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Effective Population Size, Demography, and Viability of Eastern Massasaugas (*Sistrurus catenatus*) in Southwest Michigan

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Effective Population Size, Demography, and Viability of Eastern Massasaugas
(*Sistrurus catenatus*) in Southwest Michigan

Danielle Rochelle Bradke

A Thesis Submitted to the Graduate Faculty of

GRAND VALLEY STATE UNIVERSITY

In

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Biology

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Dedication

To Eric

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Abstract

As humans increasingly exploit natural areas, wildlife populations face a growing number of threats that often result in population decline and isolation. Small, isolated populations are vulnerable to extirpation due to both genetic and demographic factors. Yet, low detectability of many imperiled species often precludes the collection of population-level data important for assessing population viability and implementing successful conservation. The eastern massasauga (*Sistrurus catenatus*) is a cryptic pitviper that has been extirpated throughout much of its historic range due to agricultural conversion of wetland habitat and other synergistic threats. Consequently, this species is federally listed as threatened in both the United States and Canada, and most remnant populations are believed to be small and isolated. However, most extant populations lack data on population size and long-term survival rates, making effective management, monitoring, and viability assessments difficult. To address these data deficiencies, I estimated the genetic effective population size (N_e) and census population size (N_c) for eastern massasaugas at two sites in southwest Michigan. My results revealed small N_c , with approximately 108 (95% CI = 87–165) and 148 (95% CI = 102–295) adults estimated at the study sites in Cass County and Barry County, respectively. Estimates of N_e were even smaller: approximately 29.5 (95% CI = 22.2–40.5) for Cass County and 44.2 (95% CI = 29.7–73.4) for Barry County. Additionally, N_e/N_c ratios were similar across study sites. Secondly, for the Barry County population, I used mark-recapture data spanning 2008–2016 to estimate annual apparent survival rates of adults. Using these estimates and other parameter values obtained from my site and a nearby population, I modeled population viability over the next 100 years. I also performed a sensitivity analysis to assess the relative influence of model parameters on extinction risk. I estimated annual apparent survival rates of 0.79 (95% CI = 0.68–0.87) for adult males and 0.78

(95% CI = 0.68–0.86) for adult females. Results of my sensitivity analysis suggest that actions promoting high survival of adult females should be a management priority, followed by activities that facilitate high reproductive output and neonate survival.

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Key to Symbols

Φ – Apparent survival

\mathcal{L} – Model likelihood

α_n/SE_n – Standardized regression coefficient

\hat{c} – Variance inflation factor

Abbreviations

AIC_c – Akaike’s information criterion adjusted for small sample size

b – Behavioral effect

bp – Basepairs

c – Recapture probability (Chapter 2.1)

CI – Confidence Interval

DNA – Deoxyribonucleic acid

EV – Standard deviation due to environmental variation

FIS – Inbreeding coefficient

GPS – Global Positioning System

H_e – Expected heterozygosity

H_o – Observed heterozygosity

K – Number of parameters

LHS – Latin Hypercube Sampling

N_a – Effective number of alleles

N_c – Census population size

N_e – Effective population size

p – Capture probability (Chapter 2.1) or recapture probability (Chapter 2.2)

PIT – Passive integrated transponder

PVA – Population viability analysis

r – Growth rate

SD – Standard deviation

SVL – Snout-vent length

w_i – AIC_c weight

Chapter 1 – Introduction

Introduction

Small, isolated wildlife populations are increasingly common as habitat destruction and fragmentation becomes ever more pervasive. Once a population has declined in size, it is more vulnerable to extirpation from stochastic processes, even if the initial threats to habitat are mitigated. Genetically, small populations tend to have higher rates of inbreeding, lower genetic diversity, and increased fixation of deleterious alleles, compared to large populations (Lynch et al. 1995; Lande 1995; Frankham 1995a). Additionally, small, isolated populations have greater sensitivity to natural random variation in vital rates and sex ratios (i.e., demographic stochasticity; Lande 1993; Mills 2012). Moreover, when a population is too small, genetic and demographic influences may generate positive feedback between one another and interact with environmental stressors to produce an “extinction vortex” (Gilpin and Soulé 1986; Fagan and Holmes 2006).

Low detectability of many imperiled species often precludes the collection of adequate population-level data needed to accurately estimate population parameters. However, long-term demographic and genetic estimates specific to the focal population are important for making informed decisions, monitoring the impacts of management actions, and evaluating population extinction risk (White et al. 2002, Williams et al. 2002). For instance, population viability analysis is a common tool for evaluating population extinction risk in vulnerable species, but incorporating inaccurate or non-site-specific data can yield unrealistic and misleading results (Lande et al. 2003; Hileman 2016).

The eastern massasauga (*Sistrurus catenatus*) is a cryptic pitviper that has been extirpated from many localities throughout its range due to agricultural conversion of wetland habitat and other synergistic threats (Szymanski 1998; U.S. Fish and Wildlife Service 2016). Consequently, this species is federally listed as threatened in both the United States and Canada (Environment Canada 2012, U.S. Fish and Wildlife Service 2016), and most remnant populations are believed to be small and isolated. Yet, most extant populations lack data on population size and long-term survival rates, making effective management, monitoring, and viability assessments difficult.

Purpose

The purpose of this project was to address data deficiencies in eastern massasauga populations within southwest Michigan. Through the collection of genetic and demographic data, I aimed to provide population-level estimates important to effective monitoring, management, and extinction risk assessment. Additionally, using my estimates and other parameter values obtained from a nearby population, my goal was to model population viability and assess the relative influence of model parameters on extinction risk.

Scope

This study focuses on two populations of eastern massasaugas in Southwest Michigan, near the center of the species' range (Harding 1997). While the results of this study are most relevant to land managers at these two sites, they can also inform conservation of this species throughout its distribution. For instance, estimates from this study can be compared with other populations range-wide. These comparisons can lend insight into the viability and management of this species as a whole. Furthermore, the methods used here are relevant to assessing the viability of small populations of any imperiled species.

Assumptions

In Chapter 2.1 I assume that the population is approximately closed (i.e., no recruitment, deaths, or migration) during the period of time used to estimate adult abundance (i.e., census population size). I believe this is a reasonable assumption given the length of time used to estimate abundance (approximately 3.5 months). Significant mortality during this period is unlikely, given the high estimates of annual adult survival estimated in Chapter 2.2. To minimize the likelihood of immigration and emigration, I attempted to survey the majority of suitable habitat. Additionally, closed capture models assume no tag loss and that all animals are correctly identified. These models also assume animals are independent of one another and have equal catchability; however, violations of these assumptions can be accounted for by using grouping variables or covariates.

In calculating ratios of effective population size (N_e) and census population size (N_c), I assumed that that N_c has been stable for at least one generation (i.e., approximately five years; Sovic et al. 2016), since estimates of N_e apply to the parent generation of the samples used to estimate them. I believe this is reasonable given both sites are actively managed to maintain eastern massasauga populations and based on no detectable changes in estimated abundance from 2011-2016 at the Cass County site (Hileman 2016) and from 2013-2017 at the Barry County site (D. Bradke, unpublished data).

In Chapter 2.2 I use Cormack-Jolly-Seber models (Cormack 1964, Jolly 1965, Seber 1965) to estimate apparent survival. These models assume equal recapture and survival probabilities for all marked animals and that animals are independent of one another; however, violations of these assumptions can be accounted for using grouping variables or covariates and

by adjusting the variance inflation factor (i.e., \hat{c}). Additional assumptions include no tag loss, instantaneous or short sampling occasions, no temporary emigration, and marked animals are a representative sample of the population.

The population viability analysis I conducted incorporates assumptions regarding population dynamics and current threats. These include the assumption of a stable age distribution, no reproductive senescence, no environmental variation correlation between survival and reproduction, and no density dependence in reproduction. Additionally, I assumed that model parameters obtained from other eastern massasauga populations are representative of my study population and that all extant threats to persistence of the study population are included in my model.

Objectives

For Chapter 2.1 my objectives were to 1) provide baseline estimates of effective population size (N_e) and census population size (N_c) for two populations of eastern massasaugas in southwest Michigan, 2) compare N_e/N_c ratios across sites, and 3) qualitatively assess whether small N_e in these populations has a perceptible effect on genetic diversity or rates of inbreeding. For Chapter 2.2 my objectives were to 1) provide long-term estimates of annual apparent adult survival for a population of massasaugas in Barry County, Michigan, 2) use this and other pertinent demographic data to estimate extinction probability over the next 100 years, and 3) determine the relative influence of model parameters on extinction probability, to guide management and future research.

Significance

This study is important for addressing genetic and demographic data deficiencies in eastern massasauga populations within southwest Michigan. Few studies have estimated population size or long-term survival rates using mark-recapture data for this species across its distribution. Additionally, my study is the first I know of to estimate the genetic effective population size for this species within Michigan. Also, there are no published studies I am aware of that estimate a ratio of N_e/N_c in eastern massasaugas. The estimates provided in this study may serve as a comparison for other populations throughout this species' range. Furthermore, they provide baseline data for monitoring the study populations over time. Results from the population viability assessment sensitivity analysis can be used to prioritize future management, monitoring, and research goals.

Definitions

Apparent survival: $1 - (\text{probability of mortality} + \text{probability of permanent emigration})$.

Census population size (N_c): Number of adults in the population.

Genetic effective population size (N_e): Number of individuals in an 'idealized' population (e.g., one with constant population size, equal family sizes, and a 1:1 sex ratio) experiencing genetic drift or inbreeding at the same rate as the population of interest.

Chapter 2.1

Effective Versus Census Population Size for a Threatened Pitviper (*Sistrurus catenatus*) in Southwest Michigan

Abstract

Destruction and fragmentation of wildlife habitat often results in small, isolated populations that are highly susceptible to extirpation. However, in many cases estimates of population size are lacking, precluding accurate assessments of population viability and sound conservation management recommendations. The eastern massasauga (*Sistrurus catenatus*) is a federally threatened pitviper that has been extirpated throughout much of its historic range due to agricultural conversion of wetland habitat and other synergistic threats. Further, population size is generally unknown among extant massasauga populations, making site-specific effective management difficult. In this study, we focused on the importance of estimating genetic effective population size (N_e) and census population size (N_c) for eastern massasaugas at two sites in southwest Michigan. For each population, we used mark-recapture models to estimate N_c and the linkage disequilibrium method to estimate N_e . Our results revealed small N_c , with approximately 108 (95% CI = 87–165) and 148 (95% CI = 102–295) adults estimated at our study sites in Cass County and Barry County, respectively. Estimates of N_e were even smaller: approximately 29.5 (95% CI = 22.2–40.5) for Cass County and 44.2 (95% CI = 29.7–73.4) for Barry County. Additionally, N_e/N_c ratios were similar across study sites, suggesting some stability in this ratio for eastern massasaugas, at least for populations in close proximity. Although we did not detect high levels of inbreeding or relatedness in either population, we caution that these small populations could become increasingly vulnerable to extirpation due to unpredictable threats such as disease and climate change.

Key words: Eastern massasauga rattlesnake, Effective population size, Census population size, Habitat loss, Genetic diversity, Endangered species

Introduction

As human populations rapidly grow and exploit natural areas, wildlife populations across the globe are increasingly impacted by habitat loss and fragmentation (Lande et al. 1999). One consequence of this is a growing number of small and isolated populations (Allendorf and Luikart 2009), which are often vulnerable to extirpation due to a variety of factors. Specifically, small, isolated populations tend to have higher rates of inbreeding, lower genetic diversity, and increased fixation of deleterious alleles compared to large populations (Lynch et al. 1995; Lande 1995; Frankham 1995a). Additionally, small populations have greater sensitivity to demographic stochasticity (i.e., natural random variation in vital rates and sex ratios; Lande 1993; Mills 2012). Moreover, when a population is too small, genetic and demographic influences may generate positive feedback between one another and interact with environmental stressors to produce an “extinction vortex” (Gilpin and Soulé 1986; Fagan and Holmes 2006).

Reptiles represent some of the most imperiled species on the planet, with habitat destruction, fragmentation, and degradation among their greatest threats (Gibbons et al. 2000; Ananjeva et al. 2015; Tingley et al. 2016). Other forces driving reptile extinctions include over-exploitation, climate change, disease, invasive species, and pollution (Gibbons et al. 2000; Tingley et al. 2016). Narrow geographic distributions, ties to specialized habitat, slow life histories, and temperature-dependent sex determination are among the factors that make many species particularly susceptible to these threats (Tingley et al. 2013; Böhm et al. 2016a, b). Additionally, reptiles are the least understood group of terrestrial vertebrates in terms of viability, with less than half of documented species evaluated by the International Union for Conservation of Nature (Tingley et al. 2016). Of the 45% of reptile species evaluated, an estimated 20% are at risk of extinction while another 19% lack enough data to determine their status (Tingley et al. 2016).

Population-level data, in particular, are generally lacking among reptiles and especially for snakes (Böhm et al. 2013).

Collecting population-level data can be difficult, especially when species are cryptic in nature, which is true of many snakes (Fitch 1987). However, effective conservation depends on having sufficient data on which to base decisions and is crucial in the case of rare and endangered species. For instance, population viability analysis is a common tool for evaluating population extinction risk in vulnerable species, but incorporating inaccurate or non-site-specific data can yield unrealistic and misleading results (Lande et al. 2003; Hileman 2016).

The eastern massasauga (*Sistrurus catenatus*) is a small pitviper with an affinity for shallow wetland habitat and is primarily distributed across the Great Lakes region of North America (Harding 1997). Wetlands in this region have been a major target for agricultural conversion (Dahl and Johnson 1991), making habitat loss and fragmentation the principal cause of population decline in this species (Szymanski 1998; U.S. Fish and Wildlife Service 2016). Additionally, eastern massasauga populations are often subject to synergistic threats of human persecution, road mortality, and an emerging snake fungal disease caused by *Ophidiomyces ophiodiicola* (Shepard et al. 2008; Baker et al. 2016; Lorch et al. 2016; Allender et al. 2016). Because of population declines, the eastern massasauga is currently federally listed as threatened under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service 2016) and Canada's Federal Species at Risk Act (Environment Canada 2012). Due to their secretive nature and cryptic coloration, it is generally difficult to collect data on and monitor massasauga populations. Across the species' known historical distribution, only 46% of populations have been confirmed extant, while the rest are either extirpated (26%) or have unknown status (28%; Szymanski et al.

2015). Even known extant populations generally lack data pertaining to their population size, which is important for assessing long-term viability (Szymanski et al. 2015).

The population-level parameter often of greatest interest to managers is population census size (N_c), which represents the number of reproductively mature adults in a population (Luikart et al. 2010). N_c is important to monitor because it indicates how vulnerable a population is to demographic stochasticity (Lande 1993; Mills 2012). Additionally, biological interactions such as competition, mating, and cooperative social behaviors may be influenced by N_c .

A second and, arguably, equally important parameter to monitor is the genetic effective size of a population (N_e). This parameter represents how many individuals would be in an ‘idealized’ population (e.g., one with constant population size, equal family sizes, and a 1:1 sex ratio) that is experiencing genetic drift or inbreeding at the same rate as the population of interest (Wright 1931). Therefore, any life-history attributes leading to deviations from an idealized population can cause N_e to be lower than N_c (Hare et al. 2011). N_e is of interest to managers because it indicates how vulnerable a population is to genetic drift. Populations with smaller N_e experience a higher rate of drift, which decreases genetic diversity via random changes in allele frequencies that result in fixation of alleles (Wright 1931). With less genetic diversity to act on, the process of selection is constrained and populations are less adaptable to environmental change. Furthermore, populations with small N_e generally experience high levels of inbreeding, which can lead to reduced fitness (Keller and Waller 2002; Reed 2005).

Monitoring both N_e and N_c is ideal because each one is important in understanding population viability and the impacts of management decisions. If there is a consistent relationship between these parameters within a particular species or taxa, managers may be able to collect data on only

one and infer the other, making monitoring efforts more efficient (Luikart et al. 2010). Comparing N_e/N_c ratios across populations will help determine whether any stability in this ratio exists. Assessing variation in this ratio and its relationship to life history, demography, and the environment can also help elucidate the relative influence of these factors on N_e (Cooper et al. 2009; Luikart et al. 2010; Waples et al. 2013) and be used to guide management.

Here, we collected data on two populations of eastern massasauga rattlesnakes located in southwest Michigan. At each site, we used mark-recapture models and microsatellite genotypes to obtain estimates of N_c and N_e , respectively. We also calculated basic measures of genetic diversity (e.g., H_o , H_e) and inbreeding (F_{IS} and relatedness). Our main objectives were to 1) provide baseline estimates of population size, important to monitoring this protected species, 2) compare the two sets of population size estimates for consistency in the N_e/N_c ratio across sites, and 3) qualitatively assess whether small N_e in these populations has a perceptible effect on genetic diversity or rates of inbreeding.

Methods

Study Sites and Field Methods

Our two study sites are located in southwest Michigan and are approximately 86 km apart (Figure 1). The first site, in Cass County, is on 1,052 ha of private property composed of prairie, meadow, wetland, forest, open water, cropland, private roads, and buildings. The second site, in Barry County, is located on approximately 277 ha of privately owned land that includes forest, wetland, open water, old-field, prairie, and buildings and is bisected by a public dirt road. Both sites are actively managed for eastern massasaugas (e.g., controlled burns, invasive species removal) and a large proportion of the land cover surrounding both sites is agricultural or

otherwise developed. Within a 5km radius measured from the center of each study area, approximately 72% of land in Cass County and 48% of land in Barry County is classified as either “developed” or as “planted/cultivated” for livestock or crop production (based on the 2011 National Land Cover Database; Homer et al. 2015).

We used a combination of visual encounter surveys, drift fences with funnel traps, and artificial cover objects to capture snakes within an approximately 64.3-ha survey area at the Cass County site and an approximately 19.9-ha survey area at the Barry County site. Surveys at the Cass County site were conducted between 28 March–8 October 2012 and surveys at the Barry County site were conducted between 28 April–30 August 2015. During surveys, all surveyors recorded their search effort (i.e., time spent looking for snakes). When we found a snake, we captured it using snake tongs, secured it in a cloth bag, and held it within a bucket until processing. We recorded all capture locations using handheld GPS units.

We measured each snake’s total length to the nearest 0.1 cm using a squeezebox (Quinn and Jones 1974) and flexible measuring tape. Once the snake was restrained in a clear plastic tube, we measured tail length (starting at the posterior end of the anal plate) with a ruler and subtracted it from total length to obtain the snout-vent length (SVL; Fitch 1987). While snakes were restrained, we also determined sex via cloacal probing (Schaefer 1934) and palpated females for the presence of embryos. We classified females as adults if they had SVLs ≥ 45.1 cm, which is the SVL of the smallest gravid female observed during studies conducted concurrently with this one, spanning 2010-2016 at the Cass County site and 2013-2016 at the Barry County site. We based adult male size on the smallest male with motile sperm detected via cloacal smear at the Cass County site (43.3 cm SVL; Richard B. King, pers. obs). We marked all individuals with a subdermal passive integrated transponder (PIT) tag (Gibbons and Andrews 2004). When

possible, we drew blood from the caudal vein and stored it in either 95% or 100% ethanol. After processing, we released snakes at their respective capture locations.

Laboratory Methods

We extracted DNA from ~10 µl of blood using Qiagen DNEasy kits, following standard manufacturer protocols. We used primers developed by Anderson et al. (2010) to amplify 17 microsatellite loci from each DNA sample. Each 10 µl PCR reaction consisted of 20–100 ng DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 1 µl of 0.5 mg/ml bovine serum albumin, 0.2 µl deoxynucleotide solution mix (0.2 mM of each), 1 unit Taq DNA Polymerase, 0.6 µl primers (2 µM of each, with fluorescently labeled forward primer), and 5 µl double-distilled H₂O. We amplified markers using an Eppendorf Mastercycler nexus gradient thermal cycler and followed the protocol in Anderson et al. (2010), except for modified annealing temperatures. Loci and respective annealing temperatures were as follows: *Scu200* (62°C), *Scu201* (62°C), *Scu202* (60°C), *Scu203* (60°C), *Scu204* (62°C), *Scu205* (60°C), *Scu206* (62°C), *Scu208* (62°C), *Scu209* (62°C), *Scu210* (56°C), *Scu211* (56°C), *Scu212* (56°C), *Scu213* (56°C), *Scu214* (56°C), *Scu215* (50°C), *Scu216* (56°C), and *Scu217* (50°C). For all PCR runs, we included a negative control for each amplified locus to detect any contamination. Following amplification, fragment analysis was performed with an ABI3730 DNA Analyzer (Applied Biosystems) at the University of Arizona Genetics Core. We scored fragments using PeakScanner v 2.0 (Applied Biosystems).

Genetic Analyses

To assess genotype accuracy, we re-amplified and genotyped 12% of individuals (15 out of 124) from our full data set (which included juveniles and adults captured in years not included for this study). Based on this, we calculated an allele scoring error rate (i.e., incorrect alleles/total

alleles). We used Micro-Checker v 2.2.3 (Van Oosterhout et al. 2004) to check the full data set for null alleles, large allele dropout, and stuttering. We also used FreeNA (Chapuis and Estoup 2007) to estimate null allele frequencies. For all other descriptive statistics and analyses, we included only genotypes from adults captured during the years of this study.

We calculated the number of alleles (N_a), effective number of alleles (effective N_a), observed heterozygosity (H_o), and expected heterozygosity (H_e) for each locus using GenAlEx v 6.503 (Peakall and Smouse 2006, 2012). In addition, we used GenAlEx to estimate mean pairwise relatedness according to Queller and Goodnight (1989). Using GENEPOP v 4.6 (Rousset 2008) we calculated F_{IS} according to Weir and Cockerham (1984) and tested for deviations from Hardy-Weinberg equilibrium based on probability tests. We also performed pairwise exact tests in GENEPOP v 4.6 to test for linkage disequilibria. We performed all analyses separately for each population. For Hardy-Weinberg and linkage disequilibria tests, we used the default settings, *a priori* set $\alpha = 0.05$ to evaluate significance, and applied a sequential Bonferroni correction to account for multiple tests (Holm 1979; Rice 1989).

To estimate contemporary effective population size (N_e) we used the linkage disequilibrium method in NeEstimator v 2.01 (Do et al. 2014). This single-sample method estimates N_e based on the frequency of correlations between alleles located at separate loci (i.e., linkage disequilibrium; Hill 1981). Linkage disequilibrium is inversely related to N_e because in physically unlinked neutral loci it results mainly from genetic drift, which becomes a stronger evolutionary force as population size decreases (Waples 1991). We used a random mating model and excluded alleles with frequencies < 0.02 as recommended by Waples and Do (2010) to best balance the bias associated with keeping rare alleles and the precision lost by removing them. We report jackknife 95% confidence intervals (CIs), which have reduced bias compared to

parametric CIs due to the lack of independence inherent in pairwise comparisons of loci (Waples and Do 2008).

Census Size Estimation

Our estimates of census size (N_c) represent abundance estimates of reproductively mature adults within each study area during the respective survey period. We used Huggins' closed-capture models in Program MARK version 8.1 (White and Burnham 1999), which allowed us to censor individuals during occasions where they were unavailable for capture. This was necessary because 11 gravid females were held for captive parturition at the Cass County site. We truncated the data sets to include no more than four months of data to better approximate the assumptions of closure (i.e., no births, deaths, immigration, or emigration). In order to maximize the data retained for this analysis, we selected time periods that included the greatest number of captures. For the Cass County site, we used captures from 17 April–14 August and, for the Barry County site, we used captures from 28 April–14 August. For each site, we pooled our data into seven capture occasions.

We built a set of candidate models that included different combinations of variables expected to affect detection rates (Table 1). Capture probability (p) and recapture probability (c) were constrained to be constant or allowed to vary with sex, search effort, or time (Table 1). We also modeled additive and interactive effects of sex with effort and sex with time. This resulted in eight models with equal capture and recapture probabilities. Additionally, we duplicated these eight models, this time including an additive effect of behavior, for total set of 16 candidate models. In the behavior models, p and c differed from one another by a constant, presumably due to a handling effect (i.e., 'trap happy' or 'trap shy'). We ranked models using Akaike's

information criterion adjusted for small sample size (AIC_c ; Akaike 1973; Burnham and Anderson 2002). To be conservative in our abundance estimates and to account for model selection uncertainty, we model averaged our candidate models according to AIC_c weight. We calculated lognormal 95% confidence intervals for each estimate.

Results

We genotyped 47 (24 female and 23 male) adult eastern massasaugas from the Cass County site and 53 (35 female and 18 male) adults from the Barry County site. One locus (*Scu200*) was removed from the data set before running any analyses. This locus was described as a tetranucleotide repeat by Anderson et al. (2010); however, scoring conflicted with the published repeat motif (e.g., some heterozygotes had alleles two base pairs apart). For the remaining 16 loci, alleles per locus ranged from 2–12 (mean = 6.5 ± 0.68 SE) for Cass County, and from 2–13 (mean = 7.2 ± 0.77 SE) for Barry County (Table 2). Observed heterozygosity for a given locus varied widely, ranging from 0.15–0.91 (mean 0.70 ± 0.05 SE) in Cass County, and from 0.08–0.94 (mean 0.69 ± 0.06 SE) in Barry County (Table 2). The F_{IS} value calculated across all loci was -0.035 for Cass County and 0.010 for Barry County. Mean pairwise relatedness was the same for each population [$R = -0.02 (\pm 0.01$ SE)].

We calculated an allele scoring error rate of 0.2%. We did not detect evidence of allelic dropout or stuttering using Micro-checker. Based on Micro-checker and FreeNA, one locus (*Scu206*) demonstrated evidence of null alleles, but only in the Barry County population, with an estimated frequency of 8.4% (Table 2). Since the estimated null allele frequency was 0% for this locus at the Cass County site, we retained it for further analyses. No loci significantly deviated from Hardy-Weinberg equilibrium. We detected evidence of linkage disequilibrium at the Cass

County site in 10 out of 120 pairs of loci and at the Barry county site in seven out of 120 pairs of loci (Table 3). Five pairs of loci with linkage disequilibria were consistent across sites (Table 3). Effective population size estimated using the linkage disequilibrium method was 29.5 (jackknife 95% CI = 22.2–40.5; Figure 2) for Cass county and 44.2 (jackknife 95% CI =29.7–73.4; Figure 2) for Barry County.

At the Cass County site, we surveyed 568.8 hours, resulting in 152 captures of 74 unique adults (31 male, 30 gravid females, 13 non-gravid females) from 17 April – 14 August 2012. Five out of the 16 candidate models included in the Cass County N_c analysis received AIC_c support (Table 4). The top-ranked model, which included additive effects of sex and search effort on detection probability, received 42% of AIC_c weight. The second, third, and fifth-ranked models were all variants of the top-ranked model, but each one included an additional parameter for an additive behavioral effect, an interaction between sex and effort, or both. However, these additional parameters were considered uninformative since the model deviance did not decrease by at least two units with the addition of either parameter (Arnold 2010; Table 4). The fourth-ranked model included an additive effect of sex and time on detection, indicating weak support for time as an informative parameter, since this model received 14% of AIC_c weight. Our model-averaged abundance estimate was 108 (95% CI = 87 – 165; Figure 2), including 62 males (95% CI = 42 – 119; Figure 3) and 46 females (95% CI = 44 – 56; Figure 3). Using the point estimates for N_e and N_c , we estimated an N_e/N_c ratio of 0.27 for this site.

At the Barry County site, we surveyed 462.3 hours, resulting in 214 captures of 80 unique adults (29 males, 45 gravid females, 6 non-gravid females) from 28 April – 14 August 2015. Nine out of the 16 candidate models included in the Barry County N_c analysis received AIC_c support (Table 4). However, only the top two models received greater than 5% of the total weight, thus

comprising the bulk of the model-averaged estimate. The top-ranked model included additive effects of sex, search effort, and behavior on detection probability, and received 55% of AIC_c weight. The second-ranked model was identical to the top model, except it included an interaction between sex and effort. However, the addition of this interaction term did not reduce the model deviance by at least two units, indicating that it was an uninformative parameter. The remaining models suggest weak support for time as an informative model parameter. Our model-averaged abundance estimate was 148 adults (95% CI = 102 – 295; Figure 2), including 85 males (95% CI = 46 – 212; Figure 3) and 63 females (95% CI = 54 – 97; Figure 3). Our estimated N_e/N_c ratio for this site was 0.30.

Discussion

Our results demonstrate that eastern massasauga populations are small at both study sites in terms of both genetic effective and census population sizes. We estimated an effective population size of approximately 30 individuals at the Cass County site and approximately 44 individuals at the Barry County site. Both of these N_e estimates are below the minimum thresholds commonly advocated to avoid extinction, where effective populations smaller than 50 are considered vulnerable to inbreeding depression in the short-term (Franklin 1980; Soulé 1980) and populations smaller than 500 risk diminished adaptive potential from genetic drift in the long-term (Franklin 1980). Moreover, recent follow-up work on Franklin's and Soulé's rules for critical N_e has provided evidence that minimum effective sizes should be even larger: at least 100 to avoid inbreeding depression within five generations and greater than 1000 for long-term viability (Frankham et al. 2014).

The effective population size estimator we used in this study has become popular in recent years due to its ability to produce precise estimates for populations with relatively small N_e (i.e., < 200 individuals), using samples taken at a single point in time (Waples and Do 2010). However, this estimator assumes discrete generations, which is rarely true in nature and is not true of eastern massasauga populations. To limit potential bias associated with violating this assumption, we included only adults in our data set, which were randomly sampled from our study site. Robinson and Moyer (2013) found this to be the best sampling approach for approximating true N_e in simulated populations of iteroparous species exhibiting various life histories, with all N_e estimates generated using this sampling strategy coming within 15% of true N_e . However, in simulating populations of species with a broader range of life-histories, Waples et al. (2014) found that even estimates based on randomly sampled adults resulted in N_e estimates that were biased low. Therefore, it is possible that N_e is underestimated in our study, but even a large percent bias in N_e (e.g. ~30%; Waples et al. 2014) would be relatively small in terms of number of individuals. Further, as long as the sampling strategy is consistent, estimates should be comparable across time and populations.

Contemporary effective population size estimates are scarce for other species of snakes but our estimates fall within the range of those published (i.e., 7.5–283.8 individuals; Holycross and Douglas 2007; Ursenbacher et al. 2009; Bushar et al. 2015; Wood et al. 2015; Levine et al. 2016). For eastern massasaugas, this parameter has been estimated in two other studies of which we are aware. In the first, estimates for three populations in Ontario, Canada ranged from approximately 36 to 52 individuals (with confidence intervals spanning approximately 19 to 106 individuals; see Supporting Information Fig. S4 from DiLeo et al. 2013). The other study estimated N_e for a single population in Illinois that varied from 19 to 30 individuals across 2002

to 2012 (with confidence intervals spanning 15 to 40 individuals; Baker 2016). These estimates are surprisingly similar to ours. Additionally, Chiucchi and Gibbs (2010) found evidence suggesting that eastern massasauga populations within 25 km of each other, located in Ohio, Illinois, and Pennsylvania, have been small and isolated for thousands of years. Therefore, it is possible that this species has long been adapted to persisting in small isolated patches of habitat. The relatively high levels of genetic diversity we observed, coupled with low inbreeding coefficients and low relatedness within each of our study populations supports potential adaptations to tolerating small N_e . For instance, massasaugas may be able to recognize and avoid mating with kin, similar to kin discrimination demonstrated in timber rattlesnakes (*Crotalus horridus*; Clark 2004), which could prevent inbreeding. The relatively high proportion of negative of F_{IS} values per locus per population we observed (50%; Table 2) may also indicate outbreeding and support kin recognition.

While this species may have adaptations to help it cope with isolation and small N_e , compounding contemporary threats likely increase the vulnerability of populations to extirpation. Snake fungal disease was recently detected at both sites in this study (Allender et al. 2016), and the population-level implications of this disease are currently unknown (Lorch et al. 2016). Furthermore, environmental stochasticity is expected to escalate with climate change, likely impacting these populations in unpredictable ways. Thus, our small N_e estimates may be of greater concern presently than they would have been under past conditions.

Broad recommendations similar to those for effective population size are not available for census population size because extinction risk from non-genetic factors is expected to be more contingent on life-history and environmental characteristics specific to each population (Lynch et al. 1995). Only two other eastern massasauga populations that we are aware of have published

abundance estimates. The first is located in Cicero Swamp, New York, where gravid female abundance estimates made between 2006–2014 ranged from 9–41 individuals (Johnson et al. 2016). Based on these estimates, the authors extrapolated to infer a maximum population size of 164 adult snakes (Johnson et al. 2016). In the other population, on Beausoleil Island, Ontario, estimated annual adult abundance ranged from 35–77 individuals during 1993–2007 (Jones et al. in press). Our estimates of 108 adults for Cass County and 148 adults for Barry County are within the range of these other populations.

Results from the multimodel inference approach we used to estimate N_c indicated that detection of snakes was influenced by sex and search effort at both study sites. This is not surprising, as we expected increased search effort to result in higher rates of capture. Additionally, gravid females generally bask in open areas to stimulate embryological development, resulting in greater female detection (Bonnet and Naulleau 1996) and, therefore, more precise estimates of population sizes for females than males (Figure 3). For the Barry County population, model selection also supported a behavioral effect on detection, where recapture probabilities were higher than capture probabilities in both sexes, but the magnitude of this effect was greatest for females. This ‘trap happy’ response is likely a consequence of surveyor bias, rather than biological, reflecting the tendency of surveyors to increase search effort in areas where snakes were previously found.

Our N_e/N_c ratios are consistent across study sites (0.27 for Cass County and 0.30 for Barry County), which may indicate some stability in this ratio for eastern massasaugas, at least for populations in close proximity (approximately 86 km in this case). However, numerous factors affect the ratio of N_e/N_c , including life history, environmental characteristics, and demography (Lee et al. 2011; Waples et al. 2013; Wood et al. 2014; Schrey et al. 2016; Waples 2016), some of which demonstrably vary across the eastern massasauga’s range (Jones et al. 2012; Hileman et

al. 2017). Indeed, N_e/N_c ratios have been found to vary across different species (Frankham 1995b; Palstra and Ruzzante 2008; Palstra and Fraser 2012), among populations of the same species (Belmar-Lucero et al. 2012), and even temporally within the same population (Ardren and Kapuscinski 2003). Population census size itself may affect the ratio if variation in reproductive success decreases as a population becomes smaller (i.e., genetic compensation; Palstra and Ruzzante 2008). Implicit in our ratios of N_e/N_c is the assumption that N_c sizes have been stable for at least one generation (i.e., approximately five years; Sovic et al. 2016), because our estimates of N_e apply to the parent generation of the samples used to estimate them (Waples and Do 2008). We believe this is reasonable given both sites are actively managed to maintain eastern massasauga populations and based on no detectable changes in estimated abundance from 2011–2016 at the Cass County site (Hileman 2016) and from 2013–2017 at the Barry County site (D. Bradke, unpublished data). Additional estimates of this ratio for eastern massasaugas will reveal whether it is consistent geographically and temporally, or help elucidate what factors have the greatest influence on N_e/N_c variation within this species.

Conclusions

Effective management of small wildlife populations is of increasing importance in conservation biology as humans continue to modify the landscape. Our results demonstrate that remnant populations of the federally protected eastern massasauga can be very small, especially in terms of genetic effective size. Despite small N_e , we did not detect high levels of inbreeding or relatedness in either study population. Still, we caution that these populations could become increasingly vulnerable to extirpation with the recent introduction of unpredictable threats such as disease and climate change.

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Figure Captions

Figure 1. Locations of the two study sites in southwest Michigan: Cass County in black and Barry County with black cross-hatching.

Figure 2. Eastern massasauga effective population size (N_e) estimates with jackknife 95% CIs and census population size (N_c) estimates with lognormal 95% CIs for the two study sites located in southwest Michigan. Data were collected during 2012 for the Cass County site and 2015 for the Barry County site.

Figure 3. Eastern massasauga census population size (N_c) estimates with lognormal 95% CIs for females and males for the two study sites located in southwest Michigan. Data were collected during 2012 for the Cass County site and 2015 for the Barry County site.

Table 1. Candidate models used to estimate abundance of eastern massasaugas at two sites in southwest Michigan. K is the number of parameters. Capture probability (p) and recapture probability (c) are modeled as equal (=) or as differing by an additive constant (+b). Variables considered for an effect on p and c include: time, sex, and effort. Effort refers to total search effort time per sampling occasion. Additional notation: (*) interaction term; (+) additive term; (.) invariant parameter.

| Model | K |
|-------------------------------|----------|
| p(.)=c(.) | 1 |
| p(sex)=c(sex) | 2 |
| p(effort)=c(effort) | 2 |
| p(time)=c(time) | 7 |
| p(sex+effort)=c(sex+effort) | 3 |
| p(sex+time)=c(sex+time) | 8 |
| p(sex*effort)=c(sex*effort) | 4 |
| p(sex*time)=c(sex*time) | 14 |
| p(.)=c(.)+b | 2 |
| p(sex)=c(sex)+b | 3 |
| p(effort)=c(effort)+b | 3 |
| p(time)=c(time)+b | 8 |
| p(sex+effort)=c(sex+effort)+b | 4 |
| p(sex+time)=c(sex+time)+b | 9 |
| p(sex*effort)=c(sex*effort)+b | 5 |
| p(sex*time)=c(sex*time)+b | 15 |

Table 2. Genetic diversity at 16 microsatellite loci in two southwest Michigan populations of eastern massasaugas. Values reported are for number of alleles (N_a), effective number of alleles (effective N_a), allele size range, observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), and estimated null allele frequency.

| Population (n) | Locus | | | | | | | | | | | | |
|------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | <i>scu202</i> | <i>scu203</i> | <i>scu204</i> | <i>scu213</i> | <i>scu205</i> | <i>scu212</i> | <i>scu214</i> | <i>scu211</i> | <i>scu209</i> | <i>scu201</i> | <i>scu215</i> | <i>scu206</i> | <i>scu210</i> |
| <u>Cass Co. (47)</u> | | | | | | | | | | | | | |
| N_a | 5 | 4 | 4 | 9 | 9 | 8 | 7 | 9 | 3 | 7 | 8 | 2 | 8 |
| Effective N_a | 2.6 | 2.7 | 2.2 | 6.4 | 4.6 | 5.7 | 4.9 | 6.5 | 1.2 | 4.8 | 5.7 | 1.6 | 5.5 |
| Size range (bp) | 171-185 | 231-255 | 147-163 | 189-225 | 189-233 | 341-369 | 167-195 | 293-329 | 181-185 | 213-245 | 111-157 | 188-194 | 176-227 |
| H_o | 0.68 | 0.72 | 0.64 | 0.85 | 0.72 | 0.89 | 0.77 | 0.85 | 0.15 | 0.77 | 0.85 | 0.47 | 0.85 |
| H_e | 0.62 | 0.63 | 0.55 | 0.84 | 0.78 | 0.82 | 0.80 | 0.85 | 0.14 | 0.79 | 0.83 | 0.38 | 0.82 |
| F_{IS} | -0.08 | -0.14 | -0.15 | 0.00 | 0.09 | -0.07 | 0.05 | 0.00 | -0.05 | 0.04 | -0.02 | -0.22 | -0.03 |
| Null alleles | 0.000 | 0.000 | 0.000 | 0.006 | 0.034 | 0.000 | 0.012 | 0.000 | 0.000 | 0.007 | 0.000 | 0.000 | 0.000 |
| <u>Barry Co. (53)</u> | | | | | | | | | | | | | |
| N_a | 6 | 4 | 5 | 12 | 8 | 9 | 7 | 10 | 5 | 7 | 11 | 2 | 5 |
| Effective N_a | 3.5 | 2.1 | 3.6 | 6.6 | 6.2 | 6.6 | 4.1 | 7.1 | 1.6 | 3.3 | 6.9 | 1.1 | 3.1 |
| Size range (bp) | 177-189 | 243-255 | 147-163 | 193-241 | 189-221 | 329-373 | 155-187 | 289-333 | 179-187 | 213-253 | 111-159 | 188-194 | 170-218 |
| H_o | 0.64 | 0.49 | 0.70 | 0.94 | 0.79 | 0.85 | 0.79 | 0.87 | 0.32 | 0.77 | 0.85 | 0.08 | 0.77 |
| H_e | 0.71 | 0.52 | 0.72 | 0.85 | 0.84 | 0.85 | 0.75 | 0.86 | 0.38 | 0.70 | 0.85 | 0.11 | 0.68 |
| F_{IS} | 0.11 | 0.07 | 0.04 | -0.10 | 0.07 | 0.01 | -0.04 | 0.00 | 0.16 | -0.10 | 0.02 | 0.30 | -0.13 |
| Null alleles | 0.016 | 0.000 | 0.026 | 0.000 | 0.023 | 0.012 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.084 | 0.000 |

| Locus | | | |
|------------------------------|---------------|---------------|---------------|
| Population (n) | <i>scu217</i> | <i>scu208</i> | <i>scu216</i> |
| <u>Cass Co. (47)</u> | | | |
| N_a | 4 | 5 | 12 |
| Effective N_a | 2.5 | 1.5 | 8.0 |
| Size range (bp) | 167-181 | 176-192 | 200-268 |
| H_o | 0.64 | 0.38 | 0.91 |
| H_e | 0.60 | 0.33 | 0.87 |
| F_{IS} | -0.06 | -0.14 | -0.04 |
| Null alleles | 0.002 | 0.000 | 0.000 |
| <u>Barry Co. (53)</u> | | | |
| N_a | 6 | 5 | 13 |
| Effective N_a | 3.2 | 2.6 | 9.1 |
| Size range (bp) | 173-185 | 160-192 | 196-332 |
| H_o | 0.70 | 0.66 | 0.79 |
| H_e | 0.69 | 0.62 | 0.89 |
| F_{IS} | 0.00 | -0.05 | 0.12 |
| Null alleles | 0.000 | 0.000 | 0.028 |

Table 3. Pairs of loci exhibiting significant linkage disequilibria (LD) in eastern massasauga populations at two sites in southwest Michigan. Bold values indicate pairs of loci that with significant LD at both sites.

| Population | Locus # 1 | Locus # 2 | P-value |
|-------------------|------------------|------------------|----------------|
| Cass Co. | Scu204 | Scu205 | < 0.00001 |
| | Scu213 | Scu201 | < 0.00001 |
| | Scu202 | Scu210 | < 0.00001 |
| | Scu202 | Scu217 | < 0.00001 |
| | Scu212 | Scu216 | < 0.00001 |
| | Scu214 | Scu216 | < 0.00001 |
| | Scu201 | Scu216 | < 0.00001 |
| | Scu215 | Scu216 | < 0.00001 |
| | Scu210 | Scu217 | < 0.00001 |
| | Scu213 | Scu216 | < 0.00001 |
| | Barry Co. | Scu204 | Scu205 |
| Scu213 | | Scu212 | < 0.00001 |
| Scu213 | | Scu201 | < 0.00001 |
| Scu202 | | Scu217 | < 0.00001 |
| Scu212 | | Scu216 | < 0.00001 |
| Scu202 | | Scu210 | 0.00028 |
| Scu212 | | Scu201 | 0.00038 |

Table 4. Model selection for estimating abundance of adult eastern massasaugas at **(A)** a site in Cass County, Michigan in 2012 and **(B)** a site in Barry County, Michigan in 2015. Only models garnering $\geq 1\%$ of AIC_c weight (w_i) are shown and models are ranked in ascending ΔAIC_c order. K is the number of parameters. Deviance is the difference in $-2\log(\mathcal{L})$ of the current model and $-2\log(\mathcal{L})$ of the saturated model, where \mathcal{L} is the maximized likelihood for each model. Capture probability (p) and recapture probability (c) are modeled as equal (=) or as differing by an additive constant (+b). Explanatory variables considered for p and c include time, sex, and effort. Effort refers to total search effort time per sampling occasion. Additional notation: (*) interaction term; (+) additive term.

A) Cass County, Michigan

| Model | AICc | ΔAIC_c | w_i | \mathcal{L} | K | Deviance |
|-------------------------------|--------|----------------|-------|---------------|---|----------|
| p(sex+effort)=c(sex+effort) | 497.15 | 0.00 | 0.42 | 1.00 | 3 | 501.42 |
| p(sex+effort)=c(sex+effort)+b | 498.58 | 1.44 | 0.21 | 0.49 | 4 | 500.83 |
| p(sex*effort)=c(sex*effort) | 499.18 | 2.03 | 0.15 | 0.36 | 4 | 501.42 |
| p(sex+time)=c(sex+time) | 499.34 | 2.19 | 0.14 | 0.33 | 8 | 493.37 |
| p(sex*effort)=c(sex*effort)+b | 500.61 | 3.46 | 0.07 | 0.18 | 5 | 500.81 |

B) Barry County, Michigan

| Model | AICc | ΔAIC_c | w_i | \mathcal{L} | K | Deviance |
|-------------------------------|--------|----------------|-------|---------------|----|----------|
| p(sex+effort)=c(sex+effort)+b | 628.85 | 0.00 | 0.55 | 1.00 | 4 | 584.78 |
| p(sex*effort)=c(sex*effort)+b | 630.28 | 1.44 | 0.27 | 0.49 | 5 | 584.18 |
| p(sex+time)=c(sex+time) | 633.75 | 4.91 | 0.05 | 0.09 | 8 | 581.49 |
| p(sex)=c(sex)+b | 634.21 | 5.36 | 0.04 | 0.07 | 3 | 592.17 |
| p(sex+time)=c(sex+time)+b | 634.37 | 5.52 | 0.03 | 0.06 | 9 | 580.04 |
| p(sex+effort)=c(sex+effort) | 635.16 | 6.31 | 0.02 | 0.04 | 3 | 593.12 |
| p(sex*time)=c(sex*time) | 635.71 | 6.86 | 0.02 | 0.03 | 14 | 570.94 |
| p(sex*effort)=c(sex*effort) | 636.09 | 7.24 | 0.01 | 0.03 | 4 | 592.02 |
| p(sex*time)=c(sex*time)+b | 636.34 | 7.50 | 0.01 | 0.02 | 15 | 569.46 |

Figure 1.



Figure 2.

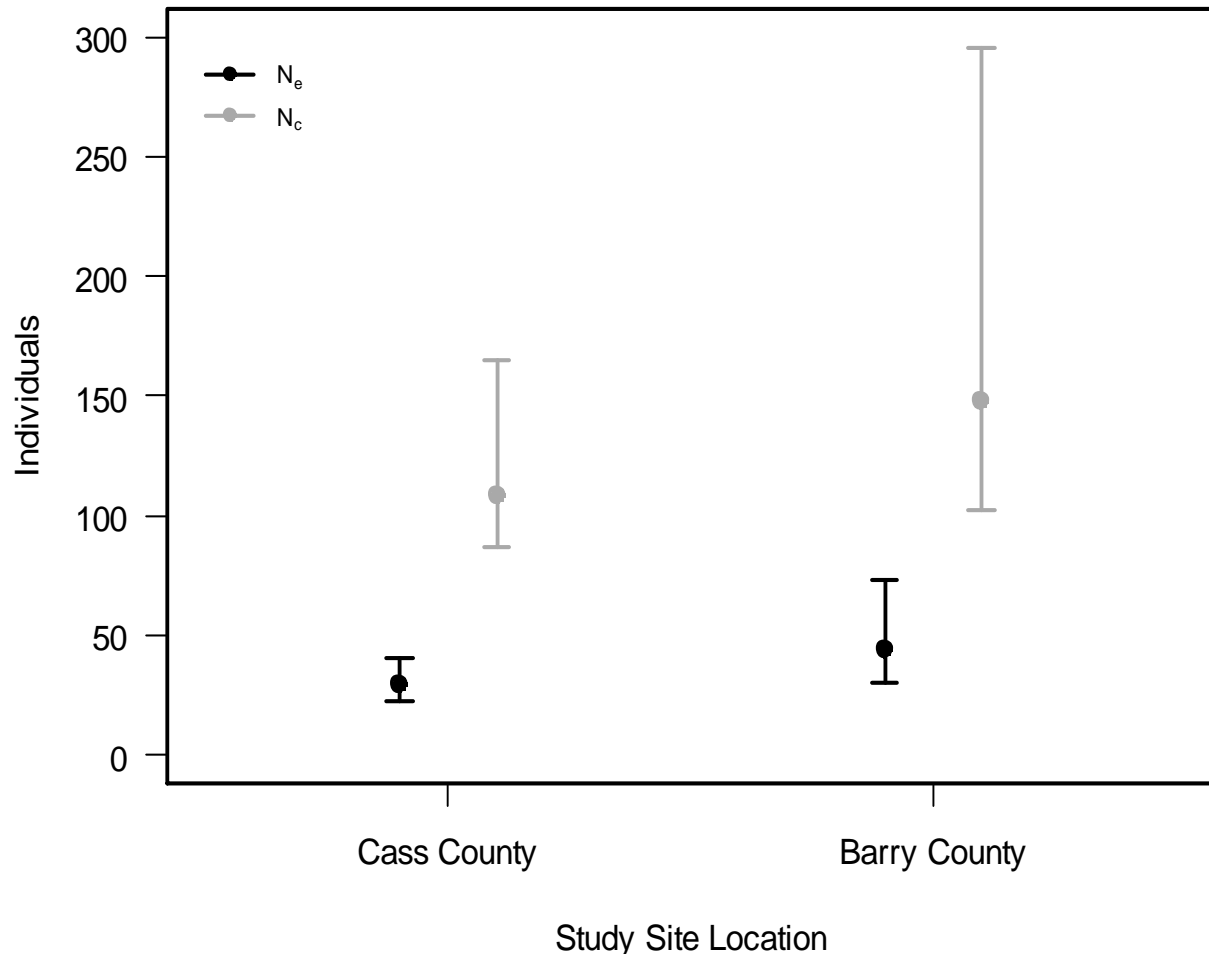
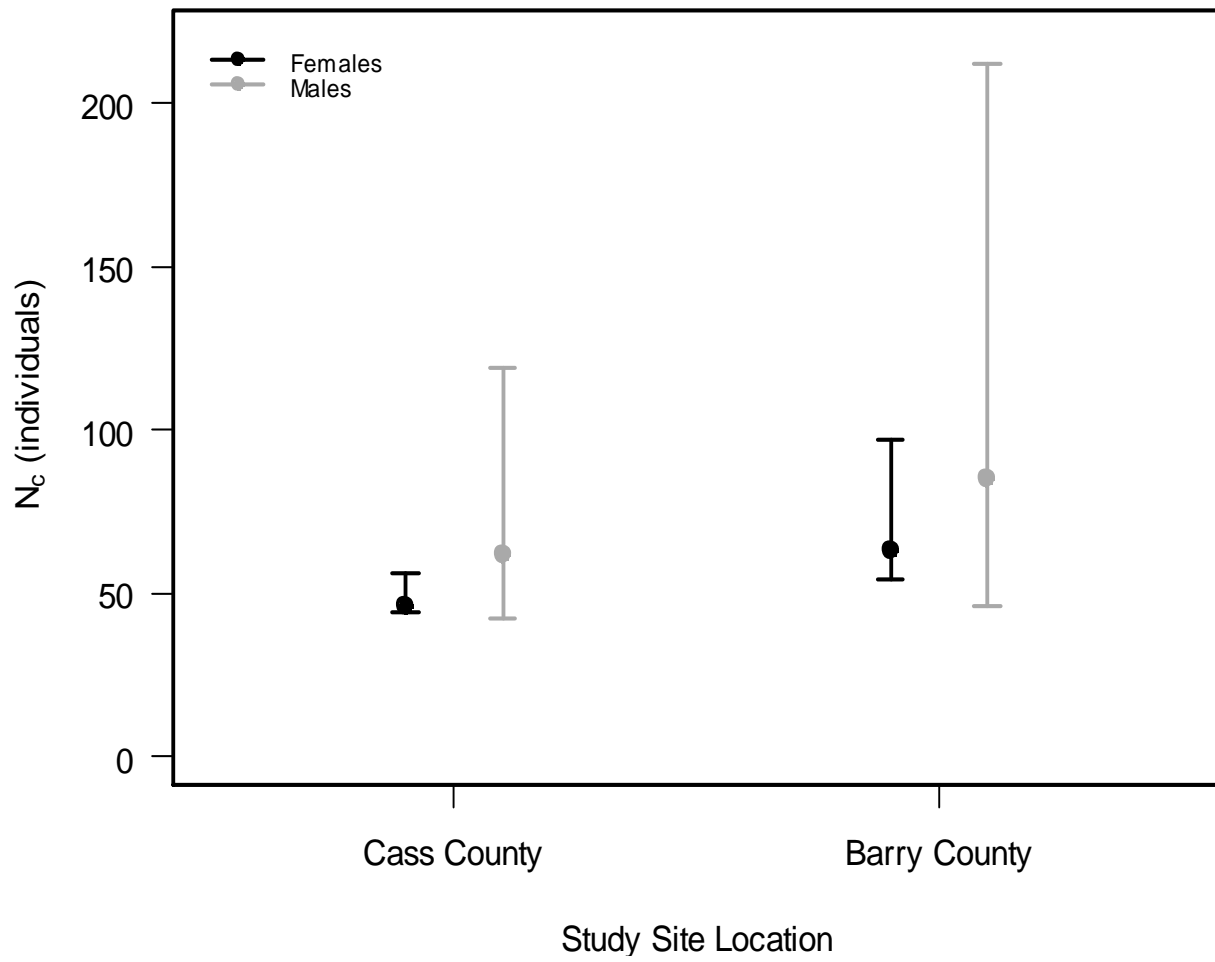


Figure 3.



Chapter 2.2

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RH: Bradke • Eastern Massasauga Viability and Survival

Population Viability and Survival of Threatened Eastern Massasaugas (*Sistrurus catenatus*)

ABSTRACT Long-term demographic data are important for conserving populations of threatened and endangered species. Collecting data specific to the population of interest can improve conservation success by allowing managers to make informed decisions, monitor impacts of management activities, and evaluate extinction risk. For instance, population viability analysis (PVA), a tool commonly used to estimate extinction risk in threatened populations, is most accurate when precise, population-specific data are available. However, even when population parameters are uncertain, PVA may still be useful in predicting the relative impacts of conservation efforts. In this study, we used mark-recapture data spanning 2008–2016 to estimate annual adult survival rates in a population of threatened eastern massasauga rattlesnakes, located in Barry County, Michigan. Using these estimates and other parameter values obtained from our site and a nearby population, we modeled population viability over the next 100 years. We also performed sensitivity analysis using Latin Hypercube Sampling, followed by logistic regression to assess the relative influence of model parameters on extinction risk. We estimated annual apparent survival rates of 0.79 (95% CI = 0.68–0.87) for adult males and 0.78 (95% CI = 0.68–0.86) for adult females, which are higher than expected given the population’s locality. Results of our sensitivity analysis suggest that actions promoting high survival of adult females should be a management priority, followed by activities that facilitate high reproductive output and neonate survival. We also recommend focusing future research and monitoring efforts on these parameters.

KEY WORDS population viability analysis, survival, threatened and endangered species, Latin Hypercube Sampling, sensitivity analysis, demography, synchronous reproduction, snakes

INTRODUCTION

Success of threatened and endangered species recovery is often contingent on having sufficient and accurate population-level data. For instance, long-term demographic estimates specific to the focal population are important for making informed decisions, monitoring the impacts of management actions, and evaluating population extinction risk (White et al. 2002, Williams et al. 2002). However, generating precise estimates of demographic parameters such as vital rates, population size, and population growth is generally difficult due to the low detectability of many threatened and endangered species, which necessitates extensive survey effort to obtain adequate data (Williams et al. 2002).

Reaching threatened or endangered status generally results from deterministic drivers (e.g., habitat loss, over-exploitation; Lacy 1993). However, once a population has declined in size it becomes more susceptible to stochastic processes, which can cause further damage even if the initial threat is mitigated. Probabilistic demographic events, including sex determination, birth, and mortality, have a greater impact in small populations because outcomes of these phenomena exhibit higher variance when there are fewer individuals to act on (Shaffer 1981, Engen et al. 1998). Furthermore, effects of environmental fluctuations add to the variation in vital rates and sex ratios produced by demographic stochasticity (Caughley 1994, Engen et al. 1998). Likewise, random genetic processes of inbreeding and drift are more likely to lead to an overall reduction in population fitness and adaptive potential in small populations (Wright 1931, Lande 1995, Frankham 1996). Because these stochastic processes play such a large role in the fate of small populations, it may be crucial to account for them in assessing extinction risk.

Population viability analysis (PVA) is a common conservation tool used to estimate extinction risk in small populations by incorporating demographic, environmental, and genetic stochasticity into models of population dynamics (Boyce 1992, Beissinger and McCullough 2002). PVA can be an accurate tool for assessing extinction probability, particularly when long-term, population-specific data are available (Brook et al. 2000). However, uncertainty associated with data and future environmental conditions usually precludes reliable projections (Beissinger and Westphal 1998, Coulson et al. 2001, Ralls et al. 2002). In these cases, PVA may still be useful in predicting relative impacts of conservation efforts on population dynamics, if the model adequately represents the system and biology of the species (Beissinger and Westphal 1998). For example, alternative management strategies can be compared based on modeling their relative effects on extinction risk, population growth, or some other outcome (Beissinger and Westphal 1998, Ralls et al. 2002). Modeling the effects of alternative management strategies can be useful in incorporating PVA into an adaptive management framework (Bakker and Doak 2009). Additionally, exploring sensitivity of model output to parameter input (e.g., via incremental changes or using variation representative of parameter uncertainty) can reveal the relative importance of each factor to population persistence (Mills and Lindberg 2002). Managers can use this relative importance to prioritize management goals and direct future research and monitoring efforts to obtain precise estimates of parameters deemed most important (Cross and Beissinger 2001, Mills and Lindberg 2002, Reed et al. 2002).

A classic case demonstrating the usefulness of sensitivity analysis involves reevaluating management priorities for the loggerhead sea turtle (*Caretta caretta*; Crouse et al. 1987, Crowder et al. 1994). By examining the sensitivity of population growth to changes in model parameters, studies determined that the existing conservation approach, which focused solely on egg success,

was unlikely to result in this species' recovery (Crouse et al. 1987, Crowder et al. 1994). Instead, decreasing mortality in older juveniles was determined to be most important, shifting the focus of management efforts to reduce juvenile bycatch from trawl nets (Crouse et al. 1987, Crowder et al. 1994).

Often the factors expected to have the greatest impact on population persistence are those with the largest contribution to individual fitness and, consequently, population growth (Mills 2012). In studies comparing the response of population growth rates to proportional changes in demographic parameters (i.e., elasticity analysis), survival rates were generally the most influential parameters in species with "slow" life history traits (i.e., long-lived, delayed sexual maturity, small broods; Heppell et al. 2000, Sæther and Bakke 2000, Oli and Dobson 2003). In contrast, reproductive parameters were more influential on population growth within species with "fast" life-history traits (i.e., short-lived, fast maturation, large broods). In addition, changes in female vital rates should have a larger influence on population growth than changes in male vital rates, particularly in species with polygynous mating systems (but see Rankin and Kokko 2007, Lee et al. 2011, and Wedekind 2012 for negative effects of skewed sex ratios).

The eastern massasauga (*Sistrurus catenatus*) is a federally listed, threatened rattlesnake (Environment Canada 2012, U.S. Fish and Wildlife Service 2016) with moderate longevity and fecundity. Mean brood size across the species' distribution is approximately nine offspring (including stillbirths; Hileman et al. 2017), with females in most populations approximating biennial reproduction (Szymanski et al. 2015). Although longevity in the wild is unknown, captive individuals can live at least 20 years (Snider and Bowler 1992). Range-wide population declines, which led to federal listing, are primarily due to habitat loss and fragmentation caused by agricultural conversion and other human modification of wetland habitat (Dahl and Johnson

1991, Szymanski 1998, Szymanski et al. 2015). Additional threats, including human persecution (Szymanski 1998) and road mortality (Shepard et al. 2008, Baker et al. 2016), have also contributed to declines in some localities. Consequently, most remaining eastern massasauga populations are small and isolated (Szymanski 1998).

The geographic range of the eastern massasauga extends throughout the Midwest and Great Lakes regions of the United States and into southern Ontario, Canada (Harding 1997). Survival rates, litter size, and other life-history and demographic parameters demonstrably vary across this range in response to environmental gradients, such as precipitation, temperature, and anthropogenic landscape modification (Aldridge et al. 2008, Jones et al. 2012, Pomara et al. 2014, Hileman et al. 2017). Therefore, obtaining data specific to the population of interest, or a similar population, is important for assessing population viability. Here, we used mark-recapture data spanning nine years to estimate annual adult apparent survival rates in an eastern massasauga population located in Barry County, Michigan. Based on these estimates and other parameter values primarily obtained from data collected at our site or from a nearby population, we modeled population viability over the next 100 years. Specifically, our objectives were to 1) provide estimates of survival valuable for monitoring this population of threatened rattlesnakes, 2) use this and other pertinent demographic data to estimate extinction probability over the next 100 years, and 3) determine the relative influence of model parameters on extinction probability, to guide management and future research.

STUDY AREA

We conducted our study in Barry County, Michigan (Figure 1) on a 277-ha privately owned parcel of land bisected by a public dirt road. The study area was primarily composed of prairie

fen, upland prairie, and old-field habitats surrounding a third-order stream and a small lake (ca. 5 ha). The remainder of the parcel includes forest, prairie, and developed sites and has trails open to the public. Open wetlands and adjacent uplands, including the study area, are actively managed to control invasive species and promote early successional communities. From 2003 to 2012, prescribed burns were conducted opportunistically in these areas, but with caution to avoid the eastern massasauga's active season (i.e., typically in March or December). Additional management activities performed in the study area between 2003 and 2016 include mechanical removal of woody and invasive species, targeted herbicide application, and the introduction of biological control agents (i.e., *Galerucella californiensis*).

METHODS

We collected mark-recapture data during 2008, 2009, and 2011–2016. In all years, we primarily located snakes using visual encounter surveys, but we also used drift fences and artificial cover objects as supplemental capture methods during 2015 and 2016. In 2008 and 2009 surveys were conducted in conjunction with a radio-telemetry study (Bailey et al. 2011, 2012) and 19 out of the 21 individuals marked within our study area over these two years were surgically implanted with radio transmitters. Surveys associated with the telemetry study used two surveyors and spanned May through October, and we only included captures made without radio tracking in our data set. In 2011 and 2012, we conducted short-duration, high-effort mark-recapture surveys that included five days spanning 21–25 June 2011 and 12 days spanning 8–12 May, 19–23 June, and 7–8 August in 2012. These shorter surveys relied heavily on volunteer effort with up to 20 surveyors in the field at a time. For the remainder of the study period, surveys spanned a larger portion of the active season (approximately late April/early May through mid-August), and two

to four people regularly conducted surveys with occasional volunteer assistance. For all years, we used handheld GPS units to record the locations of captured snakes.

After capturing a snake, we restrained it within a clear PVC tube and determined its sex via cloacal probing (Schaefer 1934). For females, we used x-ray, ultrasound, or palpation to detect developing embryos, with palpation being the sole method from 2011–2016. To determine age class, we measured the snout-vent length (SVL) of each individual using a flexible measuring tape, either while it was restrained in the snake tube (2008 and 2009), or using a squeezebox (2011–2016; Quinn and Jones 1974). We considered females to be adults if SVL was \geq the smallest observed female with developing embryos (45.1 cm). We classified males as adults if SVL was \geq the smallest observed male with motile sperm detected from cloacal smear at a nearby site (43.3 cm SVL; Richard B. King, pers. obs). Upon first capture, we marked snakes with a subdermal passive integrated transponder (PIT) tag (Gibbons and Andrews 2004). After processing, we released each snake at its capture location.

Survival Analysis

We used Cormack-Jolly-Seber models (Cormack 1964, Jolly 1965, Seber 1965) implemented in Program MARK version 8.1 (White and Burnham 1999) to estimate annual apparent survival of reproductively mature adults from 2008–2016. This parameter is termed “apparent” survival because it incorporates the confounded effects of mortality and permanent emigration. For this analysis, we allowed expansions but not contractions in the study area across years, as reductions to survey area may bias survival estimates low. While increases to survey area between years can induce heterogeneity in recapture probabilities, this expansion may also reduce the effects of temporary emigration on estimates of apparent survival.

We included only captures made between 1 May and 17 August of each survey year and created binary individual capture histories, which specified whether each snake was captured (“1”) or not captured (“0”) during each year. Three individuals that were part of the radio telemetry study in both 2008 and 2009 were treated as unavailable in 2009 by entering a “.” in their capture history, as they were unlikely to be encountered by chance. Additionally, we treated two snakes with surgery-induced mortalities as known removals so these deaths would not influence survival estimates.

We included 16 models in our candidate set (Table 1), ranked models using Akaike’s information criterion adjusted for small sample size (AIC_c), and generated model-averaged estimates of survival based on AIC_c weight (Akaike 1973, Burnham and Anderson 2002). In each model, we either treated survival probability (Φ) as constant or allowed it to differ between males and females. To account for potential effects of different survey methods on detection, we allowed recapture probabilities (p) to vary by survey type (i.e., radio-telemetry study vs. short-duration, high-effort mark-recapture vs. full-season mark-recapture), to vary by time (i.e., each year with a different recapture probability), or to be constant across all years. We also modeled sex differences in recapture rates and included model variations that tested for additive vs. interactive effects between sex and time or sex and survey type. To test for overdispersion, we used a goodness-of-fit bootstrap procedure with 1,000 iterations on our global model:

$\Phi(\text{sex})p(\text{sex}*\text{time})$.

As a post-hoc analysis, we assessed whether transience had a significant effect on apparent survival. Transient snakes are individuals that are not part of the resident population, but pass through the study area (Pradel et al. 1997). Therefore, if there is a significant effect of transience, our survival estimates may be biased low. To assess this we used a likelihood ratio test,

comparing our top ranked model to one that was identical except it included a transience effect. We considered a p-value < 0.05 to be significant.

Population Viability Analysis

We used Vortex v. 10.2.6.0 (Lacy and Pollak 2016) to assess the probability of population extinction within the next 100 years under current conditions at our study site. Vortex incorporates environmental, demographic, and genetic stochasticity, with probabilistic events (e.g., mortality, sex determination) occurring at the individual level and the fate of each individual is tracked through time (Lacy and Pollak 2016). The majority of parameter values used in our model were derived from data collected at our site or from a population located approximately 86 km away in Cass County, Michigan (Hileman 2016).

We calculated adult mortality rates ($1 - \text{survival}$) from the survival analysis in this study. A mean litter size of 7 ($SD = 2.9$) viable offspring was also determined using data from our site (Bailey 2010). We set an initial population size of 284 and a carrying capacity of 569 based on our 2015 adult abundance estimate and its upper 95% confidence interval (CI) limit, respectively (Chapter 2.1), which we extrapolated to total population size by assuming a stable age distribution.

Mortality estimates of sub-adult age classes, mean percent of adult females breeding each year (and associated environmental variation; EV), and age at first reproduction were set at values reported from the Cass County site (Hileman 2016; Table 2). We estimated environmental variation for mortality rates by taking 11% of total variance associated with each respective survival estimate. We used this proportion because Jones et al. (in press), estimated process variance to be approximately 11% of the total variance in adult male eastern massasauga survival. Maximum age of reproduction and maximum lifespan are unknown for wild

populations; however eastern massasaugas can live up to 20 years in captivity (Snider and Bowler 1992), and reproductive senescence is not apparent in captive individuals (Miller 2006). We recognize that animals likely have shorter lifespans in the wild than in captivity. Therefore, to put a reasonable restraint on these parameters, but allow mortality rates to be the principle determinant of longevity, we limited lifespan and reproduction to a maximum age of 15 years. We did not include inbreeding effects in our model, because the inbreeding coefficient calculated at this site in 2015 was low (0.010; Chapter 2.1). Additionally, we did not include an EV correlation between survival and reproduction or incorporate density dependent reproduction, as these effects are unknown for this species. Remaining parameters were set based on life history data from the literature (Table 2).

Finally, we uploaded known allele frequencies for 16 microsatellite loci so that we could track changes in genetic diversity. These frequencies were calculated in GenAlEx v 6.503 (Peakall and Smouse 2006, 2012) for 75 individuals sampled at our site between 2013 and 2015. Loci, methods of DNA extraction, and protocol for PCR amplification and microsatellite genotyping are described in Chapter 2.1. We ran 10,000 iterations of the Vortex simulation and specified that population extinction occurred when only individuals of one sex remained.

Sensitivity Analysis

To evaluate the relative effects of each model parameter's uncertainty on extinction risk, we used Latin Hypercube Sampling (LHS) in Vortex followed by logistic regression in R v 3.3.2 (R Core Team 2016). Latin Hypercube Sampling takes incremental samples from the range of uncertainty designated for each parameter (Lacy et al. 2017). Random combinations of these incremental parameter values are generated to create unique scenarios within the overall space of parameter

uncertainty (Lacy et al. 2017). Consequently, this technique can generate high coverage of the parameter space using fewer samples than a strictly random sampling approach, while including interactions between model parameters that cannot occur in a single-factor sensitivity analysis.

In our sensitivity analysis, we included potential inbreeding effects by varying lethal equivalents from 0–6.29 per individual, with percent due to recessive lethal alleles constant at 50% (Lacy et al. 2017). Here, the upper bound represents the estimated average lethal equivalents affecting fecundity and age 0–1 survival in a meta-analysis of wild species (O’Grady et al. 2006). We allowed mean litter size to vary \pm one neonate from our baseline value (i.e. 6–8 offspring), based on rounding the standard error of the estimate to the nearest whole individual. We varied initial population size from 198–569 individuals based on the upper and lower 95% CI bounds of the 2015 adult abundance estimate (Chapter 2.1), which we extrapolated by assuming a stable age distribution. Our uncertainty range for carrying capacity was set at 284–854 individuals (i.e., baseline initial population value to 150% baseline carrying capacity). We allowed mean annual percent of adult females breeding and mortality rates specific to each sex and age class to vary within \pm 0.05 of their respective baseline rate. We maintained all additional parameters at the values specified in our baseline PVA (Table 2) and ran 3,000 samples with 10 iterations each for 100 years, producing 30,000 observations of 3,000 unique scenarios.

We generated simple logistic regression models using our LHS output, where the binary response variable indicated whether a population went extinct and each explanatory variable was one of the 13 model parameters varied for sensitivity testing. Since all parameters fluctuated simultaneously and their values were combined randomly during LHS, we were able to assess their relative influence while accounting for interactions, but without building interactions into our models (Cross and Beissinger 2001). To assess the relative importance of each explanatory

variable on population extinction risk, we compared their standardized regression coefficients (Cross and Beissinger 2001).

RESULTS

Survival

Our apparent survival analysis included 246 captures of 160 unique adults (67 males and 89 females), spanning 2008–2016. Overdispersion was not evident based on the goodness-of-fit bootstrap procedure ($P = 0.42$), so we did not adjust the variance inflation factor (i.e., \hat{c}) in program MARK. Our model averaged apparent survival estimate was 0.79 (95% CI = 0.68–0.87) for males and 0.78 (95% CI = 0.68–0.86) for females. The top ranked model, receiving 44% of AIC_c weight (Table 1), supported equal survival rates for males and females and included an interaction effect between time and sex on recapture probability (Figure 2). The likelihood ratio test did not support a transience effect on survival ($\chi^2 = 1.425$, $df = 1$, $P = 0.23$).

Population Viability and Sensitivity Analysis

Our baseline PVA yielded a 0% probability of extinction over the next 100 years. The mean stochastic growth rate ($r = 0.0185$, $SD = 0.0769$) was slightly lower than the deterministic growth rate ($r = 0.0199$), and the mean population size at year 100 was 534.55 ($SD = 45.16$). At the beginning of the simulation (year zero), expected heterozygosity (H_e) was 0.69 ($SD = 0.0032$), observed heterozygosity (H_o) was 0.69 ($SD = 0.0062$), and mean number of alleles (N_a) was 7.55 ($SD = 0.03$). We observed reductions in all three measurements by year 100 ($H_e = 0.65$, $SD = 0.018$; $H_o = 0.65$, $SD = 0.019$; $N_a = 6.18$, $SD = 0.24$).

Our sensitivity analysis indicated that variation in adult female mortality has the strongest influence on extinction probability, with mean litter size, percent of adult females breeding each

year, and age 0–1 female mortality also conveying a relatively strong influence (Table 3; Figure 3). Male mortality rates were least important among the parameters examined (Table 3).

DISCUSSION

Few studies on threatened snakes have used population viability analysis to inform conservation efforts, likely due to a usual shortage of demographic data (Dorcas and Wilson 2009). In this study, we conducted a PVA for a population of federally threatened rattlesnakes, with the majority of parameter values derived from data collected at our site or a relatively close population, approximately 86 km away. Our results indicate that under our model of population dynamics and current environmental conditions, this population is likely to persist over the next 100 years. However, as is typical of PVAs, this model only accounts for known threats and processes currently affecting the population and, therefore, may underestimate extinction risk (Boyce 1992, Ludwig 1999, Beissinger and McCullough 2002).

Density dependent processes and threats related to climate change and disease are some of the factors not considered in our PVA that may be significant now or in the future. For instance, flooding and drought are strong predictors of variation in adult survival rates across the eastern massasauga's range (Pomara et al. 2014). Thus, as climate change intensifies, these catastrophes are more likely to influence the trajectory of this population. Additionally, the fungal pathogen *Ophidiomyces ophiodiicola*, which causes snake fungal disease (Allender et al. 2015), was recently confirmed in our study population, although detection of the fungus was low (i.e., one of 43 individuals tested positive in 2014; Allender et al. 2016). Since the influence of environmental conditions on snake fungal disease dynamics is unknown, it is difficult to predict future impacts (Lorch et al. 2016). Furthermore, while extinction probability for our baseline

PVA was 0%, we still observed a slight loss in mean genetic diversity, with the most notable change being a decrease in mean number of alleles from 7.55 (SD = 0.03) to 6.18 (SD = 0.24).

We cannot translate these results to a loss in adaptive potential, but a link between genetic diversity and evolutionary potential has been made in other studies (England et al. 2003, Swindell and Bouzat 2005, Reed 2008). Lowered adaptive potential could reduce the population's ability to respond to threats like disease or climate change. This reduction in response, combined with a limited ability to seek refuge or more suitable conditions due to population isolation, could easily create a very different scenario than modeled here.

Despite the inherent uncertainty associated with PVAs, sensitivity testing can still help guide management, research, and monitoring efforts. Following the approach of Cross and Beissinger (2001), we used a global sensitivity analysis and logistic regression to determine the relative effects of model parameters on extinction risk. In this analysis, all parameter values varied simultaneously, allowing for interactions among them. This method improves model realism compared to the single-factor analyses more commonly used in PVAs, where each parameter is varied individually across its specified range of values while other parameters are held constant (Fieberg and Jenkins 2005, Cariboni et al. 2007). Of the model parameters tested, standardized regression coefficients indicated that variation in adult female mortality had the strongest impact on population persistence with mean litter size, percent of adult females breeding each year, and age 0–1 female mortality also relatively important. These results are consistent with Miller (2006) who found high sensitivity to uncertainty in the same four factors in his PVA of eastern massasaugas in the Bruce Peninsula, Ontario. On the other hand, our results differ slightly from Baker (2016) who found that reproductive rates, including percent breeding females, litter size, and offspring sex ratio were most important in predicting extinction probability in Clinton

County, Illinois. We did not consider model sensitivity to offspring sex ratio in this study, since we have no reason to believe the probability of producing male versus female offspring differs from 1:1.

Results of our sensitivity analysis suggest that actions promoting high survival of adult females should be a management priority, followed by activities that facilitate high reproductive output and neonate survival. However, it is also important to consider the capacity for management actions to achieve each of these goals (Manlik et al. 2016). Adult survival in this population is already higher than average (discussed below), but mean litter size is low compared to other populations (Aldridge et al. 2008, Hileman et al. 2017; but note that most litter sizes in the literature include nonviable offspring). Management that reduces resource limitations could enhance fecundity through increased reproductive frequency, brood size, or offspring viability (Ford and Seigel 1989, Naulleau and Bonnet 1996, Shine and Bonnet 2009). Additionally, prey abundance has been linked to adult survival rates in studies of other snakes (Forsman and Lindell 1997, Sperry and Weatherhead 2008, Olson et al. 2015). Maintaining suitable basking sites is also important for successful embryonic development and may result in fitter offspring or earlier parturition, which provides more time for mother and offspring to forage and increase body reserves prior to ingress (Charland and Gregory 1990, Burger 1991, Lourdais et al. 2004). However, management conducted to maintain basking habitat should be cautious to limit direct mortality by scheduling prescribed burns to avoid the active season (Johnson et al. 2000, Cross et al. 2015) or by using methods such as cutting rather than burning to remove woody vegetation (e.g., Johnson et al. 2016a). Leaving sufficient cover for crypsis from predators is also recommended to minimize indirect mortality (Shoemaker and Gibbs 2010); otherwise, costs associated with female mortality could outweigh the benefits of habitat management. For

example, re-running the baseline PVA with a 0.05 higher rate of adult female mortality increases the probability of population extinction from 0% to approximately 10%, demonstrating how important this parameter is.

The same parameters identified as important management targets can also be the focus of research and monitoring. Adult female survival was estimated in the current study but should continue to be monitored over time. Fortunately, adult females tend to be the easiest class to observe due to increased basking behavior while gravid (Bonnet and Naulleau 1996), so this is also a practical goal. Litter sizes should also be monitored by ultrasound or captive parturition. Other methods of monitoring such as palpation or x-ray can include non-viable embryos, and litter observations in the field are likely to be underestimates and should not be used. We suggest directing future research to obtain estimates of reproductive frequency and neonate survival because we used surrogate estimates from the Cass County site that might not be representative of our population. Multistate Cormack-Jolly-Seber models can be used to estimate female reproductive frequency if reliable records of reproductive status are kept (Hileman 2016). To estimate neonate survival, mark-recapture surveys should extend past parturition, which generally occurs in late July and throughout August (Bailey et al. 2012). Research on predation and diet in this age class could also inform how management might increase neonate survival rates.

Our apparent survival estimates are among the few generated for eastern massasaugas using relatively long-term mark-recapture data, rather than short-term telemetry data (but see Jones et al. in press, Hileman 2016, Johnson et al. 2016*b*). In addition to allowing for studies of longer duration, mark-recapture methods generally produce a larger sample size than telemetry studies, yielding estimates that are more representative of the population. Our survival rates of 0.79 and

0.78 for adult males and females, respectively, are higher than the mean estimate of 0.67 from a meta-analysis of telemetry data by Jones et al. (2012), who found that survival increased across the southwest to northeast range distribution of the species. Based on our population's location at approximately the center of the species' range, we would expect survival to be closer to this mean, or perhaps below it since human-induced mortalities were censored out of the Jones et al. (2012) dataset. However, a telemetry-based estimate of active season survival at our site from 2008–2009 was also exceptionally high (i.e., 0.95; Bailey et al. 2011). These results may reflect high quality habitat at our site (Bailey et al. 2011). Additionally, despite a public dirt road that bisects suitable massasauga habitat, few road mortalities were observed over the course of this study (Bailey et al. 2011; pers. obs), compared to other massasauga populations (e.g., Shepard et al. 2008, Baker et al. 2016). Furthermore, managers at our site are very cautious in limiting prescribed burns conducted within massasauga habitat to pre-spring emergence or post-fall ingress, potentially avoiding burn mortalities observed at other sites (e.g. Durbian 2006, Moore and Gillingham 2006, Cross et al. 2015). Finally, private ownership and regular presence of staff near the study area may deter poaching.

Although our estimates are for apparent survival, we believe they approximate true survival, since we did not detect a transience effect that would indicate a significant number of nonresident individuals (i.e., emigrants) in our sample. Expansions in our study area over time may have also reduced the effects of emigration from the study area, as individuals on the edge are more likely to be encountered across years with study area increases. Furthermore, the markedly high survival estimates within our population corroborate a limited effect of emigration.

Similar to previous eastern massasauga mark-recapture studies in Michigan and Ontario, we did not detect a difference in the survival rates of males and females (Jones et al. in press, Hileman 2016). However, our top ranked model did support an interaction effect between time and sex on capture rates that appears to be driven by a cyclical pattern of detection probability in females, with detection in both sexes exhibiting an overall increase across years (Figure 2). The overall trend of increasing capture rates likely relates to a general increase in surveyor effort and experience across years, rather than biological effects. Cyclical detection within females however, is presumably a consequence of their reproductive biology. Female eastern massasaugas primarily reproduce biennially in this portion of their range (Hileman 2016) and gravid females tend to have higher detection because they spend more time basking (Reinert and Kodrich 1982, Bonnet and Naulleau 1996; pers. obs.). Therefore, the cyclic nature of female detection suggests a high proportion of females sharing the same biennial reproductive cycle (i.e., synchronous reproduction). Synchronous reproductive behavior could be driven by prey availability or antipredator mechanisms such as satiation (Knowlton 1979, Ims 1990). For example, in a population of *Crotalus viridis oregonus*, a much higher proportion of females were gravid following years of high small mammal prey availability (Diller and Wallace 2002), generating a cyclical pattern in proportion of gravid females remarkably similar the one suggested by our data.

MANAGEMENT IMPLICATIONS

Although our population viability analysis indicates that the study population will persist for the next 100 years, we believe uncertainty associated with future conditions is too great to make this prediction. Rather than relying on the baseline PVA, managers should focus on our sensitivity analysis, which indicates that activities that maintain or increase reproductive output and survival

rates of females in the adult and neonate age classes will be the most effective use of resources. Research and monitoring efforts should also target these parameters. Specifically, future research at our site should aim to estimate reproductive frequency and neonate survival, since these are influential parameters that we lack estimates for specific to our population. Further, monitoring adult female survival and litter sizes over time could provide strong signals of changes in population viability. Finally, we recommend using caution when implementing methods used to maintain sufficient basking habitat, such as burning or mowing. These techniques often cause mortality when implemented during the massasauga's active season and our results indicate that a small difference in adult female mortality could have a large impact on population extinction risk.

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Figure Captions

Figure 1. Study site location in Barry County, Michigan (black) within the eastern massasauga's historical geographic range (gray). This figure was reproduced with modifications from Jaeger et al. (2016) with permission granted by Collin P. Jaeger.

Figure 2. Estimated eastern massasauga recapture probabilities and 95% CIs for adult females and adult males at a site in Barry County, Michigan. Estimates are based on the Cormack-Jolly-Seber model $\Phi(\cdot) p(\text{sex} \times \text{time})$, which was ranked highest using AIC_c for model selection.

Figure 3. Extinction probability versus four model parameters in an eastern massasauga population predicted using simple logistic regression to model results generated by multi-factor sensitivity analysis. The four parameters with the greatest influence on extinction probability are shown.

Table 1. Model selection for estimating apparent survival (Φ) in adult eastern massasaugas from 2008–2016 in Barry County, Michigan. Models are ranked in order of AIC_c weight (w_i). K indicates the number of parameters. Additional notation: (p) recapture probability; (.) constant; (*) interaction term; (+) additive term.

| Model | AIC_c | ΔAIC_c | w_i | K | Deviance |
|---------------------------------------|---------------------------|----------------------------------|-------------------------|----------|-----------------|
| $\Phi(.)$ p(sex*time) | 353.92 | 0.00 | 0.44 | 15 | 69.51 |
| $\Phi(.)$ p(time) | 355.73 | 1.81 | 0.18 | 8 | 87.25 |
| $\Phi(\text{sex})$ p(sex*time) | 356.12 | 2.20 | 0.15 | 16 | 69.34 |
| $\Phi(.)$ p(sex+time) | 356.48 | 2.57 | 0.12 | 9 | 85.81 |
| $\Phi(\text{sex})$ p(time) | 357.67 | 3.75 | 0.07 | 9 | 86.99 |
| $\Phi(\text{sex})$ p(sex+time) | 358.68 | 4.76 | 0.04 | 10 | 85.78 |
| $\Phi(.)$ p(survey type) | 364.59 | 10.67 | 0.00 | 4 | 104.68 |
| $\Phi(.)$ p(sex+survey type) | 365.41 | 11.49 | 0.00 | 5 | 103.39 |
| $\Phi(\text{sex})$ p(survey type) | 366.07 | 12.15 | 0.00 | 5 | 104.05 |
| $\Phi(\text{sex})$ p(sex+survey type) | 367.51 | 13.59 | 0.00 | 6 | 103.37 |
| $\Phi(.)$ p(sex*survey type) | 369.45 | 15.53 | 0.00 | 7 | 103.15 |
| $\Phi(\text{sex})$ p(sex*survey type) | 371.58 | 17.66 | 0.00 | 8 | 103.11 |
| $\Phi(.)$ p(.) | 375.31 | 21.39 | 0.00 | 2 | 119.55 |
| $\Phi(.)$ p(sex) | 376.63 | 22.71 | 0.00 | 3 | 118.81 |
| $\Phi(\text{sex})$ p(.) | 376.67 | 22.75 | 0.00 | 3 | 118.84 |
| $\Phi(\text{sex})$ p(sex) | 378.53 | 24.61 | 0.00 | 4 | 118.62 |

Table 2. Parameter values included in Vortex eastern massasauga population viability analysis. SD = standard deviation; EV = SD due to environmental variation.

| Parameter | Value | Source or Justification |
|---|--------------|--|
| Inbreeding depression: | None | Low F_{IS} value reported for this study site (Chapter 2.1) |
| Reproductive system: | Polygynous | Duvall et al. 1992, Stedman et al. 2016 |
| Age of first reproduction (both sexes): | 3 | Hileman 2016 |
| Maximum lifespan: | 15 | Up to 20 years in captivity (Snider and Bowler 1992), but likely shorter for wild individuals (Miller 2006) |
| Maximum age of reproduction: | 15 | Unknown, but reproductive senescence not apparent in captive individuals (Miller 2006) |
| Maximum broods per year: | 1 | Bailey 2010 |
| Mean litter size (SD): | 7 (2.9) | Bailey 2010 |
| Sex ratio at birth: | 0.5 | Keenlyne and Beer 1973 |
| Mean % of adult females breeding/year (EV): | 44.0 (7.7) | Hileman 2016 |
| % of adult males in breeding pool: | 100 | Miller 2006 |
| Annual % mortality | | |
| Age 0–1 both sexes (EV): | 62.2 (0.7) | Hileman 2016 (EV = 11% of total variance; Jones et al. in press) |
| Age 1–2 both sexes (EV): | 34.7 (0.7) | Hileman 2016 (EV = 11% of total variance; Jones et al. in press) |
| Age 2–3 both sexes (EV): | 32.7 (0.7) | Hileman 2016 (EV = 11% of total variance; Jones et al. in press) |
| Adult males (EV): | 21.1 (0.5) | This study (EV = 11% of total variance; Jones et al. in press) |
| Adult females (EV): | 21.8 (0.5) | This study (EV = 11% of total variance; Jones et al. in press) |
| Initial population size: | 284 | Extrapolated 2015 adult abundance estimate (148; Chapter 2.1) to total population size by assuming a stable age distribution |
| Carrying capacity: | 569 | Extrapolated upper 95% CI of 2015 adult abundance estimate (295; Chapter 2.1) by assuming a stable age distribution |

Table 3. Sensitivity analysis of model parameters used in eastern massasauga population viability analysis. Range indicates the minimum and maximum values specified for Latin Hypercube Sampling in Vortex. The relative influence of each parameter on population extinction risk was determined using simple logistic regression and is indicated by its standardized regression coefficient (α_n/SE_n).

| Parameter | Range | α_n/SE_n |
|-------------------------------------|--------------|-----------------------------------|
| Adult female mortality (%) | 16.8–26.8 | 53.4 |
| Mean litter size | 6–8 | -39.4 |
| Mean adult females breeding/yr (%) | 39–49 | -36.5 |
| Age 0–1 female mortality (%) | 57.2–67.2 | 35.1 |
| Age 1–2 female mortality (%) | 29.7–39.7 | 23.0 |
| Age 2–3 female mortality (%) | 27.7–37.7 | 22.7 |
| Lethal equivalents (per individual) | 0–6.29 | 15.9 |
| Initial population size | 198–569 | -12.8 |
| Carrying capacity | 284–854 | -6.5 |
| Adult male mortality (%) | 16.1–26.1 | 3.5 |
| Age 0–1 male mortality (%) | 57.2–67.2 | 2.0 |
| Age 1–2 male mortality (%) | 29.7–39.7 | 1.7 |
| Age 2–3 male mortality (%) | 27.7–37.7 | 0.9 |

Figure 1.

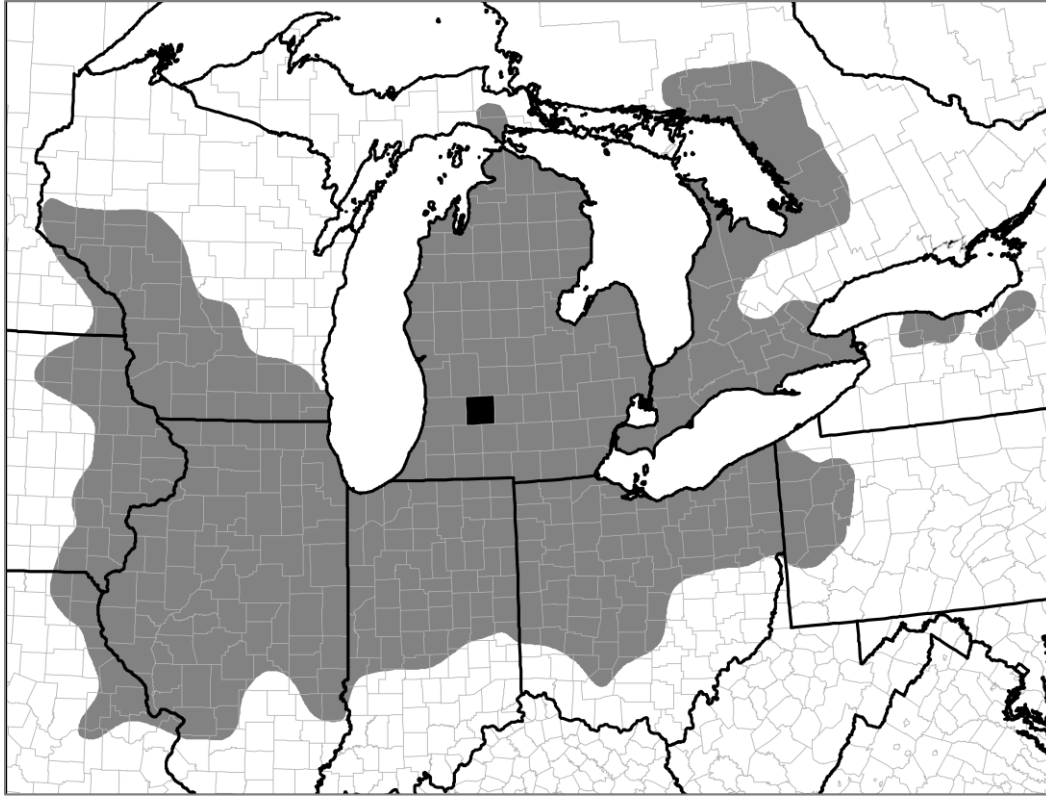


Figure 2.

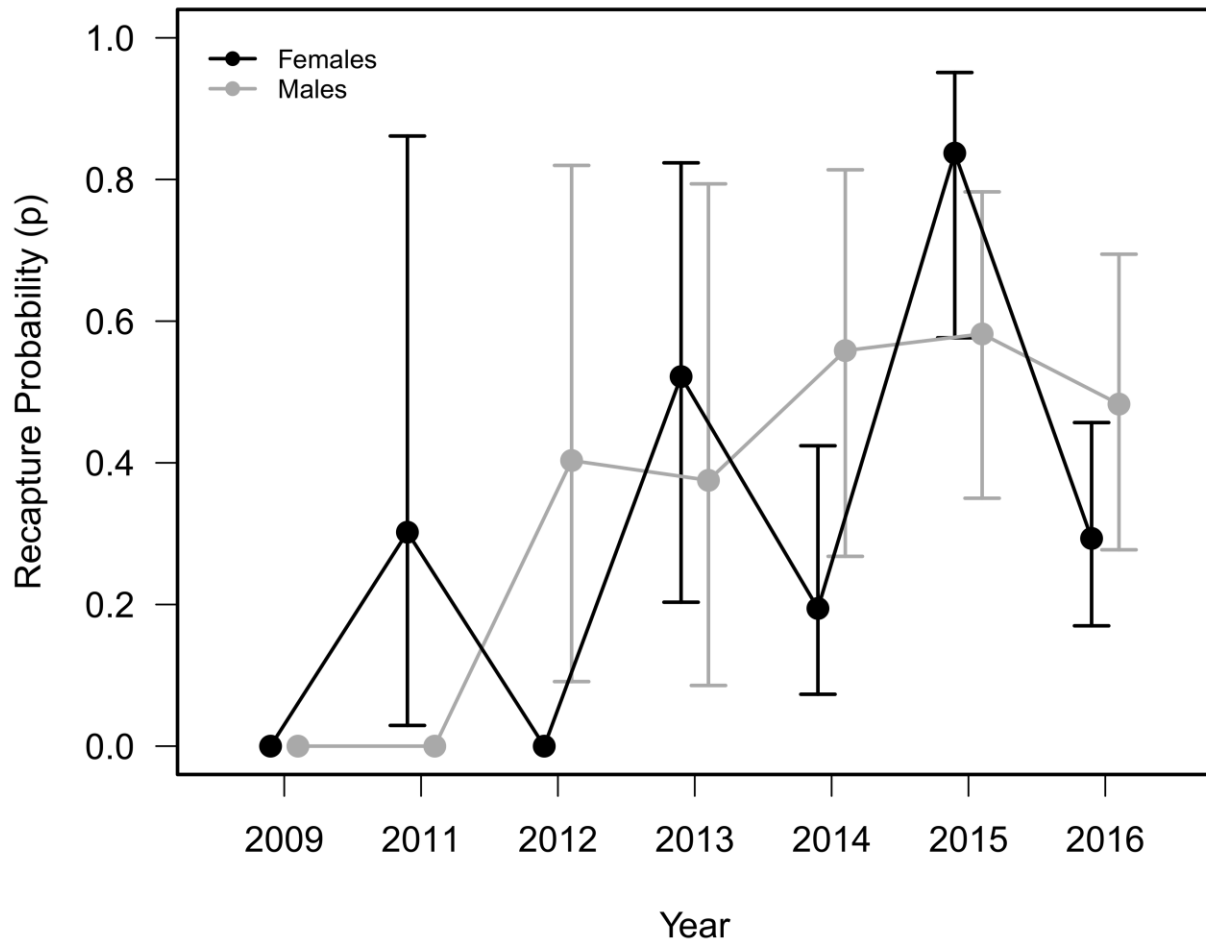
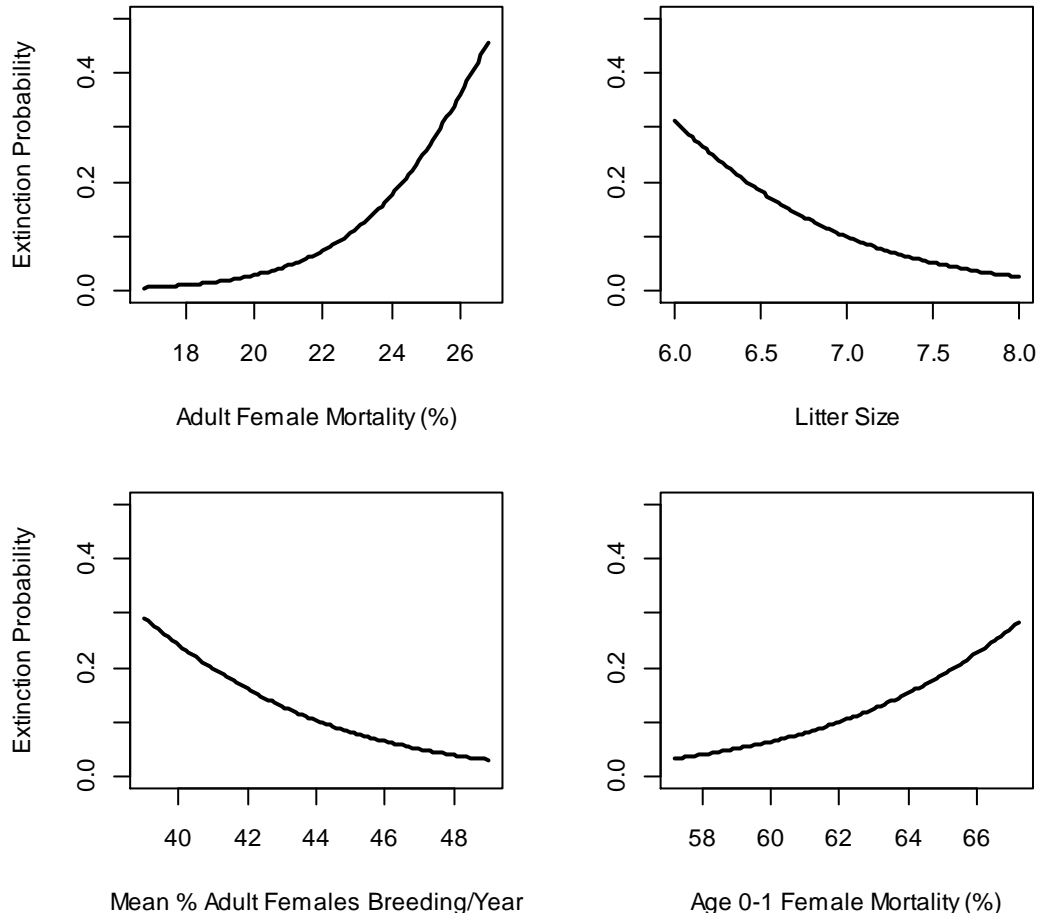


Figure 3.



Chapter 3

Extended Review of Literature

Introduction

Insight into the demography and genetics of wildlife populations is important for making conservation decisions and evaluating management success. Estimates of genetic effective population size and census population size can indicate whether a species warrants protected conservation status (Mace et al. 2008). Monitoring population size and other parameters, such as survival rates, can also reveal population-level responses to management activities or threats (Williams et al. 2002). Additionally, population-specific estimates can be used in population viability analysis to assess extinction risk and evaluate alternative management strategies (Beissinger and McCullough 2002).

Reptiles represent some of the most imperiled species on the planet, with habitat destruction, fragmentation, and degradation among their greatest threats (Gibbons et al. 2000, Ananjeva et al. 2015, Tingley et al. 2016). Other forces driving reptile extinctions include over-exploitation, climate change, disease, invasive species, and pollution (Gibbons et al. 2000, Tingley et al. 2016). Narrow geographic distributions, ties to specialized habitat, slow life histories, and temperature dependent sex determination are among the factors that make many species particularly susceptible to these threats (Tingley et al. 2013, Böhm et al. 2016*a, b*). Additionally, reptiles are the least understood group of terrestrial vertebrates in terms of viability, with less than half of documented species evaluated by the International Union for Conservation of Nature (Tingley et al. 2016). Of the 45% of reptile species evaluated, an estimated 20% are at risk of extinction while another 19% lack enough data to determine their

status (Tingley et al. 2016). Population-level data, in particular, are generally lacking among reptiles and especially for snakes (Böhm et al. 2013).

Many reptiles species are difficult to detect due to their secretive nature and cryptic coloration (Gibbons et al. 2000, Mazerolle et al. 2007). Additionally, non-invasive genetic sampling popular in mammal studies is not feasible for species that lack hair and produce scat that is hard to find and identify. For these reasons, genetic and demographic data can be challenging to collect, but are still critical to effective population management and recovery.

The eastern massasauga (*Sistrurus catenatus*) is a federally threatened reptile lacking demographic data for many populations throughout its range (Environment Canada 2012, U.S. Fish and Wildlife Service 2016). The geographic range of the eastern massasauga extends throughout the Great Lakes region of the midwestern United States and into southern Ontario, Canada (Holman 2012). Across the species' known historical distribution, only 46% of populations have been confirmed extant, while the rest are either extirpated (26%) or have unknown status (28%; Szymanski et al. 2015). Even in Michigan, where the majority of remnant populations occur, an estimated 33% of historical populations have been extirpated (Johnson et al. 2000), and the status and viability of most populations is unknown.

The primary cause of declining massasauga populations is habitat loss and fragmentation (U.S. Fish and Wildlife Service 2016). Eastern massasaugas have a strong affinity for shallow wetland habitat, which has been a prime target for agricultural conversion in the Great Lakes Region (Dahl and Johnson 1991). Additional threats including human persecution (Szymanski 1998) and road mortality (Shepard et al. 2008, Baker et al. 2016) have also contributed to

population declines. Consequently, most remaining eastern massasauga populations are believed to be small and isolated (Szymanski 1998).

Genetic Diversity and Effective Population Size

Genetic diversity is important to a population's ability to respond and adapt to changes in the environment. Small, isolated populations risk losses of genetic variation via inbreeding and genetic drift (Frankham 1996). Low genetic variation leaves populations vulnerable because they are less able to adapt to environmental changes including extreme weather events or disease (Frankham 1996). Additionally, inbreeding and drift can lead to diminished fitness through the buildup and increased expression of deleterious alleles (Lynch et al. 1995, Reed 2005). The genetic effective size of a population (N_e) represents how many individuals would be in an 'idealized' population (i.e., one with constant population size, equal family sizes, and a 1:1 sex ratio) experiencing genetic drift or inbreeding at the same rate as the population of interest (Wright 1931, Leberg 2005). Therefore, any life-history attributes leading to deviations from an idealized population can cause N_e to be lower than census population size (N_c ; Hare et al. 2011).

Although the importance of effective population size in conservation biology is widely recognized because it indicates how vulnerable a population is to genetic drift and inbreeding (Frankham 1995, Palstra and Ruzzante 2008, Charlesworth and Willis 2009, Luikart et al. 2010), relatively few studies have estimated contemporary N_e for snakes. For eastern massasaugas, this parameter has been estimated in one published study, with three populations in Ontario, Canada ranging from approximately 36 to 52 individuals (with 95% confidence intervals, CIs, spanning approximately 19 to 106 individuals; see Supporting Information Fig. S4 from DiLeo et al. 2013). In a population of New Mexico ridgenose rattlesnakes (*Crotalus willardi obscurus*)

which, like eastern massasaugas, are federally threatened in the United States (U.S. Fish and Wildlife Service 1978), N_e was estimated to be 220 (90% credible interval=103–293; Holycross and Douglas 2007). The giant gartersnake (*Thamnophis gigas*) is another federally protected threatened snake with low estimates of N_e throughout its range (Wood et al. 2015). Out of 15 giant gartersnake populations, the smallest reported N_e was 14.6 (95% CI=10.6–32.0) and the largest was 82.0 (95% CI=54.0–260.6). In contrast, effective population size estimates for eight timber rattlesnake (*Crotalus horridus*) populations in New Jersey, where they are state listed as endangered, were highly variable (Bushar et al. 2015). Here, N_e ranged from 4.0 (95% CI=2.4–10.7) to 283.8 (95% CI=1.5– ∞) and 95% confidence intervals typically had an upper limit of infinity, possibly due to small sample sizes and using only five microsatellite loci. Likewise, estimates of this parameter using seven microsatellite loci for 16 populations of adder (*Vipera berus*) were highly variable (Ursenbacher et al. 2009). Within the geographic area that the species is believed to be locally vulnerable, adder N_e estimates ranged from 4.1 (95% CI=2.8–6.3) to infinity (95% CI=37.3– ∞). Even among non-threatened snakes, such as the copperhead (*Agkistrodon contortrix*), N_e can be relatively low (approximately 93 to 111 individuals, depending on estimation method, in a Connecticut population; Levine et al. 2016).

The N_e/N_c ratio

The ratio of effective population size to census population size can be used in conservation to evaluate and monitor population viability (Kalinowski and Waples 2002, Luikart et al. 2010). If there is a consistent relationship between these parameters within a particular species or taxa, managers may be able to collect data on only one and infer the other, making monitoring efforts more efficient (Luikart et al. 2010). Moreover, assessing variation in this ratio and its relationship to life history, demography, and the environment can help elucidate the

relative influence of these factors on N_e (Cooper et al. 2009, Luikart et al. 2010, Waples et al. 2013) and be used to guide management. Two large review studies examining ratios of N_e/N_c across wild populations of plants and animals found median values of 0.11 (Frankham 1995) and 0.14 (Palstra and Ruzzante 2008). However, between the two studies, only four N_e/N_c estimates for reptiles were included (three lizards and one snake species). Additionally, in the study by Frankham (1995), N_e estimates were primarily calculated using demographic data, and this method typically incorporates only a subset of the factors influencing N_e (i.e., population size fluctuations, variation in reproductive, and sex ratio; Luikart et al. 2010). The review by Palstra and Ruzzante (2008) consisted of only genetic estimates of N_e , but authors could not always determine whether N_e and N_c were appropriately related to one another based on the time periods to which each estimate applied. A follow up meta-analysis by Palstra and Fraser (2012), using only genetic estimates of N_e and N_c that were appropriately related to one another, obtained a median N_e/N_c ratio of 0.23, but no reptile species were included in their sample.

Genetic Studies of Eastern Massasaugas

Previous population genetic studies specific to eastern massasaugas have focused on gene flow between populations and assessed whether inbreeding is apparent within populations. Gibbs et al. (1998) and Anderson et al. (2010) developed primers to amplify polymorphic microsatellite loci specific to this species. Gibbs et al. (1997) used six of these loci to investigate genetic structure and inbreeding among five eastern massasauga populations distributed through Ohio, New York, and Ontario. They found that the five populations were genetically isolated from one another and detected potential inbreeding within populations. Gibbs and Chiucchi (2012) also examined whether inbreeding effects were evident in 14 eastern massasauga populations distributed throughout the range (but not in Michigan), finding genetic evidence of low-level

inbreeding in some populations, but failing to detect a significant relationship between amount of inbreeding and the body condition of the snakes. Chiuichi and Gibbs (2010) analyzed eastern massasauga population genetics in 19 populations throughout the species' distribution (not including Michigan), comparing historic estimates of effective population size, recent and historic migration rates, and genetic structure. They concluded that historical effective population size was highly variable between populations, populations were genetically distinct from one another even when distance between them was small (<7 km apart), and migration rates have always been low between these populations despite their proximity to each other. Similarly, DiLeo et al. (2013) analyzed the genetic structure of eastern massasaugas in Ontario, Canada and assessed what physical barriers prevent nearby populations from interbreeding, concluding that water bodies were the largest barrier, followed by roads.

In addition to studies on population structure and inbreeding, genetic data has been used to confirm multiple paternity in eastern massasaugas from Michigan, Illinois, and Pennsylvania (Stedman et al. 2016). Genetic studies have also assessed the taxonomy of the eastern massasauga, providing evidence that it is a unique species from the desert massasauga (*S.c. edwardsii*) and western massasauga (*S. c. tergeminus*; Kubatko et al. 2011, Ray et al. 2013), and suggesting three range-wide management units based on mitochondrial DNA (Ray et al. 2013). Major histocompatibility complex (MHC) diversity was also examined within three Illinois populations, with results suggesting a prominent role of positive selection on MHC historically, with genetic drift recently becoming a stronger evolutionary force (Jaeger et al. 2016).

Population Demography Studies of Eastern Massasaugas

Limited demographic information has been published on the eastern massasauga throughout its range. Thus far, telemetry studies have dominated the body of literature on this species, which is not surprising given the challenges associated with collecting long-term mark-recapture data on a cryptic snake. Consequently, until very recently, demographic studies have been limited to estimates of relatively short-term survival (reviewed in Jones et al. 2012). Bailey et al. (2011) published an estimate of 95% active season survival for adult eastern massasaugas located in a managed habitat in Michigan. Jones et al. (2012) examined annual adult survival based on telemetry data from 18 data sets collected across this species' distribution (including the estimate from Bailey et al. 2011) and found that survival increased across the southwest to northeast range distribution of the species (ranging from 0.35 – 0.95), but did not typically differ by sex. Additionally, Pomara et al. (2014) demonstrated that winter drought, summer flooding, and anthropogenic landscape modification are strong predictors of the variation in adult survival rates observed in the Jones et al. (2012) study.

More recently, eastern massasauga mark-recapture data has been used to estimate longer-term survival and other demographic parameters, including process variance, population size, population growth, and reproductive frequency. In a New York population, data collected from 2006–2014 was used to estimate an adult female annual survival probability of 0.78 (95% CI = 0.67–0.86) and annual gravid female abundance that fluctuated from 9–41 individuals (Johnson et al. 2016). Additionally, mature females had an approximately 99% (95% CI = 86%–100%) probability of being non-gravid the year following a reproductive year and an approximately 71% (95% CI = 44%–88%) probability of becoming gravid the year following a non-reproductive year, indicating that reproduction is usually biennial (Johnson et al. 2016). In an

Ontario population, with data analyzed from 1992–2008, annual adult abundance ranged from 35–77 individuals, annual survival probabilities were 0.74 (95% CI = 0.67–0.80) for adult males and 0.73 (95% CI = 0.64–0.80) for adult females, and population growth was 1.02 (95% CI = 0.99–1.04; Jones et al. in press). This study was also the first to estimate a process variance for this species, with an estimate of 0.006 obtained for mature male survival over the study duration (Jones et al. in press). Finally, in a Michigan population studied from 2009–2016 age-class specific estimates of survival ranged from 0.38 (95% CI = 0.27–0.50; 0–1 year olds) up to 0.71 (95% CI = 0.61–0.80; adult [age 3+] females), and adult abundance varied from 84–140 individuals (Hileman 2016). Additionally, on average, 44% (95% CI= 29%–59%) of mature females were estimated to reproduce each year (Hileman 2016).

Additional Michigan Studies of Eastern Massasaugas

In addition to the limited genetic and demographic studies mentioned above, multiple studies of eastern massasaugas have been conducted in Michigan to investigate habitat use and spatial ecology. Bailey et al. (2012) found that massasaugas in Barry County preferred early and mid-successional habitats over forest habitats, and management promoting early successional plant communities (i.e., controlled burns and mechanical woody plant removal), influenced massasauga micro-habitat use. In contrast, Cross et al. (2015) did not perceive significant differences in movement or habitat preferences based on the controlled burn history of an area. In Lenawee County, Moore and Gillingham (2006) identified complex habitat use by eastern massasaugas that depends on multiple spatial scales ranging from microhabitat to landscape-level, and determined that wetlands and lowland forest are important habitat types. Studies in Michigan have also evaluated massasauga movement patterns and estimated home range areas, typically finding greater movement and home range sizes in males compared to females and in

non-gravid females compared to gravid females (Moore and Gillingham 2006, DeGregorio et al. 2011, Bailey et al. 2012).

Conclusions

Effective population size, census population size, and other demographic estimates are important parameters to monitor in species of conservation concern. However, collecting sufficient data for accurate and precise estimates is often difficult for cryptic reptiles such as the eastern massasauga. Because eastern massasaugas are relatively long-lived, it takes years to observe population-level responses to management activities or threats, making long-term data sets particularly valuable. Few studies on eastern massasaugas in Michigan have focused on demographic parameters important in assessing population status. Additionally, despite Michigan supporting the greatest number of remnant massasauga populations and its position at the center of the species' distribution, range-wide analyses of genetic structure and inbreeding depression have omitted Michigan populations. Addressing these genetic and demographic data deficiencies within Michigan is important to the effective conservation of this threatened species in the core of its range.

Extended Methodology

Study Sites

The two sites included in this study are located in southwest Michigan, approximately 86 km apart. The site in Cass County is on 1,052 ha of private property composed of prairie, meadow, wetland, forest, open water, cropland, private roads, and buildings. The second site, in Barry County, is located on approximately 277 ha of privately owned land that includes forest, wetland, open water, old-field, prairie, and buildings and is bisected by a public dirt road. Both sites are actively managed for eastern massasaugas (e.g., controlled burns, invasive species removal) and a large proportion of the land cover surrounding both sites is agricultural or otherwise developed. Within a 5km radius measured from the center of each study area, approximately 72% of land in Cass County and 48% of land in Barry County is classified as either “developed” or as “planted/cultivated” for livestock or crop production (based on the 2011 National Land Cover Database; Homer et al. 2015).

Effective and Census Population Size Methodology (Chapter 2.1)

We used a combination of visual encounter surveys, drift fences with funnel traps, and artificial cover objects to capture snakes within an approximately 64.3-ha survey area at the Cass County site and an approximately 19.9-ha survey area at the Barry County site. Drift fences were pre-fabricated 30.5 m long silt fencing attached to wooden posts, with the bottom (approximately 15 cm) buried into the ground. Funnel traps were constructed with hardware cloth and aluminum screening and were placed at either end of each drift fence. Artificial cover objects included carpet squares and wooden boards (various sizes and types). Surveys at the Cass County site were conducted between 28 March–8 October 2012 and surveys at the Barry County site were

conducted between 28 April–30 August 2015. During surveys, all surveyors recorded their search effort (i.e., time spent looking for snakes). When we found a snake, we captured it using snake tongs, secured it in a cloth bag, and held it within a bucket until processing. We recorded all capture locations using handheld GPS units.

We measured each snake's total length to the nearest 0.1 cm using a squeezebox (Quinn and Jones 1974) and flexible measuring tape. Once the snake was restrained in a clear plastic tube, we measured tail length (starting at the posterior end of the anal plate) with a ruler and subtracted it from total length to obtain the snout-vent length (SVL; Fitch 1987). While snakes were restrained, we also determined sex via cloacal probing (Schaefer 1934) and palpated females for the presence of embryos. We classified females as adults if they had SVLs ≥ 45.1 cm, which is the SVL of the smallest gravid female observed during studies conducted concurrently with this one, spanning 2010-2016 at the Cass County site and 2013-2016 at the Barry County site. We based adult male size on the smallest male with motile sperm detected via cloacal smear at the Cass County site (43.3 cm SVL; Richard B. King, pers. obs). We marked all individuals with a subdermal passive integrated transponder (PIT) tag (Gibbons and Andrews 2004). When possible, we drew blood from the caudal vein and stored it in either 95% or 100% ethanol. After processing, we released snakes at their respective capture locations.

We extracted DNA from ~ 10 μ l of blood using Qiagen DNEasy kits, following standard manufacturer protocols. We used primers developed by Anderson et al. (2010) to amplify 17 microsatellite loci from each DNA sample. Each 10 μ l PCR reaction consisted of 20–100 ng DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 1 μ l of 0.5 mg/ml bovine serum albumin, 0.2 μ l deoxynucleotide solution mix (0.2 mM of each), 1 unit Taq DNA Polymerase, 0.6 μ l primers (2 μ M of each, with fluorescently labeled forward primer), and 5 μ l double-distilled

H₂O. We amplified markers using an Eppendorf Mastercycler nexus gradient thermal cycler and followed the protocol in Anderson et al. (2010), except for modified annealing temperatures. Loci and respective annealing temperatures were as follows: *Scu200* (62°C), *Scu201* (62°C), *Scu202* (60°C), *Scu203* (60°C), *Scu204* (62°C), *Scu205* (60°C), *Scu206* (62°C), *Scu208* (62°C), *Scu209* (62°C), *Scu210* (56°C), *Scu211* (56°C), *Scu212* (56°C), *Scu213* (56°C), *Scu214* (56°C), *Scu215* (50°C), *Scu216* (56°C), and *Scu217* (50°C). For all PCR runs, we included a negative control for each amplified locus to detect any contamination. Following amplification, fragment analysis was performed with an ABI3730 DNA Analyzer (Applied Biosystems) at the University of Arizona Genetics Core. We scored fragments using PeakScanner v 2.0 (Applied Biosystems).

To assess genotype accuracy, we re-amplified and genotyped 12% of individuals (15 out of 124) from our full data set (which included juveniles and adults captured in years not included for this study). Based on this, we calculated an allele scoring error rate (i.e., incorrect alleles/total alleles). We used Micro-Checker v 2.2.3 (Van Oosterhout et al. 2004) to check the full data set for null alleles, large allele dropout, and stuttering. We also used FreeNA (Chapuis and Estoup 2007) to estimate null allele frequencies. For all other descriptive statistics and analyses, we included only genotypes from adults captured during the years of this study.

We calculated the number of alleles (N_a), effective number of alleles (effective N_a), observed heterozygosity (H_o), and expected heterozygosity (H_e) for each locus using GenAlEx v 6.503 (Peakall and Smouse 2006, 2012). In addition, we used GenAlEx to estimate mean pairwise relatedness according to Queller and Goodnight (1989). Using GENEPOP v 4.6 (Rousset 2008) we calculated F_{IS} according to Weir and Cockerham (1984) and tested for deviations from Hardy-Weinberg equilibrium based on probability tests. We also performed pairwise exact tests in GENEPOP v 4.6 to test for linkage disequilibria. We performed all

analyses separately for each population. For Hardy-Weinberg and linkage disequilibria tests, we used the default settings, *a priori* set $\alpha = 0.05$ to evaluate significance, and applied a sequential Bonferroni correction to account for multiple tests (Holm 1979; Rice 1989).

To estimate contemporary effective population size (N_e) we used the linkage disequilibrium method in NeEstimator v 2.01 (Do et al. 2014). This single-sample method estimates N_e based on the frequency of correlations between alleles located at separate loci (i.e., linkage disequilibrium; Hill 1981). Linkage disequilibrium is inversely related to N_e because in physically unlinked neutral loci it results mainly from genetic drift, which becomes a stronger evolutionary force as population size decreases (Waples 1991). We used a random mating model and excluded alleles with frequencies < 0.02 as recommended by Waples and Do (2010) to best balance the bias associated with keeping rare alleles and the precision lost by removing them. We report jackknife 95% confidence intervals (CIs), which have reduced bias compared to parametric CIs due to the lack of independence inherent in pairwise comparisons of loci (Waples and Do 2008).

Our estimates of census size (N_c) represent abundance estimates of reproductively mature adults within each study area during the respective survey period. We used Huggins' closed-capture models in Program MARK version 8.1 (White and Burnham 1999), which allowed us to censor individuals during occasions where they were unavailable for capture. This was necessary because 11 gravid females were held for captive parturition at the Cass County site. We truncated the data sets to include no more than four months of data to better approximate the assumptions of closure (i.e., no births, deaths, immigration, or emigration). In order to maximize the data retained for this analysis, we selected time periods that included the greatest number of captures. For the Cass County site, we used captures from 17 April–14 August and, for the Barry

County site, we used captures from 28 April–14 August. For each site, we pooled our data into seven capture occasions.

We built a set of candidate models that included different combinations of variables expected to affect detection rates. Capture probability (p) and recapture probability (c) were constrained to be constant or allowed to vary with sex, search effort, or time. We also modeled additive and interactive effects of sex with effort and sex with time. This resulted in eight models with equal capture and recapture probabilities. Additionally, we duplicated these eight models, this time including an additive effect of behavior, for total set of 16 candidate models. In the behavior models, p and c differed from one another by a constant, presumably due to a handling effect (i.e., ‘trap happy’ or ‘trap shy’). We ranked models using Akaike’s information criterion adjusted for small sample size (AIC_c ; Akaike 1973; Burnham and Anderson 2002). To be conservative in our abundance estimates and to account for model selection uncertainty, we model averaged our candidate models according to AIC_c weight. We calculated lognormal 95% confidence intervals for each estimate.

Survival Rates and Population Viability Analysis Methodology (Chapter 2.2)

We collected mark-recapture data during 2008, 2009, and 2011–2016 for the Barry County population only. In all years, we primarily located snakes using visual encounter surveys, but drift fences and artificial cover objects were employed as supplemental capture methods during 2015 and 2016. In 2008 and 2009 our surveys were conducted in conjunction with a radio-telemetry study (Bailey et al. 2011, 2012) and 19 out of the 21 individuals marked within our study area over these two years were surgically implanted with radio transmitters. Surveys associated with the telemetry study used two surveyors and spanned May through October, and

only captures made without radio tracking were included in our data set. In 2011 and 2012, we conducted short-duration, high-effort mark-recapture surveys that included five days spanning 21–25 June 2011 and 12 days spanning 8–12 May, 19–23 June, and 7–8 August in 2012. These shorter surveys relied heavily on volunteer effort, with up to 20 surveyors in the field at a time. For the remainder of the study period, surveys spanned a larger portion of the active season (approximately late April/early May through mid-August), and two to four people regularly conducted surveys with occasional volunteer assistance. For all years, we used handheld GPS units to record the locations of captured snakes.

After capturing a snake, we restrained it within a clear PVC tube and determined its sex via cloacal probing (Schaefer 1934). For females, we used x-ray, ultrasound, or palpation to detect developing embryos, with palpation being the sole method from 2011–2016. To determine age class, we measured the snout-vent length (SVL) of each individual using a flexible measuring tape, either while it was restrained in the snake tube (2008 and 2009), or using a squeezebox (Quinn and Jones 1974; 2011–2016). We considered females to be adults if SVL was \geq the smallest observed female with developing embryos (45.1 cm). We classified males as adults if SVL was \geq the smallest observed male with motile sperm detected from cloacal smear at a nearby site (43.3 cm SVL; Richard B. King, pers. obs). Upon first capture, we marked snakes with a subdermal passive integrated transponder (PIT) tag (Gibbons and Andrews 2004). After processing, we released each snake at its capture location.

We used Cormack-Jolly-Seber models (Cormack 1964, Jolly 1965, Seber 1965) implemented in Program MARK version 8.1 (White and Burnham 1999) to estimate annual apparent survival of reproductively mature adults from 2008–2016. This parameter is termed “apparent” survival because it incorporates the confounded effects of mortality and permanent

emigration. For this analysis, we allowed expansions but not contractions in the study area across years, as reductions to survey area may bias survival estimates low. While increases to survey area between years can induce heterogeneity in recapture probabilities, this expansion may also reduce the effects of temporary emigration on estimates of apparent survival.

We included only captures made between 1 May and 17 August of each survey year and created binary individual capture histories, which specified whether each snake was captured (“1”) or not captured (“0”) during each year. Three individuals that were part of the radio telemetry study in both 2008 and 2009 were treated as unavailable in 2009 by entering a “.” in their capture history, as they were unlikely to be encountered by chance. Additionally, we treated two snakes with surgery-induced mortalities as known removals so these deaths would not influence survival estimates.

We included 16 models in our candidate set, ranked models using Akaike’s information criterion adjusted for small sample size (AIC_c), and generated model-averaged estimates of survival based on AIC_c weight (Akaike 1973, Burnham and Anderson 2002). In each model, we either treated survival probability (Φ) as constant or allowed it to differ between males and females. To account for potential effects of different survey methods on detection, we allowed recapture probabilities (p) to vary by survey type (i.e., radio-telemetry study vs. short-duration, high-effort mark-recapture vs. full-season mark-recapture), to vary by time (i.e., each year with a different recapture probability), or to be constant across all years. We also modeled sex differences in recapture rates and included model variations that tested for additive vs. interactive effects between sex and time or sex and survey type. To test for overdispersion, we used a goodness-of-fit bootstrap procedure with 1,000 iterations on our global model:

$\Phi(\text{sex})p(\text{sex}*\text{time})$.

As a post-hoc analysis, we assessed whether transience had a significant effect on apparent survival. Transient snakes are individuals that are not part of the resident population, but pass through the study area (Pradel et al. 1997). Therefore, if there is a significant effect of transience, our survival estimates may be biased low. To assess this we used a likelihood ratio test, comparing our top ranked model to one that was identical except it included a transience effect. We considered a p-value < 0.05 to be significant.

We used Vortex v. 10.2.6.0 (Lacy and Pollak 2016) to assess the probability of population extinction within the next 100 years under current conditions at our Barry County study site. Vortex incorporates environmental, demographic, and genetic stochasticity, with probabilistic events (e.g., mortality, sex determination) occurring at the individual level and the fate of each individual is tracked through time (Lacy and Pollak 2016). The majority of parameter values used in our model were derived from data collected at our site or from a population located approximately 86 km away in Cass County, Michigan (Hileman 2016).

We calculated adult mortality rates ($1 - \text{survival}$) from the survival analysis in this study. A mean litter size of 7 ($SD = 2.9$) viable offspring was also determined using data from our site (Bailey 2010). We set an initial population size of 284 and a carrying capacity of 569 based on our 2015 adult abundance estimate and its upper 95% confidence interval (CI) limit, respectively (Chapter 2.1), which we extrapolated to total population size by assuming a stable age distribution. Mortality estimates of sub-adult age classes, mean percent of adult females breeding each year (and associated environmental variation; EV), and age at first reproduction were set at values reported from the Cass County site (Hileman 2016). We estimated environmental variation for mortality rates by taking 11% of total variance associated with each respective survival estimate. We used this proportion because Jones et al. (in press), estimated process

variance to be approximately 11% of the total variance in adult male eastern massasauga survival. Maximum age of reproduction and maximum lifespan are unknown for wild populations; however eastern massasaugas can live up to 20 years in captivity (Snider and Bowler 1992), and reproductive senescence is not apparent in captive individuals (Miller 2006). We recognize that animals likely have shorter lifespans in the wild than in captivity. Therefore, to put a reasonable restraint on these parameters, but allow mortality rates to be the principle determinant of longevity, we limited lifespan and reproduction to a maximum age of 15 years. We did not include inbreeding effects in our model, because the inbreeding coefficient calculated at this site in 2015 was low (0.010; Chapter 2.1). Additionally, we did not include an EV correlation between survival and reproduction or incorporate density dependent reproduction, as these effects are unknown for this species. Remaining parameters were set based on life history data from the literature.

Finally, we uploaded known allele frequencies for 16 microsatellite loci so that we could track changes in genetic diversity. These frequencies were calculated in GenAIEx v 6.503 (Peakall and Smouse 2006, 2012) for 75 individuals sampled at our site between 2013 and 2015. Loci, methods of DNA extraction, and protocol for PCR amplification and microsatellite genotyping are described in Chapter 2.1. We ran 10,000 iterations of the Vortex simulation and specified that population extinction occurred when only individuals of one sex remained.

To evaluate the relative effects of each model parameter's uncertainty on extinction risk, we used Latin Hypercube Sampling (LHS) in Vortex followed by logistic regression in R v 3.3.2 (R Core Team 2016). Latin Hypercube Sampling takes incremental samples from the range of uncertainty designated for each parameter (Lacy et al. 2017). Random combinations of these incremental parameter values are generated to create unique scenarios within the overall space of

parameter uncertainty (Lacy et al. 2017). Consequently, this technique can generate high coverage of the parameter space using fewer samples than a strictly random sampling approach, while including interactions between model parameters that cannot occur in a single-factor sensitivity analysis.

In our sensitivity analysis, we included potential inbreeding effects by varying lethal equivalents from 0–6.29 per individual, with percent due to recessive lethal alleles constant at 50% (Lacy et al. 2017). Here, the upper bound represents the estimated average lethal equivalents affecting fecundity and age 0–1 survival in a meta-analysis of wild species (O’Grady et al. 2006). We allowed mean litter size to vary \pm one neonate from our baseline value (i.e. 6–8 offspring), based on rounding the standard error of the estimate to the nearest whole individual. We varied initial population size from 198–569 individuals based on the upper and lower 95% CI bounds of the 2015 adult abundance estimate (Chapter 2.1), which we extrapolated by assuming a stable age distribution. Our uncertainty range for carrying capacity was set at 284–854 individuals (i.e., baseline initial population value to 150% baseline carrying capacity). We allowed mean annual percent of adult females breeding and mortality rates specific to each sex and age class to vary within \pm 0.05 of their respective baseline estimates. We maintained all additional parameters at the values specified in our baseline PVA and ran 3,000 samples with 10 iterations each for 100 years, producing 30,000 observations of 3,000 unique scenarios.

We generated simple logistic regression models using our LHS output, where the binary response variable indicated whether a population went extinct and each explanatory variable was one of the 13 model parameters varied for sensitivity testing. Since all parameters fluctuated simultaneously and their values were combined randomly during LHS, we were able to assess their relative influence while accounting for interactions, but without building interactions into

our models (Cross and Beissinger 2001). To assess the relative importance of each explanatory variable on population extinction risk, we compared their standardized regression coefficients (Cross and Beissinger 2001).

Appendix I - Copyright Permission Letter for Chapter 2.2 Figure 1

April 23, 2017

Dear Dr. Collin Jaeger:

I am requesting permission to reprint and modify Figure 1 from the following work:

Jaeger CP, Duvall MR, Swanson BJ, Phillips CA, Dreslik MJ, Baker SJ, et al. Microsatellite and major histocompatibility complex variation in an endangered rattlesnake, the Eastern Massasauga (*Sistrurus catenatus*). Ecology and Evolution. 2016. 6:3991-4003.

This request is for permission to include the above content as part of my master's thesis:

Effective Population Size, Demography, and Viability of Eastern Massasaugas (*Sistrurus catenatus*) in Southwest Michigan

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I would greatly appreciate your permission. If you require any additional information, do not hesitate to contact me. If you agree with the terms as described above, please sign the letter where indicated below.

Sincerely,

Danielle Bradke

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Permission is hereby granted:

Signature: 

Name & Title: Collin Jaeger

Company/Affiliation: Department of Biological Sciences, Northern Illinois University

Date: April 25, 2017

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