source (i.e., journal entries, trapper/hunter logs, public survey data, death assemblage/fossil records) and timeframe, and will vary depending on an investigator's objectives. Nevertheless, historic biogeographical data use can present a suite of challenges when making comparisons to contemporary species data. When comparing current species data to historic studies of distribution issues can arise in incompatible methodologies, species misclassifications, imprecise geographic locations, or failing to account for false absences (i.e., assuming a species was absent from a location when it was not) (Tingley and Beissinger, 2009). Although using these historic data presents challenges, identifying the ways in which the distributional records have changed over time could allow us to model how a species range may shift under different future climate and land-use change scenarios (Williams and Blois, 2018).

Surveying the entirety of a species' geographic distribution is both a time and resource intensive endeavor, especially for species with a widespread range. This issue is compounded by the fact that species are often detected imperfectly when present due to cryptic coloration or behaviors, seasonal changes in habitat use, or a surveyor's inability to detect the species when present (Guimarães et al., 2014). Occupancy models allow for detectability of a species to be explicitly incorporated into estimates of occupancy through repeat surveys at multiple sites (MacKenzie et al., 2002). These models offer a framework that can estimate associations between parameters of interest and specific user-defined habitat or survey characteristics. Given their flexibility in study design and their ability to incorporate auxiliary data, occupancy models can be particularly useful when dealing with animals with naturally low detection probabilities and broad geographic ranges (MacKenzie et al., 2017).

Reptiles represent one of the most imperiled, yet least studied, taxa in scientific literature (Gibbon et al., 2000; Saha et al., 2018; Tingley et al., 2016). There are approximately 11,136 described reptile species in the scientific community, yet only 7,833 have been assessed by the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species. Of the species assessed by the IUCN, 1,160 species remain data deficient, in which data regarding population demographics, abundance, status or geographic distribution are missing (IUCN, 2020). Although distributional and range size data are critical for assessing population demographics and allocating conservation resources, they are particularly useful as a predictor of extinction risk for squamate reptiles (Böhm et al., 2016). Reptile species that fall into the category of data deficient therefore require large-scale effective surveying and monitoring strategies to address this knowledge gap, which is additionally complicated by the cryptic nature of many reptile species (Mazerolle et al., 2005).

The eastern massasauga rattlesnake (*Sistrurus catenatus*; hereafter massasauga) is a wetland-dependent pit viper distributed throughout the Great Lakes region of North America (Harding and Mifsud, 2017). Massasauga populations have markedly declined throughout their range as open-canopied wetlands, their primary habitat type, are lost or degraded through agricultural conversion, urbanization, or vegetative succession (Bailey et al., 2011; Szymanski et al., 2015). Maintaining these open-canopied wetland types has additionally been hindered by encroachment of both native and non-native invasive plant species (e.g., glossy Buckthorn, purple loosestrife, Autumn olive, Japanese honeysuckle, Aspen), which can dramatically alter wetland community structure, function, and thermal regime. Additional threats to their continued survival include road mortality, human persecution, and snake fungal disease (Szymanski et al. 2015). These synergistic threats have led to the massasaugas status as a threatened species under the U.S. Endangered Species Act and the Canadian Species at Risk Act (COSEWIC, 2013; Federal Register, 2016). Throughout their range, the lower peninsula of Michigan has the most extant populations of any other state or province (Szymanski et al., 2015). Thus, conservation of Michigan populations is crucial for eastern massasauga recovery. However, a status assessment conducted by Szymanski et al. (2015) identified 84 populations with an unknown status, 67 of which occurred in the analysis unit that contained Michigan’s lower peninsula. As such, it is critical to understand their current distribution throughout the state and determine what factors promote population persistence.

In this study, we surveyed sites with historic occurrences of eastern massasaugas throughout Michigan's lower peninsula to estimate occupancy and detection probabilities and associated covariates. Our objectives were specifically to, 1) determine what local-scale site and survey characteristics best predict occupancy and detection probabilities, respectively, and 2) describe patterns of occupancy in relation to multiple landscape-scale variables of canopy and habitat quality.

Methods

Study Design and Field Methods

We used 264 historic occurrences of massasauga sighting data from the Michigan Natural Heritage Database (Michigan Natural Features Inventory; MNFI) as a preliminary guide in site selection. These occurrences were comprised of confirmed sightings of massasaugas with varying degrees of geographic certainty, distributed across Michigan's lower peninsula. These historic occurrence data also contained a "last observed" date (i.e., the most recent date in which a massasauga was confirmed to be present in the area) which ranged from 1938 to 2014. We used these data in conjunction with a species distribution model to identify potential survey sites that contained potential massasauga habitat (E. McCluskey, unpublished data). Potential survey sites were additionally restricted to only include sites that were present on public land, thus assuring access and potential for long-term management and protections. We optimized our occupancy-based sampling design using software GENPRES8 (Bailey et al., 2007). Using a previously estimated detection probability for massasaugas of 0.31 (Harvey, 2005), we developed a study design that maximized both estimates of occupancy and detection of 45-50 survey sites, with four to five repeated surveys per site.

In an idealized study design, sites are surveyed in a completely randomized order. However, the time and logistical constraints of surveying the requisite number of sites across the entire peninsula, a sufficient number of times, within an active season necessitated a semi-random survey order. Thus, we grouped sites that were less than 30 kilometers from each other and treated these groupings of sites as a cluster. Clusters were then assigned a random survey order, and sites within the clusters were also surveyed in a random order.

We performed visual encounter surveys to detect massasaugas at sites over two field seasons during portions of the active season of massasaugas, 13 May through 16 August of 2018 and 2019. Each site was surveyed on a minimum of two separate occasions and a maximum of seven occasions. Surveys occasions were conducted with varying numbers of surveyors (2-10) in the field for a minimum of one hour of active searching and were separated by a minimum of 24 hours between surveys at the same site. Surveys were completed under appropriate climatic conditions to detect massasaugas (no precipitation, <15 mph wind speed, and temperature range of 50-90º F; see Casper et al. 2001). For each snake encountered, we recorded the location using a handheld Garmin GPS unit, along with microhabitat conditions (shaded air and soil temperatures, cloud cover, and litter depth).

Local-Scale Covariates

We collected microhabitat data in the field to create site- and survey-specific covariates. To inform detectability, we recorded survey-specific variables during each survey, including search effort (person hours), survey start and stop times of day, shaded air and substrate temperatures (C°) at the beginning and end of each survey, day of year, cloud coverage (estimated to the nearest 25%) at the beginning and end of surveys, and any precipitation (rain, hail, snow). Search effort accounted for how many surveyors were present during each survey and was measured by recording survey start and stop times minus the time spent not actively searching for snakes. Each surveyor was also equipped with a handheld Garmin GPS unit to record their survey tracks in the field. Area of actively searched habitat was calculated from projected GPS tracks of every surveyor using the Feature to Polygon function in ArcMap 10.4.1.

Community-level vegetation structure was measured through vegetation surveys with a semi-randomized subplot design. A location that contained vegetation and structural characteristics considered representative of the site was chosen to act as a centroid location of the plot. From this centroid location surveyors determined a random number of paces in each cardinal direction using a stopwatch (constrained to 0-30 paces). At the terminus of each cardinal direction surveyors took subplot measurements, resulting in a total of four subplots per vegetation survey. Subplot (1 x 1 m in size) measurements included counts or estimates of: stem density, diameter at breast height (DBH) of any stem greater than five cm in circumference, measurements of litter depth (cm), and percent canopy cover. Litter depths were measured at the corners of each subplot, giving a total of four measurements per subplot. Canopy cover was recorded at the center of each subplot using a hemispherical lens (Apexel 6, Aipai Optic) and a Sunpak 5400DLX Tripod at approximately 30 cm. All stems of woody plants within the angle of view of the hemispherical photograph were counted for stem density and measured for DBH. This process was conducted twice at each site (apart from two sites), giving a total of eight subplots per site. Hemispherical measurements of canopy cover were processed with Gap Light Analyzer 2.0 (Frazer, 1999).

Landscape-Scale Variables

We used remotely sensed data to collect broader, landscape-scale data on canopy cover and habitat suitability. We characterized canopy using various measures of the Lidar Point Cloud database on the National Map (V1.0, U.S.G.S.). Measures of canopy height average (CHA), canopy cover (CC), and canopy density (CD), were downloaded at a buffer distance of 250 and 1,000 meters around the centroid of all sites in which the data were available. Average canopy metrics were taken at an 18.3 x 18.3 m resolution and were limited to all stems greater than or equal to 1.5 m in height. Canopy Lidar data were not publicly available for seven of our northern Michigan sites, thus limiting our inference to a qualitative description of these data.

Habitat suitability data were used from an ongoing massasauga niche modeling project in which several subregions throughout the state have been modeled for massasauga habitat suitability (E. McCluskey, unpublished data). We used the full state model in which various measures of scrub wetland availability, soil drainage, canopy range, and topographic position index were determined to be of highest importance for massasauga habitat suitability. Proportions of suitable habitat were determined at each site through maximizing the sum of sensitivity and specificity (maxSSS), a conservative threshold selection method (Liu et al., 2016). Suitable habitat was taken at buffer sizes of 250, 500, and 1,000 m from the centroid of each site and were measured as the proportion of suitable habitat compared to total buffer zone area.

Statistical Analyses

Prior to analysis, all covariates were tested for correlations using the Pearson's correlation coefficient. Any correlation value of ≥ 0.65 between two variables resulted in excluding one of the variables from final analysis. We used maximum likelihood methods and single-season, single-species occupancy models to account for imperfect detection in program PRESENCE 2.12.43 (MacKenzie et al., 2002). In this model type two main parameters are estimated; the probability that a species is present at a site (ψ), and the probability that the species of interest will be detected if present (p). These probabilities were constrained between 0 and 1 using the logit link function. We *a priori* developed 20 candidate models that included 14 combinations of univariate models, five bivariate models, and one null model. Candidate models were built from previous knowledge of massasauga habitat characteristics and variables known to affect detection (Casper et al., 2001; Crawford et al., 2020; Shoemaker, 2007). Bivariate models for the occupancy parameter included an additive effect between site-specific covariates (*i*) (Equation 1):

$$logit\ (\psi) = \beta_0 + \beta_1\ (covariate_i) + \beta_2\ (covariate_i) \qquad (1)$$

Univariate models for occupancy and detection were reduced forms of the bivariate model that included one covariate to estimate the occupancy or detection parameter. Two models were added *a posteriori* to assess the model fit of the top-ranked model. Due to small sample size, we limited the number of parameters considered to a maximum of four per model. All covariates included in modeling were normalized using a z-transformation. Models were ranked using Akaike Information Criterion values adjusted for small sample size (AIC_c; Akaike, 1998). We set the effective sample size to the total number of sites sampled (33) to decrease the chance of overfitting the data (MacKenzie et al. 2017). We approximated the conditional standard error of the top-ranked model from the candidate set using the delta method.

Landscape data were analyzed in an exploratory manner to determine if any patterns of occupancy in relation to surrounding site canopy and habitat suitability metrics were apparent.

All canopy and habitat suitability data were tested for correlations using Pearson's correlation coefficient. Associations with a r value greater than 0.70 resulted in excluding one of the variables. We then used multiple one-way ANOVA tests in software R Studio (R Core Team, 2016) to test for significant differences between groups of 'high'(ψ = 0.41-0.60), 'medium'(ψ = 0.21-0.40), and 'low'(ψ = 0.0-0.20) occupancy probabilities as defined by the top-ranked model estimates. Additionally, we tested if there were significant differences in canopy and habitat quality metrics at sites in which we did encounter a massasauga (1), and sites in which we did not encounter a massasauga (0). We further divided these groups based on if they were located in the southern portion of Michigan's lower peninsula (S) or located in the northern portion of the state (N). If an ANOVA test revealed significant differences between groups, we used a *post hoc* Tukey's test to compare means.

Results

Descriptive summary

Due to logistical constraints and limited site access from seasonal flooding, we were only able to survey 34 sites. We performed 138 visual encounter surveys at 34 sites, totaling 851.38 person hours across all sites. Site distribution spanned broadly across the entirety of Michigan's lower peninsula, apart from one site located on Bois Blanc Island, Mackinac County (Figure 1). We encountered 44 massasaugas at ten of the 34 sites, yielding a naïve occupancy probability (not accounting for detection) of 0.29. All sites in which massasaugas were encountered during the first field season had encounters again in the second season, apart from one southwestern site. Area of surveyed habitat ranged from 0.44 ha to 14.83 ha per site, with an average site area of 5.22 ha. Percent canopy cover was averaged across subplot measurements per site and ranged from 0.83% to 61.18%, except for one site in which equipment malfunction prevented us from taking measurements. Shaded air temperature ranged from 10.22° C to 28.78° C, with a mean of 20.78° C (0.41 SE) across all sites. Shaded substrate temperature ranged from 8.50° C to 22.22° C, with a mean of 15.96° C (0.51 SE) across all sites. Average search effort across all sites ranged from 1.93 to 11.85 person hours, with a mean of 5.8 total person hours per site (0.49 SE).

Local-Scale Occupancy

Due to missing site-specific covariate information we omitted one site in final analysis, thus adjusting our effective sample size to 33. Our analysis indicated that total search effort expended during a survey has the strongest effect on massasauga detection probability (Table 3). Of the five variables investigated for effects on the detection parameter, 'Effort' was present in all supported models (Table 3). The top-ranked model, ψ (Canopy), p (Effort), received 27% of AIC_c weight, while the second model, including only 'Effort' as an explanatory variable for detection, received approximately 16% of AIC_c weight (Table 3). The top-ranked model estimated an average detection probability of 0.65 (CI = 0.43 – 0.83), exhibiting a strong, positive relationship between detection probability and total hours spent surveying during a survey. Detection probability approaches 1 as search effort for a single survey reaches 20 total person hours spent actively searching for massasaugas (Figure 3).

The top-ranked covariate explaining occupancy probability of massasaugas was average percent canopy coverage (Table 3). Occupancy probability decreased as average canopy cover increased from 0 to 60% coverage (Figure 2). Average occupancy probability derived from the top-ranked model is 0.33 (CI = 0.12 – 0.64). This pattern was also evident in the model in which detection probability was held constant and 'Canopy' was the only covariate used to explain occupancy. Although, -2 log likelihood values did not support good model fit (Table 3).

Landscape-Scale Analysis

Although we intended to include Lidar-derived canopy variables in occupancy models we had insufficient publicly available Lidar coverage to provide enough power to perform robust statistical analysis. However, Lidar data have the potential to accurately, and remotely, characterize canopy cover for massasauga sites, so we present them here. No statistically significant differences existed in canopy or habitat suitability between sites grouped into the 'Low', 'Medium' or 'High' occupancy probabilities. Groupings based on site locality (north or south), and whether we encountered a massasauga at the site (1 or 0), yielded two results in which there were significant differences in habitat suitability. One-way ANOVAs revealed a significant difference in habitat suitability at the 250 and 500 m buffer (p = 0.01, 0.04) only between southern and northern sites in which we did not encounter a massasauga, with northern sites showing significantly higher amounts of suitable habitat even when a massasauga was not encountered. Using the sites in which Lidar data were available, we averaged the occupancy probabilities of groups based on site locality and encounter data (Table 4). Average occupancy probabilities at northern sites in which we did not encounter a massasauga was 0.43 (0.16 SE), while occupancy probabilities averaged 0.17 (0.10 SE) at southern sites in which we did not encounter a snake (Table 3).

Discussion

Here we provided a robust analysis of occupancy patterns of eastern massasaugas in Michigan, and show occupancy and detection are most affected by canopy cover and search effort, respectively. With the top five ranked models accounting for approximately 74% of AIC_c weight, models that included search effort as an explanatory variable for detection probability were strongly supported (Table 3). Detection probability of massasaugas approaches one after surveying for 20 person hours, the equivalent of five surveyors searching for four hours, during a singular survey period. Even with a total of 7.5 person hours expended during a survey yields a detection probability of 0.76, whereas exerting approximately one-person hour per survey results in a much lower probability of 0.41. Our results indicate that investing greater amounts of search effort during survey occasions will result in significantly greater chances of encountering a massasauga.

Eastern massasaugas are cryptic, both in coloration and behavior, therefore it is not surprising that finding them using visual surveys requires considerable effort. One previous study investigated the effects of surveyor effort on detection probability of eastern massasaugas in Michigan (Shaffer et al., 2019). Shaffer et al. (2019) found that detection probability approaches 1 as an individual surveyor spends approximately 90 minutes actively searching. However, notable differences exist between Shaffer et al. (2019) and our study, including the number, locality, and area of sites and the known occupancy status and snake abundance of each site. Additional support of our results is apparent in Crawford et al (2020), in which search effort exhibited a strong positive relationship with detection probabilities of eastern massasaugas in Illinois. In the only other known study of massasauga detectability, Harvey (2005) was able to investigate the level of surveyor experience on the detection probability of massasaugas within an area- and time-confined survey event, which resulted in an average detection probability of 0.31.

Of the four covariates we investigated, average canopy cover had the highest explanatory power for occupancy probabilities of eastern massasaugas. We estimated an average occupancy probability of 0.33 from areas that have been historically occupied by massasaugas throughout

Michigan's lower peninsula. This estimate is only slightly above our naïve occupancy estimate of 0.29, suggesting that even when detection probability is accounted for, occupancy is relatively low throughout the massasaugas core range. From the top-ranked model estimates, occupancy probability peaked at $\psi = 0.6$ at a site with nearly 0% average canopy coverage, indicating that maintaining open-canopied patches is critical for persistence of massasauga populations. It is important to note that measurements of canopy cover were taken from a height of approximately 30 cm from the ground, meaning we were able to capture the effects of both tree and low shrub structure. Moreover, numerous studies have supported this association between reduced canopy cover and continued site use by snakes (Bailey et al., 2012; Harvey and Weatherhead, 2006; Robillard and Johnson, 2015). Maintaining open-canopy basking sites is what allows for an ectothermic species, like the massasauga, to regulate their internal body temperatures.

Encroachment of both invasive and native woody plant species has been cited as a threat to the habitat suitability for massasaugas in several past studies throughout the massasaugas range. Dovčiak et al. (2013) show prescribed fires at a degraded early-successional ecosystem increased habitat use for eastern massasaugas. Results of this study indicated a positive relationship between massasauga presence and increased percentage of bare ground availability after treatment of a prescribed burn. An additional study that examined the relationship between woody encroachment and basking availability took place in central New York, in which they found that both the thermal quality and crypsis potential were significant determinants in the quality of massasauga basking sites (Shoemaker and Gibbs, 2010). Canopy removal has been shown to increase habitat quality for additional squamate species, particularly in landscapes in which the natural fire regime has been suppressed. Webb et al. (2005) found that decreasing canopy cover by as little as 15% restored habitat quality for the endangered Broad-headed snake

(*Hoplocephalus bungaroides*) by creating basking sites for both the snakes and their prey items. Sites that support a high degree of basking site availability are typically used more frequently, particularly by gravid females, during the mid-to-late active season. These basking sites are not only critical for allowing massasaugas to regulate their internal body temperature, but additionally for enabling gravid females to decrease their overall gestational period (Harvey and Weatherhead, 2010). Results of our analysis further support the need for adequate basking sites to maintain population persistence at sites that have been historically occupied by massasaugas.

Although our landscape analysis provided only limited inference, we did detect a significant difference between the proportion of suitable habitat at 250 and 500 m site buffers at southern and northern sites in which we did not encounter massasaugas. This pattern likely points to geographic differences in how historically suitable massasauga habitats degrade. In northern sites, vegetative succession appears to be more common in previously open-canopied habitats, whereas in flooding or cattail monoculture invasions appeared more common in southern sites (Figure 4). Our lack of power for this analysis likely stems from our inability to include these data for our complete dataset. Additional sources of discrepancies may be due to a restricted Lidar canopy height of 1.5 m, excluding many shrub or sapling stems that may affect the overall thermal quality of a site. Future studies should aim to incorporate Lidar-derived data for all sites and include metrics related to shrub or understory plants.

Conservation and Management Implications

To our knowledge, this is the first study in which the occupancy and detection probabilities have been evaluated for massasaugas at a nearly statewide scale. Previous studies of massasauga habitat associations in Michigan have been generally limited to few sites, thus limiting the inferential power to sites that share similar characteristics as those performed in the study. Results of this study, while general in nature, are based on sites with variable habitats in the core of the massasaugas range, allowing for a high degree of inferential power.

Although our results provide insights into the factors that affect the thermal habitat characteristics that allow for massasauga population persistence, a limitation of this study includes our inability to measure historic changes in site hydrology. Three of the sites in which we attempted to survey during the first field season were unavailable due to severely flooded site conditions. Of these sites, only two were re-surveyed during the second field season and were found to be in the same condition. These flooded conditions, if prolonged throughout the massasaugas active season, could affect the amount of quality basking habitat and the availability of suitable hibernacula for overwintering. Massasaugas require sites that provide adequate hibernacula, features that are largely dependent on the stability of the water table being high enough to provide thermal protection from freezing temperatures, but low enough to allow sufficient oxygen flow. Changes in hydrology are additionally responsible for changes in vegetation community composition and structure, a feature that we show to be of vital importance to the eastern massasauga. Future studies should attempt to quantify hydrology changes at sites that have historically been occupied by massasaugas.

Of all the variables we measured, canopy cover is clearly the most important factor for maintaining habitats that will support massasaugas. Our results show that if a site approaches 50-60% canopy closure, that site is likely no longer suitable for massasaugas. Thus, a management goal should be to keep overall canopy coverage (due to both shrubs and trees) well below 50%. We recommend maintaining patches of open-canopied habitat throughout a site to ensure an adequate thermal environment in which massasaugas can effectively thermoregulate. Exact measurements of canopy reduction would vary by site, but an overall recommendation would be in reducing canopy cover in portions of high shrub or tree cover to 5-10% average canopy cover, interspersed with areas in which bare ground is exposed to provide high-quality basking sites. Although the importance of creating open-canopy basking areas is critical for maintaining high quality massasauga habitat, it is also important to maintain a high degree of habitat heterogeneity in which refugia from predators and harsh conditions are in adequate supply.

In terms of prioritizing management, efforts aimed at reducing canopy cover should be implemented first at sites that have confirmed massasauga populations. Secondary to these sites are sites with historic populations of massasaugas wherein suitable habitat is still present but may be in a degraded condition. Sites that fit this description and possess some degree of connectivity to sites with extant populations of massasaugas should then be prioritized for restoring habitat quality. Doing so may allow for population expansion or dispersal into restored habitats.

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Figure Captions

Figure 1. Distribution of the 34 eastern massasauga survey sites throughout Michigan's lower peninsula and Bois Blanc island. Sites are depicted by the small circle located adjacent to the values, which represent the estimated occupancy probability from the top-ranked model in which average canopy cover best explained patterns of occupancy. Sites in which a massasauga was encountered during surveys are represented by red circles, while sites in which massasaugas were not encountered are represented by white circles. The 'N/A' indicates a site with missing covariate data, for which occupancy probability was not estimated.

Figure 2. The relationship between eastern massasauga occupancy probability and average canopy cover from data collected throughout the lower peninsula of Michigan between 2018-2019. Shaded regions represent 95% confidence intervals.

Figure 3. The relationship between estimated eastern massasauga detection probability and the total amount of search effort expended in person hours per survey. Survey data were collected throughout the lower peninsula of Michigan between 2018-2019. Shaded regions represent 95% confidence intervals.

Figure 4. Relative quality of habitats in northern and southern sites in which we did or did not encounter massasaugas. Starting in the upper left corner and continuing in a clockwise direction is a northern site in which we did not encounter snakes, a southern site in which we did not encounter snakes, a southern site in which we did encounter snakes, and a northern site in which we did encounter snakes. All photos were taken by A. Thacker between 2018-2019.

Figure 1.

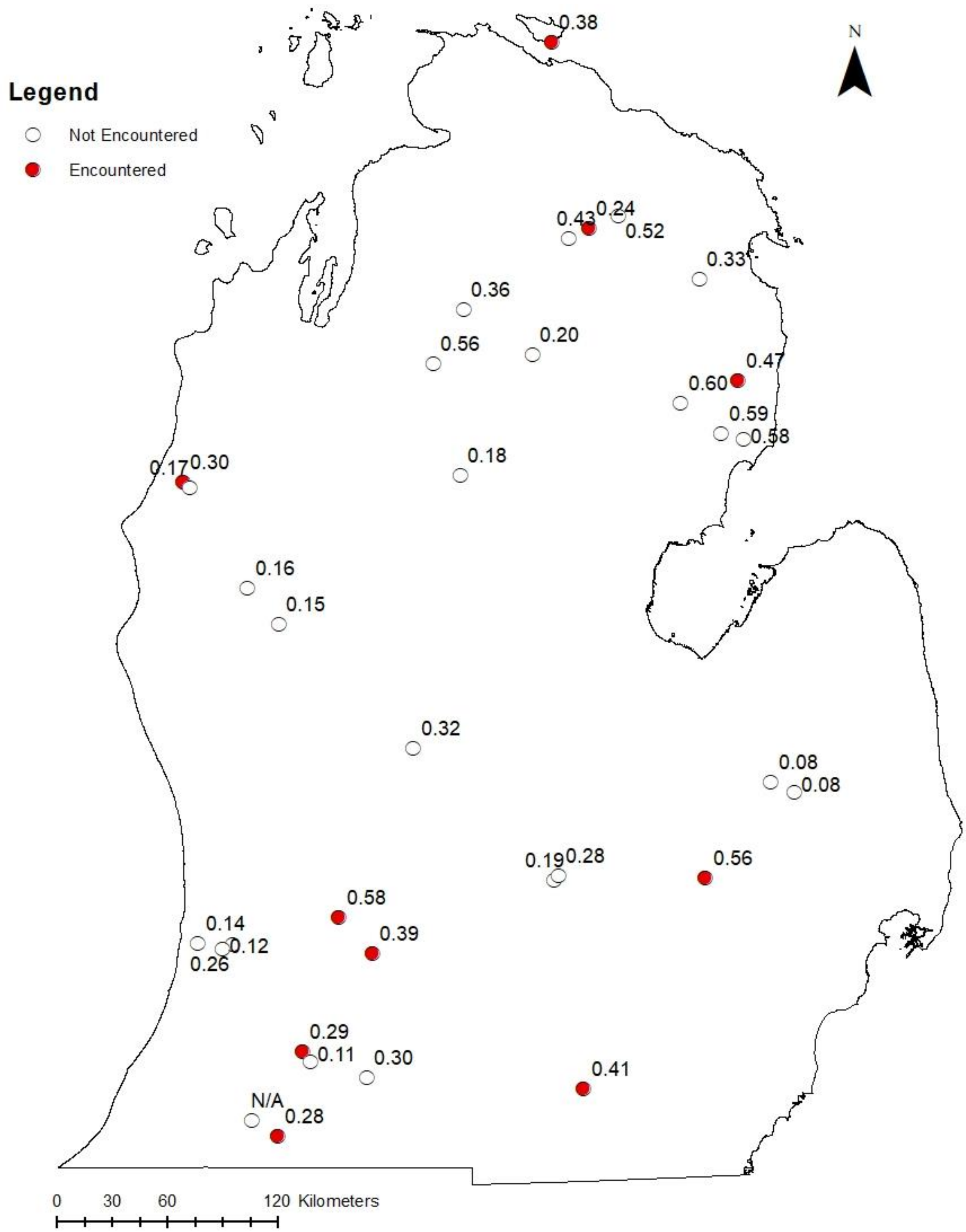

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Figure 2.

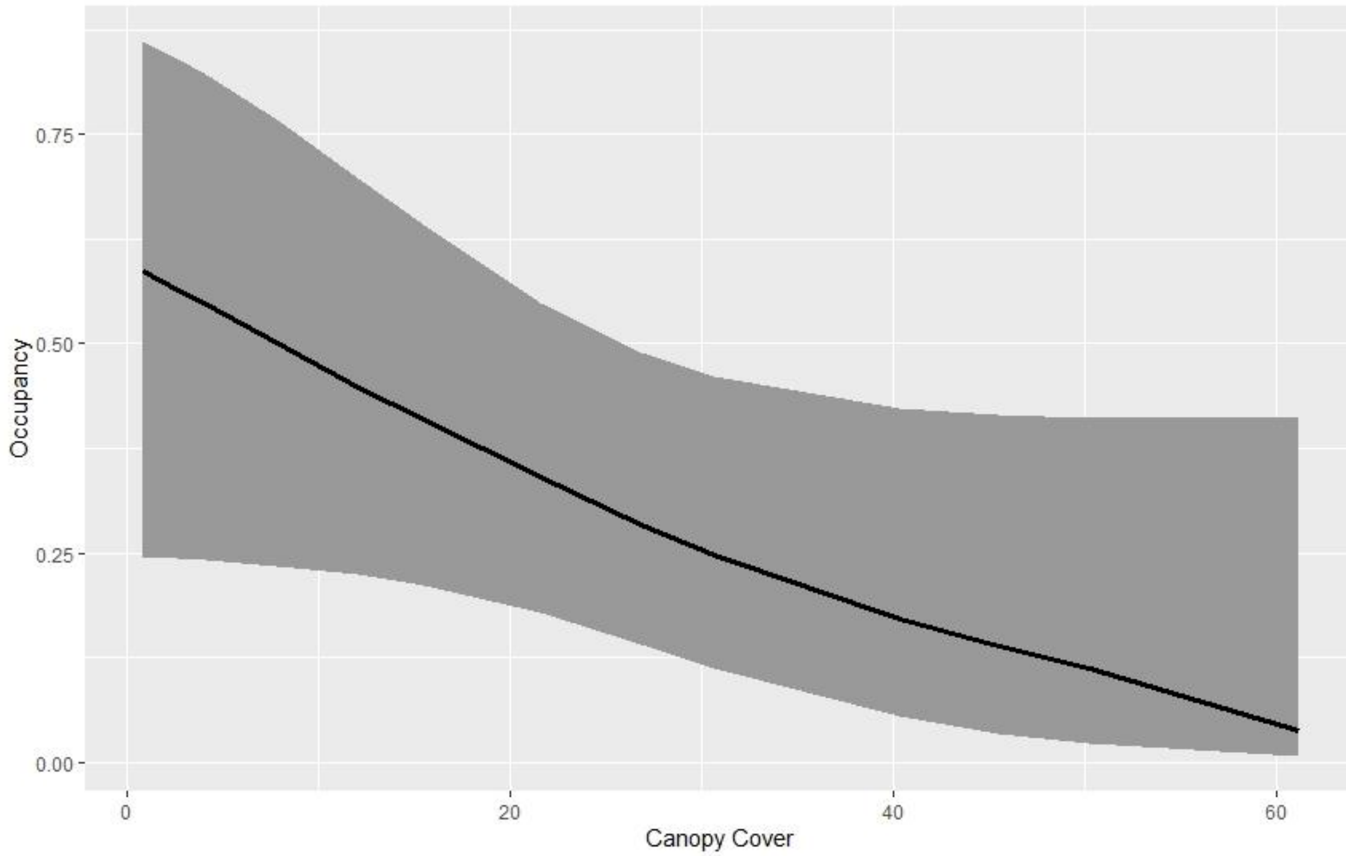

Figure 3.

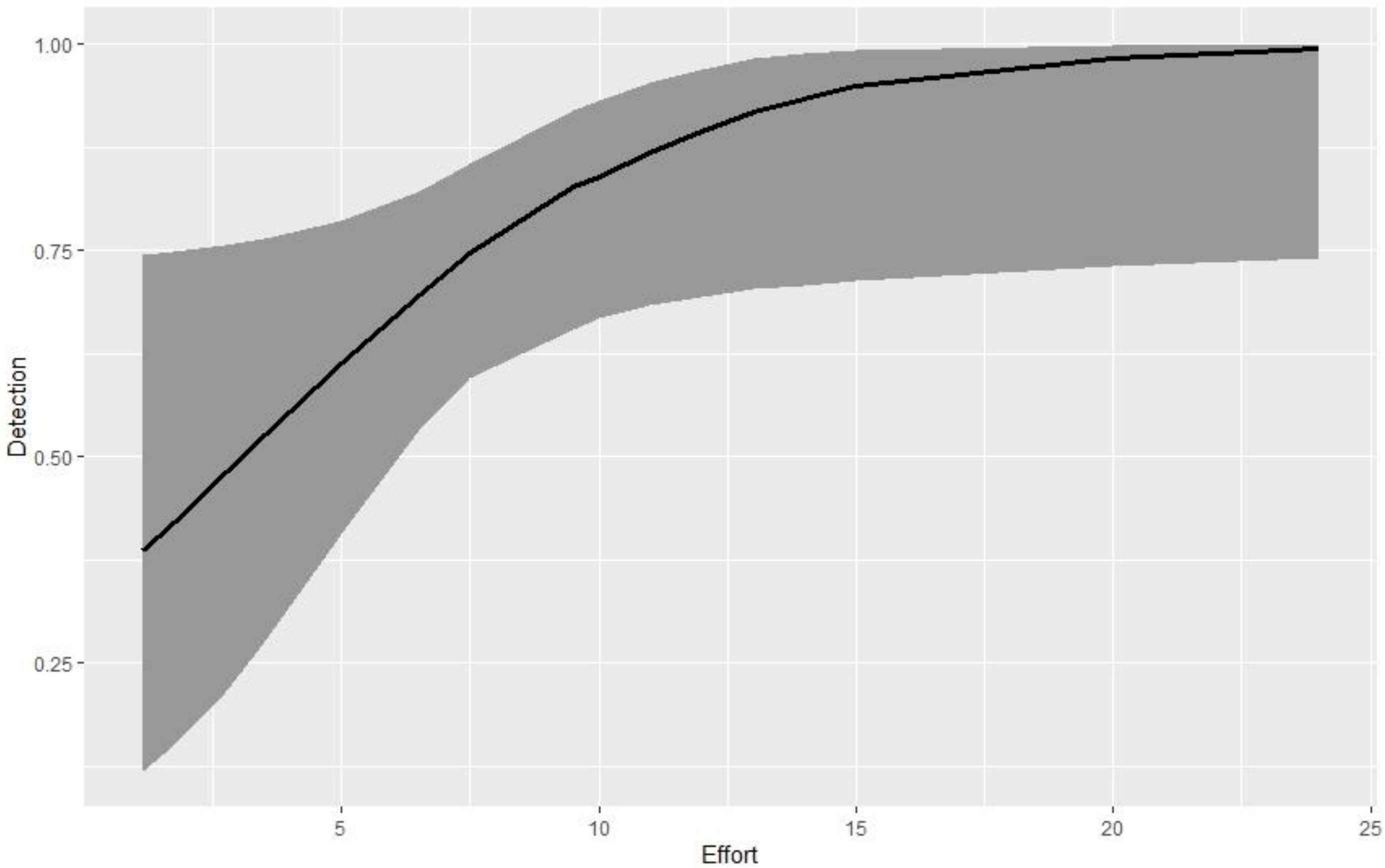

Figure 4.

Table 1. Local and landscape-scale covariates used in the analyses of occupancy and detection of Eastern Massasaugas throughout Michigan's lower peninsula from 2018-2019.

Variable	Description	Unit	Source
Local-Scale Covariates			
Canopy	Mean measurement of canopy cover	avg % coverage	Veg. plots
Area	Total area of a site	ha	Surveyor tracks
Depth	Mean measurement of litter depth	cm	Veg. plots
Density	Mean count of stems	avg count	Veg. plots
Time	Start time of the survey	minutes since 6:00AM	Individual survey
AirTemp	Shaded air temperature at the start of the survey	° C	Individual survey
SubTemp	Shaded substrate temperature at the start of the survey	° C	Individual survey
Effort	The total number of hours spent searching	hours	Individual survey
Cloud	The estimated cloud coverage at the beginning of a survey	nearest 25%	Individual survey
Landscape-Scale Variables			
Canopy Height Average (CHA)	Average of all canopy heights above 1.5 m	m	Lidar Point Cloud, U.S.G.S. – National Map Viewer Data
Canopy Cover (CC)	The number of first returns above the cover cutoff divided by the number of all first return	Avg %	Lidar Point Cloud, U.S.G.S. – National Map Viewer Data
Habitat Suitability Model (HSM)	The proportion of suitable habitat divided by the total area of the site	Proportion	E. McCluskey – unpublished data

Table 2. Summary data of site- and survey-specific covariates collected throughout Michigan, 2018-2019. From left to right the columns represent: site name, total number of massasaugas (EMR) encountered, total number of surveys conducted (N), average person hours (Effort) per survey, the total person hours at each site, average shaded air (Air) temperature at the beginning of surveys, average shaded substrate (Sub.) at the beginning of surveys, average percent canopy cover across all subplot measurements, average stem density across all subplot measurements per site, average litter depth (cm) across all subplot measurements per site, total site area.

Site	EMR	N	Effort /Survey	Effort /Site	Air C° (SE)	Sub. C° (SE)	Avg. % Canopy	Stem Density	Litter Depth	Area (ha)
NE1	11	4	11.85	47.38	17.81 (1.01)	12.42 (1.63)	19.54	14.63	3.57	5.41
NE10	7	7	4.38	30.65	22.28 (1.38)	15.62 (0.64)	33.41	12.25	4.32	7.96
NE11	0	4	3.37	13.48	23.25 (1.93)	13.88 (2.00)	15.47	0.00	19.98	2.74
NE12	0	4	3.65	14.6	23.85 (0.70)	17.42 (0.57)	7.84	0.63	19.53	11.91
NE3	0	3	4.5	10.8	21.56 (1.99)	18.72 (1.74)	2.60	0.00	3.10	5.03
NE4	0	4	3.74	14.95	20.71 (1.13)	13.49 (0.80)	2.12	2.63	12.89	12.51
NE5	0	3	2.68	8.03	17.78 (0.91)	14.13 (0.27)	0.83	6.63	4.12	4.77
NE6	17	7	7.41	51.9	19.23 (0.83)	12.98 (0.50)	12.10	0.00	12.04	2.81
NE7	0	4	10.98	43.9	16.72 (0.57)	8.44 (0.13)	38.64	21.13	9.45	14.37
NE9	0	3	7.02	21.07	18.24 (1.23)	16.61 (0.77)	24.16	9.00	5.21	8.49
NW1	0	4	6.08	24.32	17.24 (1.31)	15.54 (0.61)	21.58	13.63	9.77	1.78
NW11	0	3	3.69	11.07	23.82 (1.26)	17.43 (0.88)	43.01	6.00	6.11	5.49
NW12	0	5	3.77	18.83	23.22 (0.99)	17.30 (0.28)	45.50	3.13	12.77	3.21
NW13	0	4	6.93	27.72	23.13 (0.67)	17.94 (0.27)	24.77	3.00	17.37	7.39
NW4	0	4	4.13	16.53	21.66 (1.13)	18.50 (0.49)	4.04	7.50	3.12	14.83
NW5	0	3	4.26	12.77	20.43 (1.72)	12.26 (2.28)	40.32	11.38	5.49	5.91
NW7	0	4	3.59	14.37	16.03 (2.44)	13.42 (1.76)	42.54	16.88	6.50	2.01
NW8	8	7	4.71	32.97	19.78 (0.98)	12.85 (1.01)	26.82	14.38	6.14	3.22
SE10	0	3	3.71	11.12	21.35 (1.47)	19.21 (1.05)	61.18	0.00	16.84	1.34
SE11	0	2	3.16	6.32	20.94 (0.39)	17.36 (0.96)	60.19	0.25	14.94	0.59
SE12	0	5	2.98	14.92	24.49 (1.25)	19.86 (0.78)	29.06	0.50	7.13	4.71
SE13	0	3	2.71	8.13	20.41 (2.34)	19.00 (0.74)	39.72	5.00	8.00	0.58
SE2	11	6	9.53	57.18	21.21 (0.79)	21.02 (0.29)	16.68	0.25	9.71	5.21
SE9	22	6	9.6	57.58	22.66 (1.49)	19.77 (0.42)	4.14	1.50	17.96	6.86
SW1	11	7	10.89	65.33	18.85 (0.88)	12.83 (1.59)	N/A	4.13	16.30	5.34
SW10	13	6	6.37	38.23	19.19 (1.10)	18.71(1.09)	28.45	10.50	11.55	1.17
SW11	0	3	3.92	11.75	22.07 (0.24)	18.37 (0.28)	26.61	0.00	23.49	3.27
SW12	0	2	8.61	17.23	26.00	16.92 (0.49)	52.25	6.38	8.87	1.59
SW13	21	5	11.43	57.15	22.14 (0.95)	17.90 (0.41)	28.12	5.50	10.25	4.44
SW15	1	3	8.82	26.47	21.54 (0.92)	14.22 (0.93)	2.59	5.13	15.58	12.96
SW6	0	3	8.34	25.02	20.72 (2.96)	11.25 (0.02)	30.65	22.75	6.13	1.42
SW7	0	5	3.29	16.45	16.71 (0.95)	12.51 (0.86)	50.29	5.63	6.63	3.07
SW8	0	3	5.13	15.4	19.22 (1.49)	15.29 (1.41)	46.88	0.75	29.07	4.66
SW9	0	2	1.93	3.85	22.08 (0.53)	19.36 (0.26)	N/A	N/A	N/A	0.44

Table 3. Local-scale model selection results for eastern massasauga rattlesnake occupancy and detection throughout Michigan, 2018-2019. Header 'Model' represents the model evaluated, 'AIC_c' indicates the values derived from Akaike's Information Criterion adjusted for small sample size. Delta AIC_c is represented by ΔAICc and indicates the difference between a model and the top-ranked model. Model weight is represented by w_i. (*L)* represents model likelihood. K indicates the numbers of parameters included in each model. Relative model fit is represented by -2*Log(*L*). *Ψ* is occupancy, and *p* is detection.

Model	**AICc**	**ΔAICc**	**w_i**	**(*L)***	**K**	**-2*Log(*L*)**
Ψ(Canopy), *p*(Effort)	104.41	0.00	0.2656	1.0000	4	94.98
Ψ(.), *p*(Effort)	105.39	0.98	0.1627	0.6126	3	98.56
Ψ(Canopy + Area), *p*(Effort)	106.02	1.61	0.1187	0.4471	5	93.80
Ψ(Canopy), *p*(Time)	106.65	2.24	0.0866	0.3263	4	97.22
Ψ(Canopy), *p*(.)	107.16	2.75	0.0671	0.2528	3	100.33
Ψ(Density), *p*(Effort)	107.96	3.55	0.0450	0.1695	4	98.53
Ψ(Area), *p*(Effort)	107.99	3.58	0.0443	0.1670	4	98.56
Ψ(.), *p*(.)	108.18	3.77	0.0403	0.1518	2	103.78
Ψ(Canopy), *p*(Substrate)	108.27	3.86	0.0385	0.1451	4	98.84
Ψ(Canopy), *p*(Air)	109.41	5.00	0.0218	0.0821	4	99.98
Ψ(Density), *p*(Time)	110.00	5.59	0.0162	0.0611	4	100.57
Ψ(Litter), *p*(Time)	110.05	5.64	0.0158	0.0596	4	100.62
Ψ(Area), *p*(Time)	110.07	5.66	0.0157	0.0590	4	100.64
Ψ(Density + Area), *p*(Effort)	110.74	6.33	0.0112	0.0422	5	98.52
Ψ(Canopy + Litter), *p*(Substrate)	111.04	6.63	0.0096	0.0363	5	98.82
Ψ(Density), *p*(Substrate)	111.62	7.21	0.0072	0.0272	4	102.19
Ψ(Litter), *p*(Substrate)	111.68	7.27	0.0070	0.0264	4	102.25
Ψ(Canopy + Density), *p*(Air)	111.70	7.29	0.0069	0.0261	5	99.48
Ψ(Density), *p*(Cloud)	112.00	7.59	0.0060	0.0225	4	102.57
Ψ(Litter), *p*(Cloud)	112.03	7.62	0.0059	0.0221	4	102.60
Ψ(Area), *p*(Cloud)	112.04	7.63	0.0059	0.0220	4	102.61
Ψ(Density + Litter), *p*(Substrate)	114.41	10.00	0.0018	0.0067	5	102.19

Table 4. Descriptive statistics of landscape-level covariates in comparison to occupancy estimates derived from top-ranked local-scale model. ‘Site Locality’ indicates whether data were collected from sites located in the North of South portion of the lower peninsula of Michigan, ‘Combined’ indicates sites that were grouped based on occupancy status. Header ‘Occupancy’ represents whether a snake was encountered (1), or not encountered (0). ‘PSI (SE)’ indicates the mean occupancy probability and standard errors derived from top-ranked local-scale model, Ψ (Canopy). CHA 250, 1000 represents mean canopy height average at a 250 and 1000 m buffer from the centroid of each site, respectively. CC 250 and 1000 represents mean canopy cover at a 250 and 1000 m buffer from the centroid of each site, respectively. HSM 250 and 1000 indicate the mean proportion of suitable habitat at a 250 and 100 m buffer from the centroid of each site, respectively.

Site Locality	**Occupied**	**PSI (SE)**	**CHA 250**	**CHA 1000**	**CC 250**	**CC 1000**	**HSM 250**	**HSM 1000**
North (2)	1	0.42(0.10)	32.33	34.59	57.48	52.31	0.92	0.6
South (6)	1	0.42 (0.09)	24.56	34.13	24.78	36.88	0.8	0.36
North (9)	0	0.43 (0.16)	31.46	34.47	43.77	48.97	0.73	0.47
South (9)	0	0.17 (0.10)	30.45	34.04	36.03	33.17	0.54	0.25
Combined (8)	1	0.42 (0.09)	26.28	34.23	32.05	40.31	0.85	0.45
Combined (18)	0	0.30 (0.13)	30.9	34.23	39.47	40.19	0.65	0.39

Chapter 2.2

Factors affecting detection of a cryptic rattlesnake (eastern massasauga) in southwest Michigan.

Arin J. Thacker[1], Eric T. Hileman[2], Paul Keenlance[1], Jennifer A. Moore[1]

[1] Department of Biology, Grand Valley State University, 1 Campus Drive, Allendale, Michigan, 49401, USA

[2] Department of Wildlife, Fisheries and Aquaculture, Mississippi State University, Mississippi State, Mississippi 39762, USA

Abstract

Few species are conspicuous enough to always be successfully detected in their natural habitat. Thus, surveying for a species of interest can present a suite of challenges in devising an effective survey or trapping protocol. This is particularly true for species that possess characteristics that make them extremely difficult to detect in their environment such as cryptic coloration, reticent behaviors, and use of relatively inaccessible habitat (i.e. burrows or tree root systems). One such species is the eastern massasauga rattlesnake (*Sistrurus catenatus*), a small-bodied pit viper with a distribution centered around the Great Lakes region. We used long-term mark-recapture data (spanning 2013-2019) from a population of massasaugas located in Southwest Michigan to inform detection-specific models. We used single-season, single-species occupancy models with the occupancy parameter set to one, allocating all explanatory power to the detection parameters. We surveyed five sites on 478 occasions and encountered a minimum of one massasauga during 287 of the surveys. We found that the additive effects of total search effort, substrate temperature, the Julian day of year, and total site area best explained differences in eastern massasauga detection probabilities in Southwest Michigan. Our results suggest that detection is highest when 20 person hours are expended during a single survey period when substrate temperature is between 16-18 °C and at sites with larger total area. Additionally, detection is highest during the early and late portions of the active season, corresponding with April-May and late August in Southwest Michigan. We recommend these data only be used in habitats that are similar to those of this study population, as massasauga habitat use and spatial ecology is variable throughout its range.

Introduction

Herpetofauna represent some of the most imperiled, yet least studied, species (Bland and Böhm, 2016; Deikumah et al., 2014; Trimble and van Aarde, 2012). Within the past three decades, there has been ample evidence of significant population declines in both amphibians and reptiles due to factors including habitat destruction, overharvesting, disease, and climate change (Araújo et al., 2006; Fitzgerald et al., 2018; Gibbon et al., 2000; Pechmann et al., 1991). Snakes, in particular, have been significantly impacted by changes in habitat availability, direct persecution, and an emerging fungal pathogen, *Ophidiomyces ophiodiicola* (McKenzie et al., 2019; Reading et al., 2010; Todd et al., 2010). Many species of snakes are habitat specialists and are thus particularly sensitive to declines in suitable habitat availability. In a quantitative review, Böhm et al. (2016) found strong correlations between habitat specialization, small range size, human activity and increased extinction risk in squamate reptiles, highlighting the need for long-term studies to inform conservation efforts and development of recovery plans for federally listed species.

Herpetofauna, particularly squamates, are notoriously difficult to detect in their natural habitats due to factors such as cryptic coloration, reclusive behaviors, nocturnal activity, and sit-and-wait foraging strategies (Guimarães et al., 2014; Steen, 2010). Consequently, many species are currently data deficient for several key baseline data such as population demographic status and geographic extent (Bland et al., 2017; Bland and Böhm, 2016; Tingley et al., 2016). For example, the IUCN Red List (IUCN, 2020) identified 1,160 reptile species currently listed as ‘Data Deficient’. However, increases in survey effort may be ineffective if species-specific detection probabilities are not accounted for. Failure to account for detection probabilities and the factors that affect them can lead to biased inferences regarding a species occupancy status, its relationship with biotic and abiotic variables, and vital estimates of population demographic dynamics (e.g., local extinction, colonization, abundance) (Bailey et al., 2014; MacKenzie et al., 2017). Therefore, investing the resources in estimating detection probabilities will help in optimizing future survey and monitoring efforts, particularly for species that are difficult to detect and that occur over a broad distributional range (Sewell et al., 2012).

One such cryptic species with a broad geographic range is the eastern massasauga (*Sistrurus catenatus*; hereafter massasauga), a relatively small-bodied rattlesnake with a distribution ranging throughout the Great Lakes region of North America (Harding and Mifsud, 2017). Widespread destruction, degradation, and fragmentation of open-canopy wetlands and prairies have led to significant population declines throughout their range (Szymanski et al., 2015). Additional threats to their persistence include road mortality, human persecution, altered habitat structure and function from invasive plant species, and snake fungal disease (Allender et al., 2016; Bailey et al., 2011; Baker et al., 2016; Shepard et al., 2008). These synergistic threats have led to the massasauga's status as a threatened species on the U.S. Endangered Species Act and the Canadian Species at Risk Act (COSEWIC, 2013; Federal Register, 2016). Massasaugas are a reclusive species that are commonly camouflaged in their natural environments due to their cryptic coloration and sit-and-wait ambush foraging strategy. These characteristics, coupled with the fact that they regularly seek refuge in inaccessible locations (i.e., hummocks, root systems, sphagnum mounds, small mammal and crayfish burrows), lead to naturally low detection rates in their environments. An additional hinderance to detecting this species is the fact that passive survey techniques (drift fences, coverboards, etc.) are less effective than visual surveys (Bartman et al., 2016), thus requiring high amounts of survey effort to successfully detect them. In addition, there are a variety of biotic and abiotic variables (air temperature, wind speed, time of day, etc.) that affect the detectability of massasaugas in their natural habitat (Figure 6). Throughout their range, the lower peninsula of Michigan has the most remaining extant populations. However, a status assessment conducted by Szymanski *et al.* (2015) identified 67 populations with an unknown status in Michigan's lower peninsula. Consequently, it is critically important that we gain a deeper understanding of what variables affect detection probability of massasaugas in order to optimize survey protocols. In doing so, we will be able to gain a better understanding of their distribution throughout the state by investing higher survey efforts during times in which detection is highest.

In this study, we used long-term mark-recapture data collected from a population of eastern massasaugas on actively managed land located in southwest Michigan. The objectives of this study are to identify survey-specific environmental covariates that affect the detection probabilities of the eastern massasauga rattlesnake using data spanning from 2013-2019. Results of this study will inform a standardized survey protocol which can be used to further assess the occupancy and vital rates of populations throughout the massasaugas distributional range.

Methods

Study Site

Surveys took place at a privately-owned nature education center located in Barry County, Michigan. This center is approximately 227 ha and is primarily composed of mixed deciduous forests along with tracts of open-canopied wetlands, old fields, prairies, and open water. Efforts to preserve massasauga habitat are accomplished through controlled burns, manual removal of woody species, and herbicide applications to control for invasive plant species and prevent encroachment in open-canopied habitats. The total area of surveyed massasauga habitat is 11.23

ha, which are split into five units delineated by breaks (via dirt road, stream, or trail landcover) in the habitat. Sites were primarily made up of open-canopied wetlands interspersed with upland prairies and open fields. Two sites are separated by a creek while one is adjacent to an inland lake. Soil types are primarily composed of poorly drained loamy soils.

Field Methods

We conducted visual encounter surveys from 28 April to 30 August from 2013 through 2019, primarily to inform the results of several studies on massasauga demographics (Bartman et al., 2016; Bradke, Bailey, et al., 2018; Bradke, Hileman, et al., 2018). Surveys were constrained to conditions deemed appropriate for detecting massasaugas (no precipitation, <15 mph wind, 46-93 °F), with varying numbers of surveyors. We recorded search effort for each survey by noting start and stop times and accounting for time surveyors were not actively searching for snakes. At the beginning and end of each survey, we recorded environmental variables including shaded air temperature, shaded substrate temperature, presence and severity of any precipitation (none, drizzle, light, moderate and heavy rain), and cloud cover (estimated to the nearest 25%). Additional covariates considered included the minimum air temperature from the night before a survey, the difference between the start air and substrate temperature, the time at which the survey began (measured in minutes since 6:00 AM), and the Julian day of year in which the survey occurred. Minimum nightly air temperature data was collected from the Hastings weather station (Enviroweather, accessed April 2020). All surveyors were equipped with a handheld Garmin GPS to record their tracks while surveying. These tracks were later used to approximate the total area of each site. Massasaugas encountered in the field were collected using snake tongs and a bucket, processed in the on-site lab, and returned to the exact spot in which they were encountered.

Data Analysis

Prior to analysis we standardized all covariates using a z-transformation. Covariates that we expected to exhibit a quadratic relationship were squared before data analysis (Table 1). We tested all covariates for correlations using Pearson's correlation coefficient. Any correlation that produced a value greater than 0.65 resulted in excluding one of the variables from final analysis. Surveys were excluded if covariate data were missing or more than one site was surveyed during one survey occasion. We *a priori* developed 20 candidate models that included a null model, an additive global model, and additive models based on variables known to affect massasauga survey success. We added four null univariate models *a posteriori* to assess the explanatory power of each covariate present in the top-ranked model. We ran single-season, single-species occupancy models using software PRESENCE (MacKenzie and Hines 2002), while holding occupancy at 1 for all models thus focusing all explanatory power on factors affecting detection probabilities. We used Akaike's information criterion adjusted for small sample size (AICc) (Akaike, 1998) for model selection. We used the top-ranked model to approximate the conditional standard error and confidence intervals using the delta method.

Results

From 2013 through 2019 we conducted 472 surveys within five sites. At least one massasauga was detected in 287 of the 472 (60%) surveys. Average survey length varied from 1.8 to 12.2 person hours spent in the field, with an average across all years of 4.0 person hours and a total of 1,880.3 person hours spent surveying across all years. Total surveys per season ranged from 13 in 2018 to 145 in 2015 (Table 2). Average search effort for surveys in which a massasauga was encountered was 4.7 total person hours per survey, while average search effort for surveys in which massasaugas were not encountered was 2.9 person hours. The total area of each site ranged from 0.76 to 4.08 ha (Figure 1). Average shaded air and substrate temperatures were 21.4 and 17.6 C° in surveys in which a massasauga was detected and were 22.1 and 17.6 C° in surveys without a massasauga encounter (Table 2).

Detection was best explained by the covariates of shaded substrate temperature (C°) at the beginning of the survey, total site area, total survey search effort (measured in person hours), and the Julian day of year the survey took place on. Average detection probability was 0.60 (0.05 SE) in sites with known massasauga occupancy status. With the top ranked model accounting for approximately 49% of AIC_c weight, and the second ranked model (which included a reduced set of the variables in the top-ranked model) accounting for 10% of AIC_c weight, there was strong support for all four variables in estimating detection probabilities (Table 3). Shaded substrate temperature exhibited a strong quadratic relationship with detection probability (Figure 2) and with a peak at p = 0.60 (0.04 SE) when substrate temperature reached 17 C°.

Search effort expended during a survey exhibited a strong, positive relationship between increasing total person hours and increasing detection probabilities (Figure 3). Our results indicate that detection probability approached one as total search effort approached 24 person hours (Figure 3). Search effort was present in all models with substantial support and accounted for 3% of total AIC_c weight when ran as a univariate model (Table 3). Total site area had a relatively weak, positive association between increased area and increased detection probability (Figure 5). Day of year in which the survey took place also exhibited a quadratic relationship (Figure 4), with peaks at p = 0.90, 0.86 at the relative beginning (DOY = 92) and end (DOY = 242) of the survey period, respectively. Shaded air temperature at the beginning of a survey was present as an explanatory variable in the second-ranked model, which accounted for 10% of total

AIC_c weight (Table 3). While this variable was not present in the top-ranked model, it did present a relatively weak, negative relationship with detection probability, peaking at $p = 0.78$ when starting air temperatures were at 8°C, and decreasing to $p = 0.59$ at 34°C.

Discussion

We used seven years of visual survey data to determine the factors that significantly influence the detection probability of the eastern massasauga in southwest Michigan. Our results indicate that additive effects of shaded substrate temperature, survey Julian day of year, total search effort, and the total area of surveyed habitat have the greatest influence on detection of massasaugas. Shaded air temperature also had a modest level of support in the second-ranked model. The relatively inactive and cryptic behaviors of massasaugas, coupled with their need for conditions that maximize their thermal requirements, support the intuitive nature of these results. Massasaugas employ a sit-and-wait ambush strategy in foraging for their prey items, which leads to long periods of time in which a snake may remain in one location. This decreases the effectiveness in commonly implemented types of trapping or sampling procedures (i.e., passive traps or artificial cover boards), used for other snake species, when surveying for massasaugas. Therefore, active visual surveys with greater amounts of search effort are the preferred method for searching for massasaugas.

Our results demonstrate that the detection probability for massasaugas approaches one after surveying a site for 24 person hours during a single survey period. Even after only 6.5 person hours, the equivalent of two surveyors searching for just over three hours, detection probability reaches 0.71 (SE 0.04). Similar results of the positive relationship between increasing search effort and increased detectability have been reported for massasaugas (Crawford et al., 2020; Harvey, 2005; Shaffer et al., 2019). Factors affecting the detection probabilities have been investigated in a southern Illinois population of eastern massasaugas with similar study design and results. Crawford et al. (2020) found that substrate temperature, minimum three-day air temperature, search effort, historic solar irradiance, the time of day, and the history of prescribed fire use at the site were all significant factors in determining detection probabilities for massasaugas. Similar to our results, they found a positive linear relationship between increasing search effort and higher detectability. This pattern of increased search effort yielding higher detection probabilities has additionally been identified in two other studies of massasaugas (Harvey, 2005; Shaffer et al., 2019). Shaffer et al (2019) determined that detection probabilities approached one as a surveyor spent 90 minutes actively surveying in the field, while Harvey (2005) found a mean detection probability of 0.31 in relation to surveyor experience. However, notable differences exist between these studies and ours, including study site location, constraints on both the numbers of surveyors and area surveyed, and limits on the amount of time surveyors were allowed to actively search.

We found that both substrate temperature and, to a certain degree air temperature, at the beginning of a survey were important factors in estimating detection probabilities of massasaugas. This relationship is to be expected due to the ectothermic nature of eastern massasaugas. Our results indicate that shaded substrate temperature at the beginning of a survey exhibits a strong quadratic relationship with massasauga detectability, and peaks when substrate temperatures reach approximately 17 C° (0.04 SE). We were also able to detect a negative correlation between increased shaded air temperatures at the beginning of a survey and decreasing probability of massasauga detection. Associations between detection probabilities and the thermal environment were also investigated in Crawford et al (2020), in which they also found a quadratic relationship between detection probabilities and both the substrate temperature and mean three-day minimum air temperature. Although our results for substrate temperature are congruent with the results of Crawford et al (2020), we did not find minimum air temperature from the day prior to the survey to be a significant factor for explaining detection probability of massasaugas. A source of variation between the results of these two studies may lie in the fact that the surveys conducted in our study encompassed most of the massasaugas active season and took place in open-canopied wetland and upland old field habitat types, whereas Crawford et al. (2020) exclusively surveyed during the spring egress of an Illinois population inhabiting primarily grasslands.

In previous studies of herpetofauna both the relative time of day and the Julian day of year are significant explanatory covariates in estimating detection probabilities (Crawford et al., 2020; Erb et al., 2015), yet our results predicted only day of year as an explanatory variable in detection. This pattern likely emerged since we largely constrained our surveys to the morning hours, resulting in less variability in relative time of day. Our results indicated a quadratic relationship in which detection probability decreases and the day of year in which the survey takes place increases until it reaches the trough between the days 163-177 (e.g., June 12-25) and then steadily increases until the end of our active survey period, day 242 (e.g., August 30) (Figure 4). Our results show that detectability of eastern massasaugas is greatest during the spring egress, when snakes are more likely dependent on basking behavior due to cooler temperatures. Additionally, early-season (April – early May) leaf-out conditions may lead to increased visibility of eastern massasaugas as they employ sit-and-wait foraging techniques. The increase in detection probability observed at the end of our survey period is likely due to slightly lower air temperatures in comparison to the peak of the massasauga active season. An additional factor in this near end-season detectability increase is in the fact that gravid females are likely to give birth during this time period and display maternal attendance until the neonates postnatal ecdysis, thus increasing surveyors chances of encountering a greater number of massasaugas during a survey.

We also found a relatively weak positive association between increased surveyed area and increased detection probability. This relationship was also investigated in Crawford et al. (2020), but they did not find a statistically significant relationship between surveyed area and detectability of massasaugas. Other studies in which the association between total site area and detection probabilities of reptiles have been investigated are limited. In one such investigation of several European snake species and detection probabilities, Kéry (2002) found no association between site area and detection probability but did find a positive relationship between increased population size and increased detection probability. This pattern may be reflected in the results of our study, in which the areas of our sites varied considerably. Our results of higher detection probability in sites with larger total areas may be a simple effect of higher availability of thermally appropriate habitat, and thus a greater density of massasaugas.

Management Recommendations

Detailed and accurate baseline data are requirements to build long-term species conservation and recovery plans. The collection of these vital data are aided by efficient and effective surveying protocols, which can be particularly difficult to develop for rare or cryptic species with a broad distribution (L. L. Bailey et al., 2007; Sewell et al., 2012). Determining the biotic and abiotic factors that influence a species detection probabilities allows for stronger inferences about what types of survey methods will be effective, under what conditions surveys should be conducted, and how much effort is required to differentiate between a false absence (i.e., failing to detect a species when it is present at a site) and a true absence at a site. In this study, we have used long-term data to make recommendations regarding the survey protocols for the eastern massasauga in habitats akin to those at our study site. Massasaugas encompass a variety of habitat types throughout their range, thus the results of this study are applicable to open-canopied wetlands and upland old field habitats.

Our results support conducting surveys with high amounts of survey effort (minimum of 20 total person hours/survey) during the spring egress when shaded substrate temperatures are between 16-18 C° to maximize the likelihood of encountering a massasauga. Previous survey protocols have been either developed through expert opinion (Casper et al., 2001), or at a location at the fringe of the massasaugas range (Crawford et al., 2020). The only other study that has made survey protocol recommendations on the eastern massasauga in Michigan was conducted over two seasons at seven sites throughout southern Michigan, and surveys were constrained by area (Shaffer et al., 2019). Results from this study are partially congruent with our results, as they found a positive relationship between increased survey effort and detection probabilities and a decrease in detection probability as shaded air temperature increased. We recommend the results of our study be used to guide conservation efforts in surveying for populations with an unknown status at sites with similar habitat characteristics to those of open-canopied wetlands and upland prairies. We caution against using our findings to determine the absence of massasaugas, as our intention is to merely aid in optimizing the likelihood of detection, thus making surveys more efficient and effective.

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Figure Captions

Figure 1. Study location and site map in which surveys for eastern massasauga rattlesnakes were conducted from 2013-2019 in Barry County, Michigan. The black dot indicates the study site location in Southwest Michigan. The inset box represents the five sites in which surveys occurred. The numbers present within each polygon represent the total area of each site (ha).

Figure 2. The effect of shaded substrate temperature (C°) at the beginning of surveys on detection probabilities for eastern massasauga rattlesnakes in a southwest Michigan population. The shaded regions represent the 95% confidence intervals. Data are from surveys conducted 2013-2019 in Barry County, Michigan.

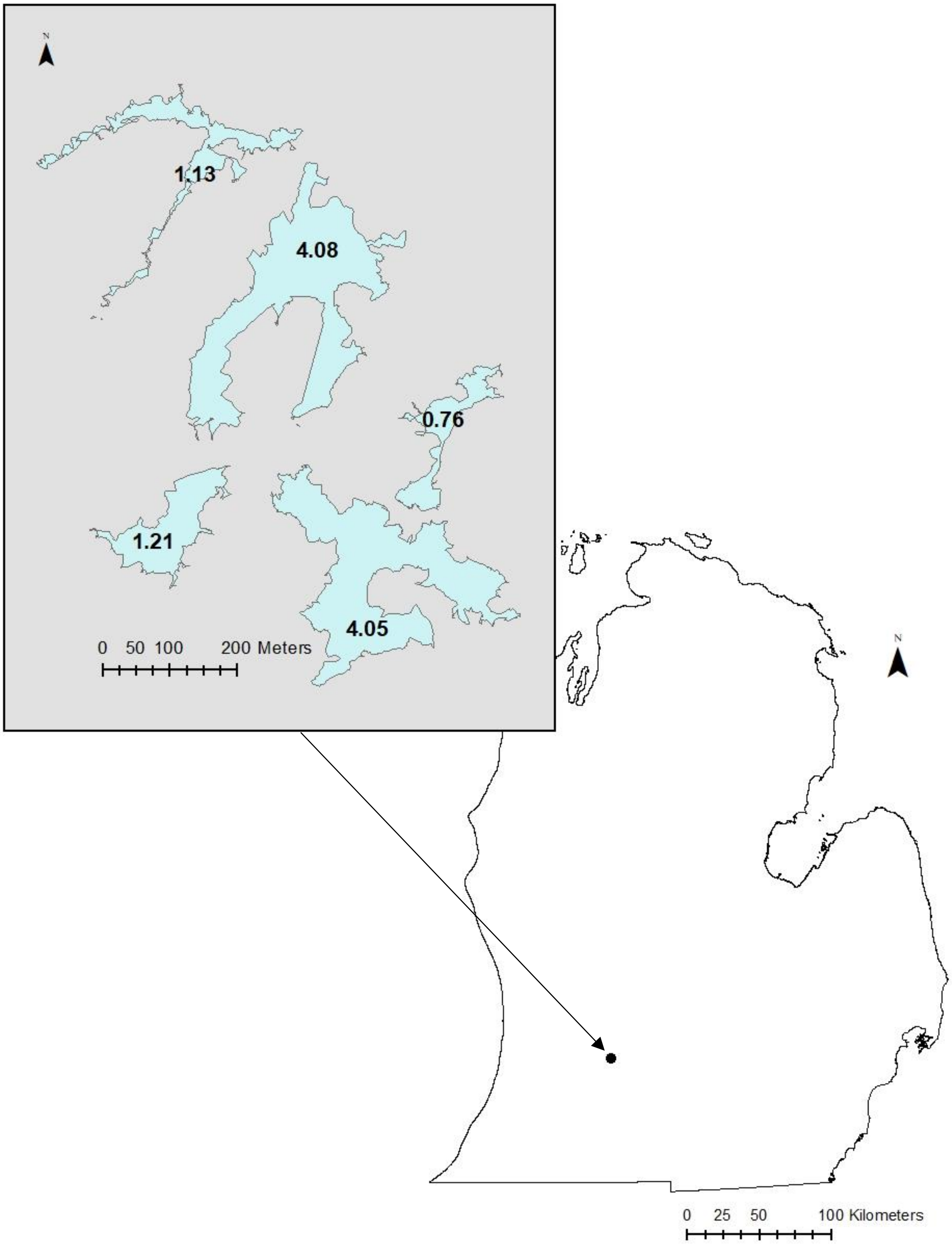
Figure 3. The effects of total survey search effort (reported in total person hours) on the detection probability of the eastern massasauga rattlesnake in a southwest Michigan population. The shaded regions represent the 95% confidence intervals. Data are from surveys conducted 2013-2019 in Barry County, Michigan.

Figure 4. The effect of day of year (reported in Julian days) on the detection probability of the eastern massasauga rattlesnake in a southwest Michigan population. The shaded regions represent the 95% confidence intervals. Data are from surveys conducted 2013-2019 in Barry County, Michigan.

Figure 5. The effects of total surveyed area (ha) on the detection probability of the eastern massasauga rattlesnake in a southwest Michigan population. The shaded regions represent the 95% confidence intervals. Data are from surveys conducted 2013-2019 in Barry County, Michigan.

Figure 6. A visual representation of variation found in eastern massasauga detectability in their natural habitat. Photos were taken in Michigan by A. Thacker.

Figure 1.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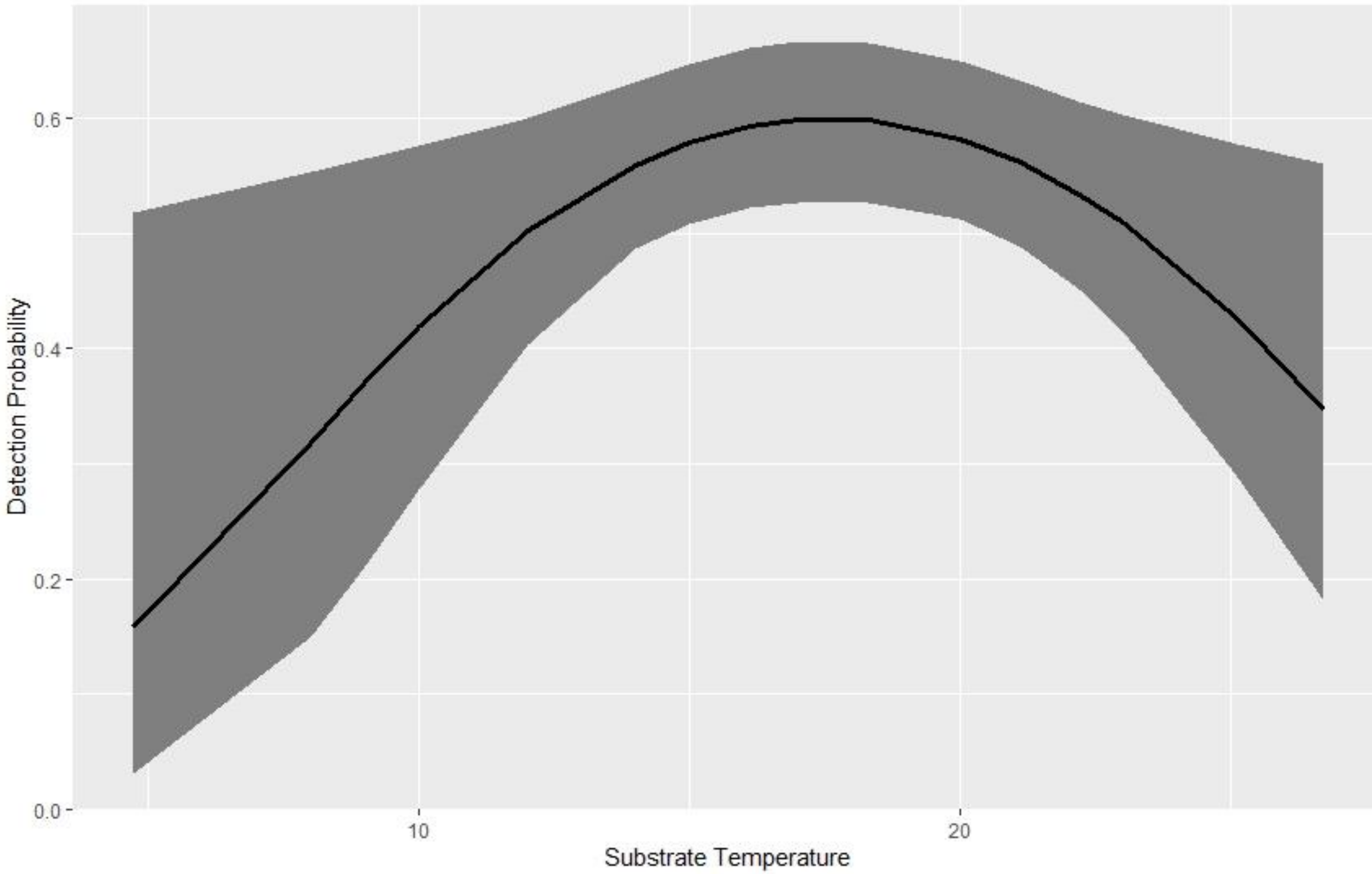
4.08
0.76
1.21
4.05
0 50 100 200 Meters
N
0 25 50 100 Kilometers

Figure 2.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0.4
0.2
0.0
Detection Probability
10
20
Substrate Temperature

Figure 3.

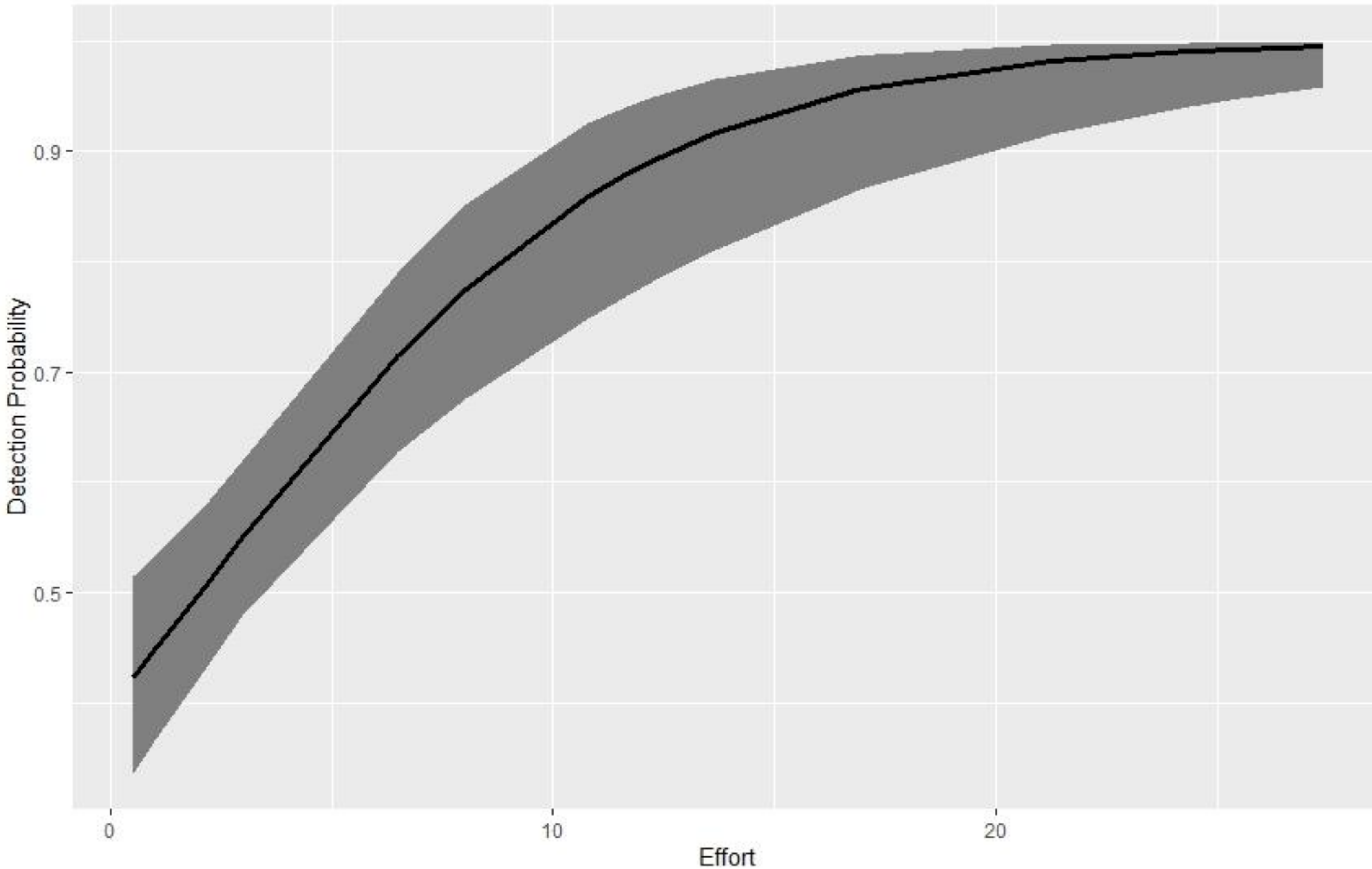
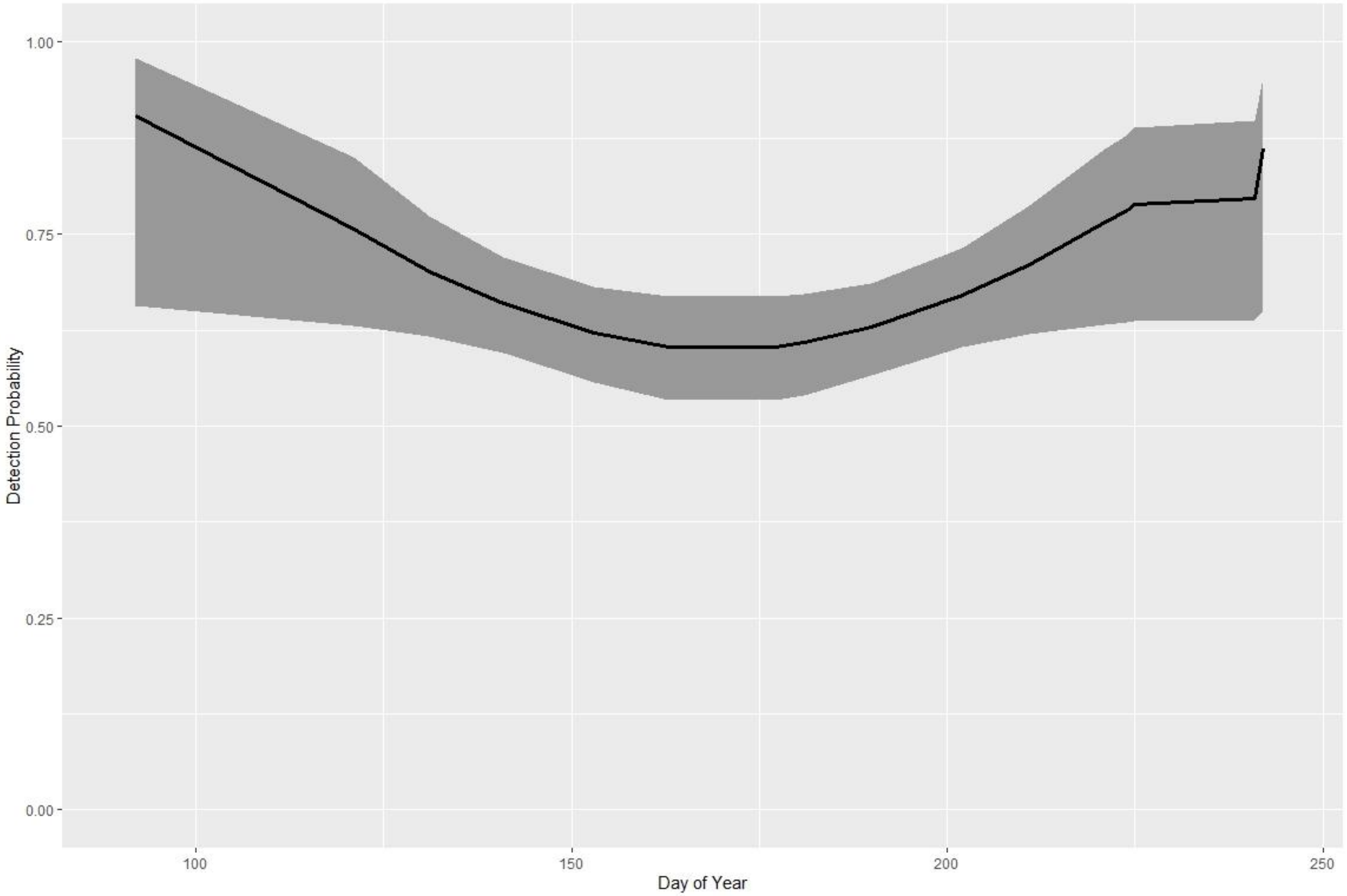


Figure 4.



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0.50
0.25
0.00
Detection Probability
100
150
200
250
Day of Year

Figure 5.

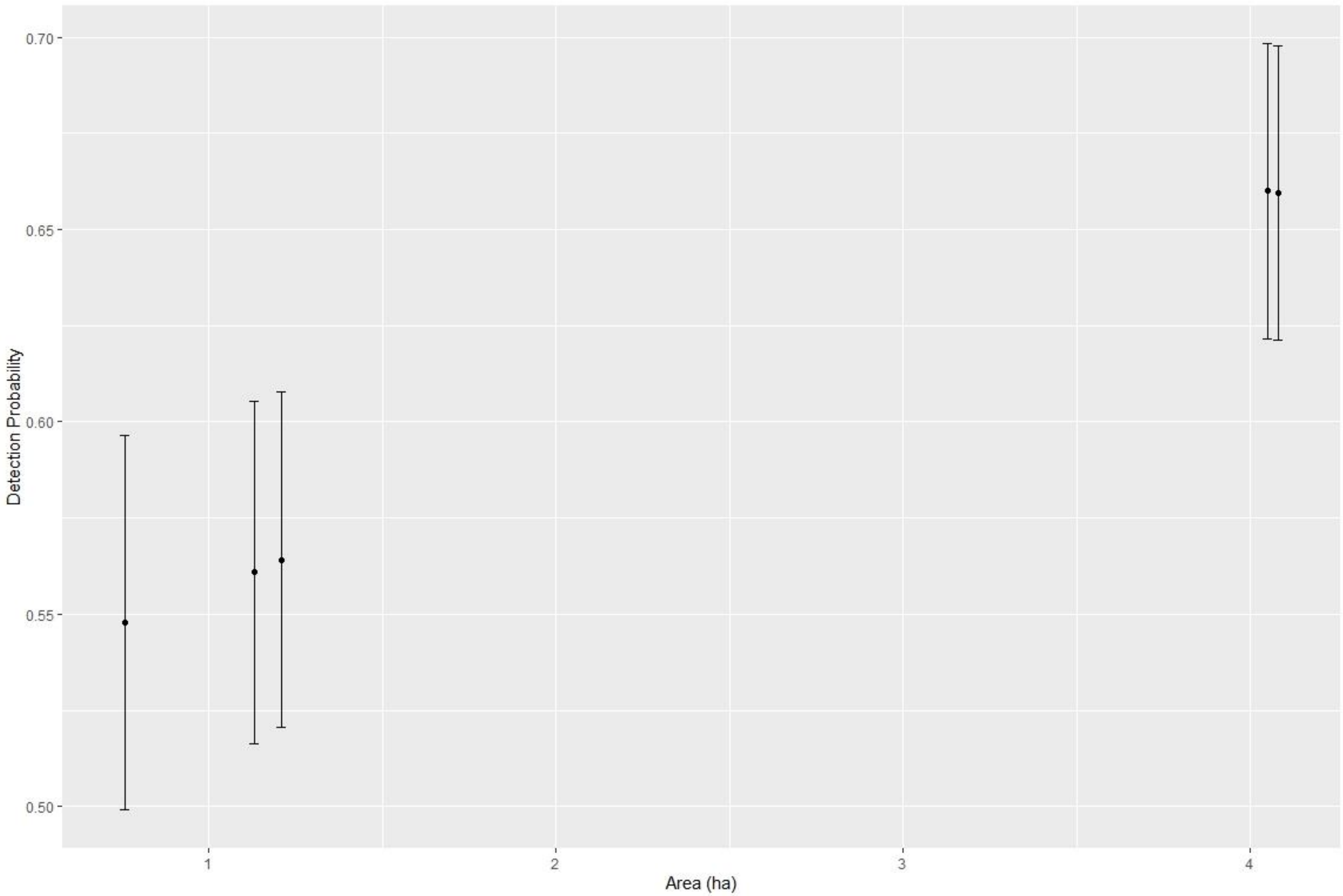

Figure 6.

Table 1. Survey-specific covariates used in the analysis of detection probabilities of the Eastern Massasauga at a managed population in Barry County, Michigan. Data were collected from 2013-2019. All values with a quadratic (squared) relationship are denoted with an asterisk.

Variable	**Description**	**Unit**	**Source**
DOY*	The day of the year the survey is taking place on	Julian date	Individual survey
Area	Total area of a site	ha	Surveyor tracks
MinAir*	The minimum air temperature from the previous day of the survey	° C	Hastings Weather Station Data
Time*	Start time of the survey	minutes since 6:00AM	Individual survey
Air	Shaded air temperature at the start of the survey	° C	Individual survey
Sub*	Shaded substrate temperature at the start of the survey	° C	Individual survey
Effort	The total number of person hours spent actively searching during a survey	hours	Individual survey
Cloud	The estimated cloud coverage at the beginning of a survey	nearest 25%	Individual survey

Table 2. Summarized data of visual encounter surveys for eastern massasauga rattlesnakes in Barry County, Michigan. Quantities listed in parentheses indicate values derived from surveys in which a massasauga was encountered. Quantities without parentheses are derived from surveys in which no massasaugas were encountered. From left to right the headings are as follows; 'Year' represents the year in which the surveys were conducted; 'Surveys' indicate the total numbers of surveys conducted; 'Total Search Effort' represents the total amount of person hours across all sites; 'Mean Search Effort' represent the average number of person hours per survey; 'Start DOY' indicates the earliest day of year in which a survey was conducted; 'End DOY' indicates the last day of year in which a survey was conducted; 'Avg. Air Temp' is the average shaded air temperature (C°) at the beginning of each survey; 'Avg. Sub Temp' is the average shaded substrate temperature (C°) at the beginning of each survey; 'Min. Air Temp' represents the average minimum air temperature (C°) the night prior to the survey; 'Avg. Cloud' is the average estimated cloud coverage at the beginning of each survey; 'Avg. Time' represents the average time in (reported in minutes since 6:00 AM).

Year	Surveys	Total Search Effort	Mean Search Effort	Start DOY	End DOY	Avg. Air Temp	Avg. Sub Temp	Min. Air Temp	Avg. Cloud	Avg. Time
2013	(41) 30	207.7	(3.5) 2.1	133	218	(22.8) 22.6	(18.1) 16.1	(12) 11.2	(50) 50	(289.3) 349.3
2014	(35) 25	227.8	(4.2) 3.2	127	220	(22.3) 22.3	(17.0) 17.0	(11.8) 10.9	(50) 50	(263.9) 300.6
2015	(91) 54	435.1	(3.4) 2.4	118	242	(22.0) 22.6	(18.2) 17.0	(12.2) 11.3	(50) 50	(271.0) 295.2
2016	(51) 43	465.9	(5.9) 3.8	123	222	(21.2) 22.5	(18.4) 20.1	(10.9) 12.0	(25) 50	(218.6) 248.2
2017	(29) 19	146.8	(3.9) 1.8	92	194	(18.6) 20.5	(16.5) 17.0	(9.3) 9.6	(50) 50	(272.6) 334.6
2018	(11) 3	149.3	(12.2) 5.1	141	207	(20.5) 18.2	(14.9) 13.0	(10.3) 10.0	(75) 100	(262.8) 231.0
2019	(29) 11	247.7	(6.6) 5.2	113	214	(20.3) 20.4	(16.8) 17.9	(11.7) 11.6	(0) 50	(243.30) 230.5
Total	(287) 185	1,880.30	(4.7) 2.9	-	-	(21.4) 22.1	(17.6) 17.6	(11.5) 11.2	(50) 50	(260.5) 228.7

Table 3. Model selection results for the eastern massasauga rattlesnake in a southwest Michigan population surveyed between 2013-2019. Models are ranked using Akaike's Information Criterion adjusted for small sample size (AIC_c). Header 'Model' represents the model evaluated, 'AIC_c' indicates the values derived from Akaike's Information Criterion adjusted for small sample size. Delta AIC_c is represented by ΔAICc and indicates the difference between a model and the top-ranked model. Model weight is represented by w_i. (*L)* represents model likelihood. K indicates the numbers of parameters included in each model. Relative model fit is represented by -2*Log(*L*).

Model	**AICc**	**ΔAIC**	**w_i**	**(*L*)**	**K**	**-2*Log(*L*)**
Ψ(.), p(Effort + Area + Sub + DOY)	597.39	0.00	0.4895	1.0000	7	582.70
Ψ(.), p(Effort + Air + Sub + DOY)	600.48	3.09	0.1044	0.2133	7	585.79
Ψ(.), p(Effort + Area + Sub + Air)	600.77	3.38	0.0903	0.1845	7	586.08
Ψ(.), p(Effort + MinAir + Sub + DOY)	601.38	3.99	0.0666	0.1360	7	586.69
Ψ(.), p(Global)	601.46	4.07	0.064	0.1307	11	577.79
Ψ(.), p(Effort + Area + Sub + MinAir)	601.55	4.16	0.0612	0.1249	7	586.86
Ψ(.), p(Effort)	602.68	5.29	0.0348	0.0710	4	594.44
Ψ(.), p(Effort + Air + Sub + Cloud)	602.81	5.42	0.0326	0.0665	7	588.12
Ψ(.), p(Effort + Air + Sub + Time)	604.14	6.75	0.0167	0.0342	7	589.45
Ψ(.), p(Effort + Time + Air + Cloud)	604.43	7.04	0.0145	0.0296	7	589.74
Ψ(.), p(Effort + Air + Sub + MinAir)	604.58	7.19	0.0134	0.0275	7	589.89
Ψ(.), p(Effort + Time + Sub + MinAir)	604.80	7.41	0.0120	0.0246	7	590.11
Ψ(.), p(Time + Area + DOY + Sub)	621.98	24.59	0.0000	0.0000	7	607.29
Ψ(.), p(Time + Area + Air + Sub)	623.59	26.20	0.0000	0.0000	7	608.90
Ψ(.), p(Time + Area + MinAir + Sub)	626.39	29.00	0.0000	0.0000	7	611.70
Ψ(.), p(Area)	627.70	30.31	0.0000	0.0000	4	619.46
Ψ(.), p(Time + DOY + Air + Sub)	631.17	33.78	0.0000	0.0000	7	616.48
Ψ(.), p(DOY + Sub + Air + Cloud)	633.65	36.26	0.0000	0.0000	7	618.96
Ψ(.), p(Time + Sub + Air + Cloud)	634.09	36.70	0.0000	0.0000	7	619.40
Ψ(.), p(Time + DOY + Cloud + Sub)	634.20	36.81	0.0000	0.0000	7	619.51
Ψ(.), p(Time + MinAir + DOY + Sub)	635.01	37.62	0.0000	0.0000	7	620.32
Ψ(.), p(Sub)	637.71	40.32	0.0000	0.0000	4	629.47
Ψ(.), p(.)	638.25	40.86	0.0000	0.0000	3	632.11
Ψ(.), p(DOY)	638.58	41.19	0.0000	0.0000	4	630.34

Chapter 3

Extended Review of Literature

Introduction

A primary component in developing sound wildlife conservation decisions and management plans is a precise and complete knowledge of population distribution throughout their geographic range. Estimates of a species use of the landscape allows investigators and managers to draw conclusions regarding critical habitat associations, population dynamics, and responses to perturbations in the environment (MacKenzie et al., 2017). Knowledge of species' distribution is also critical in examining how changes in global climate patterns, increases in urbanization, and fragmentation of remaining suitable habitat have affected wildlife populations (Boyd et al., 2008; Lenoir and Svenning, 2015; Root et al., 2003). Additionally, site-specific changes in occupancy can be used in comparisons to historic ranges, allowing us to estimate factors that led to changes in a population's occupancy status (Tingley and Beissinger, 2009)

Wildlife monitoring programs have been implemented to serve the purpose of building databases of long-term species presence data, with the overall goal of estimating the occupancy status and trends of sites throughout a broad geographic range (Sewell et al., 2012; Strien et al., 2013; Tanadini and Schmidt, 2011). Many of these monitoring programs rely heavily on simple presence-absence data, which can lead to issues of accounting for false absences (i.e., the inability to detect a species when present) (MacKenzie et al., 2002). False absences within a dataset lead to inaccurate conclusions regarding a population's dynamics, occupancy status at a site and vital habitat associations. False absences are especially prevalent in species with patchy distributions that occur at low densities over a broad geographic range. Species such as these typically have low detection probabilities, which can lead to knowledge gaps in our understanding of their life history and population demographics (MacKenzie et al., 2002, 2003).

Accounting for species-specific detection probability is a way in which we can resolve issues of false absences. Occupancy models estimate the proportion of area a focal species occupies, while accounting for site- and survey-specific detectability (MacKenzie *et al.* 2002). Implementing occupancy models can be an efficient surrogate for abundance data, especially when low detection probabilities are accounted for (Mazerolle et al., 2005). These model types are also an effective strategy in surveying the entirety of a species geographic range as they allow strong inferences to be made about species distribution and are able to guide survey protocols at sites with an unknown occupancy status (Peterman, 2013)

The eastern massasauga (*Sistrurus catenatus*), an imperiled North American rattlesnake with a distribution centered around the Great Lakes region, is a cryptic species with a broad geographic range that lacks basic distribution data throughout the core of its range. The massasauga is endangered or threatened in every state or province in which it occurs, which has led to its status as federally threatened under the U.S. Endangered Species Act (Federal Register, 2016). Massasaugas are a wetland-dependent species, and as such have severely declined in numbers and extent due to habitat degradation, destruction, and fragmentation (Syzmanski *et al.* 2015). Additional threats to massasauga population viability include disease, road kills, and direct human persecution (Allender et al., 2016; Baker et al., 2016).

While the majority of remaining massasauga habitat is present within Michigan's lower peninsula, there is still a general lack of knowledge pertaining to what factors allow for population persistence. In a status assessment conducted by Szymanski et al., (2015) 84 populations with an unknown status were identified, 67 of which occurred in Michigan's lower peninsula. Consequently, there is a need for updated distributional data in order to inform effective species survival strategies and management practices.

Population Trends in Herpetofauna

Reptiles represent one of the most imperiled, yet least studied, taxon in wildlife studies (Bland and Böhm, 2016; Deikumah et al., 2014; Trimble and van Aarde, 2012). Within the past several decades, there has been ample evidence of significant population declines in both amphibians and reptiles due to factors including habitat destruction, overharvesting, disease, and climate change (Araújo et al., 2006; Fitzgerald et al., 2018; Zipkin et al., 2020). Snakes, in particular, have been significantly impacted by changes in habitat availability, direct persecution and an emerging fungal pathogen, *Ophidiomyces ophiodiicola* (McKenzie et al., 2019; Reading et al., 2010; Todd et al., 2010). Many species of snakes are habitat specialists and are thus particularly sensitive to declines in suitable habitat availability. In a quantitative review, Böhm et al. (2016) found strong correlations between habitat specialization, small range size, human activity and increased extinction risk in squamate reptiles. These synergistic factors have led to an urgency in procuring long-term conservation and recovery plans.

Herpetofauna, particularly squamates, are notoriously difficult to detect in their natural habitats due to factors such as cryptic coloration, reclusive behaviors, nocturnal activity, and sit-and-wait foraging techniques (Guimarães et al., 2014; Steen, 2010). Consequently, many species of herpetofauna are currently data deficient regarding several key baseline data such as population demographic status and geographic extent (Bland et al., 2017; Bland and Böhm, 2016; R. Tingley et al., 2016). With 1,160 identified reptile species currently listed as ‘Data Deficient’ under the IUCN Red List (IUCN, 2020), there is an urgent need for an increased effort in gathering these baseline data. However, increases in surveying may prove to be ineffective in gathering accurate data if species-specific detection probabilities are not accounted for. Not accounting for detection probabilities and the factors that affect them can lead to biased inferences regarding a species occupancy status, its relationship with certain biotic or abiotic variable, and even vital estimates in population demographic dynamics (e.g., local extinction, colonization, abundance) (Bailey et al., 2014; MacKenzie et al., 2017). Therefore, investing the resources in estimating detection probabilities will help in optimizing future survey and monitoring efforts, particularly for species that are difficult to detect and which occur over a broad distributional range (Sewell et al., 2012).

Occupancy and Detection Studies of Herpetofauna

A detailed knowledge of the occupancy status of herpetofauna populations is important for a manager's ability to allocate resources to manage suitable habitats. Lacking this basic distributional data leaves those attempting to preserve a species persistence with potential biased or inaccurate estimates in extinction rates, critical habitat associations, and survey techniques (MacKenzie et al., 2003; Nichols et al., 2008). Additionally, not accounting for a species-specific detection probability can exacerbate these biases and lead to a dataset suffering from the effects of zero-inflation, especially in rare or extremely cryptic species, such as the majority of herpetofauna species (Guillera-Arroita et al., 2010; Guimarães et al., 2014; Sewell et al., 2012). Studies and monitoring programs that explicitly account for these variations in detection probabilities can accurately estimate the distributional patterns of species that would be otherwise resource and time-intensive endeavors.

While the importance of accounting for species-specific detection probabilities has been widely recognized due to its ability to avoid biased parameter estimates (Bailey et al., 2014; Guimarães et al., 2014; Kellner and Swihart, 2014), relatively few studies have estimated species-specific detection probabilities in herpetofauna species, particularly in snakes. For eastern massasaugas, detection probabilities have been estimated in only three studies, only one of which took place in Michigan (Crawford et al., 2020; Harvey, 2005; Shaffer et al., 2019). These studies varied in study design and years of survey data, but consistently found that factors relating to search effort and abiotic conditions, such as air and substrate temperatures, are key determinants in detection probabilities in massasaugas. Conversely, Kéry (2002) found that relative population sizes and seasonality were the greatest predictors in the detection probabilities of three European species of snakes (*V. aspis, N. natrix, C. austriaca*). From these results, Kéry was additionally able to estimate the numbers of surveys required to determine a species absent from a site. In North America, occupancy and detection probabilities along with the number of unsuccessful surveys required to declare species absence from a site were investigated in seven aquatic snake species (Durso et al., 2011). Results of this study found widespread variation in both estimates of occupancy and detection probabilities among species, ranging from ψ = 0.12-0.96 and p = 0.03-0.46, respectively. Furthermore, they found that the number of unsuccessful surveys required to declare a species absent from a site had an inverse relationship with a species' detection probability (Durso et al., 2011)

Additional studies estimating occupancy probabilities for snakes have been demonstrated largely at a landscape-scale. In a North American study of relatively widespread snake species, Steen et al., (2012) were able to identity several variables of land cover composition at multiple spatial scales that correlated to a snakes' specific occupancy probability. While these analyses were limited to snakes occurring in the relatively uninhabited portions of the Southwest United States, it illustrated the importance of gathering species-specific data regarding factors that support population persistence. Grass snake (*Natrix helvetica*) occupancy and detection probabilities along with estimates of abundance were investigated at a landscape-level to determine factors influencing each parameter (Ward et al., 2017). Results of this study show an increase in detection probabilities as search effort per transect increased and the use of artificial cover objects increased detection probabilities, $p = 0.33 \pm 0.06$ SEM. Estimates relating to occupancy probability were related to the studies transect length, indicating a potential relationship between the transect length and survey effort.

Occupancy Studies of Historical Sites

Comparisons between a species contemporary distribution and their historic range is important in predicting how species may respond to future changes in their environment (Tingley and Beissinger, 2009). Many challenges exist in making statistically valid comparisons between a species current and historic distribution including changes in species classifications, inaccurate geographic locations, and incompatible sampling techniques. However, use of explorer journal notes, trapper logs, fossil record data, among other sources, can provide insights into the relative abundance and distribution of wildlife species that are threatened in their contemporary range (Laliberte and Ripple, 2003; Miller, 2011; Munoz et al., 2014). Pearl et al., (2009) used historic breeding sites recovered through museum collections, publications, and past scientific reports, to re-assess the occupancy status and identify habitat characteristics that affected the population persistence of two species of frogs of conservation concern.

Studies of Eastern Massasauga Habitat Associations

The eastern massasauga encompasses a wide range of habitats throughout its range (Harding and Mifsud, 2017). Because of this, many studies have shown differences in preferential habitat use that additionally varies based on seasonality, snake sex, and reproductive condition. Many studies have implemented the use of radio-telemetry and capture-mark-recapture methods to investigate patterns in habitat use, spatial ecology and effects of management practices on massasauga populations. In Bruce Peninsula, located in Ontario, Canada, several studies have shown significant differences in preferential habitat use throughout their active season (Harvey and Weatherhead, 2010; Harvey and Weatherhead, 2006; Parent and Weatherhead, 2000). In an initial study conducted by Weatherhead and Prior (1992), massasaugas exhibited a strong preference for forested and open-canopied wetland habitats. while later studies conducted again by Harvey and Weatherhead (2006) resulted in massasaugas exhibiting habitat selection at the microsite scale, with massasaugas actively seeking habitats that provide both retreat sites and basking habitat regardless of sex. Although results of this study did indicate gravid females used habitats with greater amounts of rock cover and retreat sites, indicating their preferences for sites that foster warmer temperatures for sustained periods of time.

Further south, studies conducted in central New York found that patterns in shrub height and density are most likely to influence massasauga use and habitat quality (Johnson et al., 2016; Johnson and Leopold, 1998; Shoemaker and Gibbs, 2010). In one of the remaining populations of northern Illinois, Dreslik, (2005) found that massasaugas exhibited preferential use of grassland habitat followed by woodland habitat. Historic land use was linked to current habitat preferences in massasauga populations located in northeastern Ohio (McCluskey et al., 2018). Results of this study indicated that historically abandoned agricultural lands were associated with current open canopied habitats, thus supporting several extant populations of massasaugas.

Michigan Studies of Eastern Massasaugas

Multiple studies throughout the massasaugas range in Michigan have been conducted to assess population demographics, habitat use, and spatial ecology. Moore and Gillingham, (2006) identified habitat selection at multiple spatial scales in southern Michigan, finding that emergent and scrub-shrub wetlands and lowland forests are both important habitat types in massasauga use at the landscape level. Additionally, they found that various abiotic and biotic factors including soil temperatures, relative humidity, canopy, litter, and several vegetation metrics greatly influenced microsite selection. Bailey et al., (2012) observed similar patterns of preferential habitat use of early to mid-successional wetlands in southern Michigan but found that massasaugas also exhibited preferential use of early to mid-successional deciduous uplands. In northern Michigan, Degregorio et al., (2011) investigated the spatial ecology of an eastern massasauga population using radio-telemetry in a scrub-shrub wetland adjacent to red pine plantations. Results of this study found that home range size was significantly larger in male snakes as opposed to females, with average sizes of 29.8 ha and 14.4 ha, respectively.

Conclusions

Gathering distributional data throughout a geographic range is key for rare or cryptic species of conservation concern. Yet, collecting these data is often hindered by species that occur at low densities at a broad range that possess cryptic colors or behaviors and occur in relatively inaccessible habitats, all traits that many herpetofauna species, like the eastern massasauga, possess. None of the previous research on this species has attempted to estimate the factors affecting the occupancy probabilities of the massasauga, particularly on a state-wide scale. Despite Michigan possessing the greatest number of extant populations throughout the massasaugas range, efforts to reassess the status of many of these populations with unknown statuses have not occurred. Addressing these gaps in massasauga distribution throughout the Michigan range is critically important in allocating management resources and implementing long-term recovery plans.

Extended Methodology

Site Selection and Study Design (Chapter 2.1)

We used historic occurrences of massasauga sighting data from the Michigan Natural Heritage Database (Michigan Natural Features Inventory; MNFI) as a preliminary guide in site selection. These occurrences were comprised of confirmed sightings of massasaugas with varying degrees of geographic certainty, distributed across Michigan's lower peninsula. These historic occurrence data also contained a "last observed" date (i.e., the latest date in which a massasauga was confirmed to be present in the area) which ranged from 1938 to 2014. We used these data in conjunction with a species distribution model to ultimately identify potential survey sites that contained high quality massasauga habitat (E. McCluskey - unpublished data). Potential survey sites were additionally narrowed down to only include sites that were present on publicly accessible properties, thus assuring access and potential for long-term management and protections. We optimized our occupancy-based sampling design using software GENPRES8 (Bailey et al., 2007). Using a previously estimated detection probability for massasaugas of 0.31 (Harvey, 2005), we estimated a study design that maximized both estimates of occupancy and detection of 45-50 survey sites, with four to five repeated surveys per site.

In an idealized study design, sites are surveyed in a completely randomized order. However, the time and logistical constraints of surveying the requisite number of sites across the entire peninsula, a sufficient number of times, within an active season necessitated a semi-random survey order. Thus, we grouped sites that were less than 30 kilometers from each other and treated these groupings of sites as a cluster. Clusters were then assigned a random survey order, and sites within the clusters were also surveyed in a random order.

We performed visual encounter surveys to detect massasaugas at sites over two field seasons that encompassed the active season of massasaugas, April through August of 2018 and 2019. Each site was surveyed on a minimum of two separate occasions and a maximum of seven occasions. Surveys occasions were conducted with a minimum of two surveyors in the field for a minimum of one hour of active searching and were separated by a minimum of 24 hours between surveys at the same site. Surveys were completed under appropriate climatic conditions to detect massasaugas (no precipitation, <15 mph wind, and temperature range of 50-90º F; see Casper *et al.* 2001). For each snake encountered, we recorded the location using a handheld Garmin GPS unit, along with microhabitat conditions (shaded air and soil temperatures, cloud cover, and litter depth).

Field Methods (Chapter 2.1)

Microhabitat data were collected in the field to create site- and survey-specific covariates. To inform detectability, survey-specific variables were recorded during each survey and included: search effort (person hours), survey start and stop times, shaded air and substrate temperatures (C°) at the beginning and end of each survey, day of year, cloud coverage (estimated to the nearest 25%) at the beginning and end of surveys, and any precipitation (rain, hail, snow). Search effort accounted for how many surveyors were present during each survey and was measured by recording survey start and stop times minus the time spent not actively searching for snakes. Each surveyor was also equipped with a handheld Garmin GPS unit to record their survey tracks in the field. Area of actively searched habitat was calculated from projected GPS tracks of every surveyor using the Feature to Polygon function in ArcMap 10.4.1. For each snake encountered, we recorded the location using a handheld Garmin GPS unit, along with microhabitat conditions (air and soil temperatures, cloud cover, etc.). Snakes were then restrained using plastic tubing to record age class, weight, length, sex via cloacal probing, and reproductive condition. Encountered snakes were handled in compliance with GVSU IACUC permit 17-05-A under the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research. We also collected a maximum of 200 µl of blood from each snake for genetic and flow cytometry analyses. Additionally, snakes were palpated for fecal samples for diet analyses. Each snake was returned to their point of capture on the same day it was encountered. All reusable equipment that was used on an individual snake was sanitized with a 10% bleach solution between uses to prevent the spread of Snake Fungal Disease (*Ophidiomyces ophiodiicola*).

Community-level vegetation structure was measured through vegetation surveys with a semi-randomized subplot design. A location that contained vegetation and structural characteristics considered representative of the site was chosen to act as a centroid location of the plot. From this centroid location surveyors determined a random number of paces in each cardinal direction using a stopwatch (constrained to 0-30 paces). At the terminus of each cardinal direction surveyors took subplot measurements, resulting in a total of four subplots per vegetation survey. Subplots (1 x 1 m in size) measurements included counts or estimates of: stem density, diameter at breast height (DBH) of any stem greater than five cm in circumference, measurements of litter depth (cm), and percent canopy cover. Litter depths were measured at the corners of each subplot, giving a total of four measurements per subplot. Canopy cover was recorded at the center of each subplot using a hemispherical lens (Apexel 6, Aipai Optic) and a Sunpak 5400DLX Tripod in its lowest height setting (approximately 30 cm). All stems of woody plants within the angle of view of the hemispherical photograph were counted for stem density and measured for DBH. This process was conducted twice at each site (apart from two sites), giving a total of eight subplots per site. Hemispherical measurements of canopy cover were processed with Gap Light Analyzer 2.0 (Frazer, 1999).

Data Analysis (Chapter 2.1)

Prior to analysis, all covariates were tested for correlations using the Pearson's correlation coefficient. Any correlation value of 0.70 or higher between two variables resulted in excluding one of the variables from final analysis. We implemented the use of maximum likelihood methods and single-season, single-species occupancy models to account for imperfect detection in program PRESENCE 12.10 (MacKenzie et al., 2002). In this model type two main parameters are estimated; the probability that a species is present at a site (Ψ), and the probability that the species of interest will be detected if present (p). These probabilities were constrained between 0 and 1 using the logit link function. We *a priori* developed 25 candidate models that included all combinations of univariate models, three bivariate models, and one null model. Candidate models were built from previous knowledge of massasauga habitat characteristics and variables known to affect detection (Casper et al., 2001; Crawford et al., 2020; Shoemaker, 2007). Bivariate models for the occupancy parameter included an additive effect between site-specific covariates (*i*), as exemplified below (Equation 1),

$$logit\ (\Psi) = \beta_0 + \beta_1\ (covariate_i) + \beta_2\ (covariate_i) \qquad (1)$$

Univariate models for occupancy and detection were simply reduced forms of this bivariate model that included one covariate to estimate the occupancy or detection parameter. Two models were added *a posteriori* to assess the model fit of the top-ranked model. Due to the restricted sample size we limited the number of parameters considered to four per model. All covariates included in modeling were normalized using a z-transformation. Models were ranked using Akaike Information Criterion values adjusted for small sample size (AIC_c; Akaike, 1998). We set the effective sample size to the total number of sites sampled (33) to decrease the chance of overfitting the data (MacKenzie et al., 2017). We approximated the conditional standard error of the top-ranked model from the candidate set using the delta method.

Landscape data were analyzed in an exploratory manner to determine if any patterns of occupancy in relation to surrounding site canopy and habitat suitability metrics were apparent. All canopy and habitat suitability data were tested for correlations using Pearson's correlation coefficient. Associations with a r value greater than 0.70 resulted in excluding one of the variables. We then used a principal component analysis to analyze all remaining variables for trends in canopy metrics associated with sites in which a massasauga was encountered.

Study Sites (Chapter 2.2)

Surveys took place at a privately-owned nature education center located in Barry County, Michigan. This center is approximately 227 ha and is primarily composed of mixed deciduous forests along with tracts of open-canopied wetlands, old fields, prairies, and open water. Efforts to preserve massasauga habitat are accomplished through controlled burns, manual removal of woody species, and herbicide applications to control for invasive plant species and prevent encroachment in open-canopied habitats. The total area of surveyed massasauga habitat is 11.23 hectares, which are split into five units delineated by breaks (via dirt road, stream, or trail landcover) in the habitat. Sites were primarily made up of open-canopied wetlands interspersed with upland prairies and open fields. Two sites are bisected by Cedar Creek while one is adjacent to an inland lake. Soil types are primarily composed of poorly drained loamy soils.

Field Methods (Chapter 2.2)

We conducted visual encounter surveys from 28 April to 30 August from 2013 through 2019, primarily to inform the results of several studies on massasauga demographics (Bartman et al., 2016; Bradke et al., 2018). Surveys were constrained to conditions deemed appropriate for detecting massasaugas (no precipitation, <15 mph wind, 55-85 °F), with varying numbers of surveyors. We recorded search effort for each survey by noting start and stop times and accounting for time surveyors were not actively searching for snakes. At the beginning and end of each survey, we recorded environmental variables including shaded air temperature, shaded substrate temperature, presence and severity of any precipitation (none, drizzle, light, moderate and heavy rain), and cloud cover (estimated to the nearest 25%). For each snake encountered, we recorded the location using a handheld Garmin GPS unit, along with microhabitat conditions (air and soil temperatures, cloud cover, etc.). Snakes were captured using tongs and were kept in a clean pillowcase within a bucket with adequate air flow. We measured the total length of snakes to the nearest 0.1 cm using the squeezebox technique (Quinn and Jones, 1974). Snakes were then restrained using plastic tubing to record age class, weight, tail length, sex via cloacal probing, and reproductive condition. Encountered snakes were handled in compliance with GVSU IACUC permit 17-05-A under the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research. We also collected a maximum of 200 µl of blood from each snake for demographic analyses. Additionally, snakes were palpated for fecal samples for diet analyses. Each snake was returned to their point of capture on the same day it was encountered. All reusable equipment that was used on an individual snake was sanitized with a 10% bleach solution between uses to prevent the spread of Snake Fungal Disease (*Ophidiomyces ophiodiicola*).

Additional covariates considered included the minimum air temperature from the night before a survey, the difference between the start air and substrate temperature, the time at which the survey began (measured in minutes since 6:00 AM), and the Julian day of year in which the survey occurred. Minimum nightly air temperature data was collected from the Hastings weather station (Enviroweather, accessed April 2020). All surveyors were equipped with a handheld Garmin GPS to record their tracks while surveying. These tracks were later used to approximate the total area of each site. Massasaugas encountered in the field were collected using snake tongs and a bucket, processed in the on-site lab, and returned to the exact spot in which it was encountered.

Data Analysis (Chapter 2.2)

We tested all covariates for correlations using Pearson's correlation coefficient. Any correlation that produced a value greater than 0.60 resulted in excluding one of the variables from final analysis. Surveys were excluded if covariate data were missing or more than one site was surveyed during one survey occasion. We *a priori* developed 26 candidate model set that included a null model, all possible univariate models, and additive models based on variables known to affect massasauga survey success. We ran single-season, single-species occupancy models using software PRESENCE (MacKenzie et al., 2002), while holding occupancy at 1 for all models, effectively creating a series of logistic regressions. Akaike's information criterion adjusted for small sample size (Akaike, 1998) was used for model selection. We used the top-ranked model to approximate the conditional standard error and confidence intervals using the delta method.

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