LIFE AND DEATH IN A CORN DESERT OASIS: REPRODUCTION, MORTALITY, GENETIC DIVERSITY, AND VIABILITY OF ILLINOIS' LAST EASTERN MASSASAUGA POPULATION

BY

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DISSERTATION

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ABSTRACT

Biodiversity is being lost at an unprecedented rate globally, with the primary driver being habitat loss and fragmentation. As natural habitats contract, species are forced into small often fragmented populations at increased risk of extirpation. Reptile species are thought to be experiencing wide-spread declines, the extent of which may be underestimated due to lack of long-term data for most species. Snakes, especially venomous species, are particularly vulnerable because of their life-history characteristics and often specialized habitat requirements. Unfortunately, snakes are also under-studied due in part to a historical lack of interest in the taxa and the difficulty with finding large numbers of individuals. The Eastern Massasauga (*Sistrurus catenatus*) is a rattlesnake species native to prairie/grassland and bog habitats from Iowa to New York and Ontario, Canada. The Eastern Massasauga is experiencing range-wide declines primarily caused by anthropogenic habitat conversion to agriculture and was recently proposed for listing as threatened under the Federal Endangered Species Act. In Illinois, only one Eastern Massasauga population remains extant, located at Carlyle Lake, Clinton County. Capture-mark-recapture monitoring of the Carlyle Lake population has been ongoing since 1999, and radio-telemetry was conducted from 2001-2003 and 2009-2011, providing a rare long-term data set for use in conservation planning. To evaluate conservation issues in the Illinois Eastern Massasauga population, I used the long-term data set to evaluate three aspects of population biology that are of concern in small populations: population genetics, reproduction, and mortality. I then integrated those data with a population viability and sensitivity analysis to determine the population trajectory, identify which demographic parameters have the greatest impact on population persistence, and make conservation strategy recommendations. Despite being both small and isolated, the Carlyle Lake Eastern Massasauga population exhibits moderate genetic diversity, no inbreeding or evidence of recent genetic bottlenecks, but low effective population size. Thus, conservation planning should consider genetic factors, but immediate intervention specific to genetic concerns is not necessary. Reproductive output in the Eastern Massasauga is not constrained by female body size, and there is no evidence of a trade-off between offspring size and number. Reproductive output, therefore, appears to be resource dependent and has the potential to increase via targeted conservation actions. Mortality of Eastern Massasaugas is caused primarily by automobiles and predation. Seasonal or permanent closure of non-essential roads and reduction of small carnivore numbers could decrease mortality at Carlyle Lake and positively impact population persistence. Finally, the population viability analysis indicated a positive population growth rate, but also a 65% probability of extinction in 50 years. Sensitivity analyses show that reproductive characteristics (proportion of breeding females, litter size, and offspring sex ratio) and carrying capacity have the greatest impact on population trajectory. Thus, conservation strategies for the Eastern Massasauga should focus on restoring additional habitat to increase carrying capacity, and use more manipulative measures only if the population growth rate becomes negative. This study provides an important application of long-term data for the conservation of an imperiled snake species and functions as a surrogate for other Eastern Massasauga populations or species with similar life histories for which detailed data are not available.

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PREFACE

Early American conservation biology got its philosophical inception in the late nineteenth-early twentieth century from the Preservationist Ethic of John Muir and later the Ecological Land Ethic of Aldo Leopold (Primak 1993). From these early origins, modern conservation biology emerged and held its first international conference in 1978, laying out an interdisciplinary approach to the maintenance of biodiversity that incorporates wildlife management, forestry, fisheries biology, and ecology to develop new methods for species preservation (Primak 1993).

Early conservationists recognized the threats anthropogenic activities would continue to impose on biodiversity, and also the necessity of maintaining in-tact ecosystems to preserve their function both for intrinsic value and their inseparable link to human resources and well-being (Callicott 1990). Habitat loss and fragmentation are the biggest drivers of biodiversity loss (Tilman et al. 1994; 2001). As habitat is lost, species are forced into small isolated populations, which are subject to specific threats and display different dynamics than large populations, termed the small population paradigm (Caughley 1994). A central concept of the small population paradigm is the "extinction vortex" in which small populations are subject to a series of positive feedback loops where inbreeding depression, demographic stochasticity, and genetic drift combine to drive populations to extinction (Gilpin and Soulé 1986).

Current anthropogenically induced species extinction rates are up to 100 times greater than background levels (Ceballos et al. 2015) and, globally, it is estimated one in five reptile species is threatened with extinction (Böhm et al. 2013). Snake populations are thought to be in widespread decline (Reading et al. 2010), but unfortunately, long-term population data to assess most species are unavailable (Todd et al. 2010; Böhm et al. 2013). Long-term data are needed to disentangle natural variation in population size from anthropogenically caused declines (Pechmann et al. 1991). The shortage of snake-focused studies can be attributed to a historical lack of interest in the taxa (Dodd 1993), and general difficulty in capturing and studying snakes in sufficient numbers (Fitch 1987; Parker and Plummer 1987; Durso et al. 2011). Vipers are an especially vulnerable group of snakes (Böhm et al. 2013) due in part to their life history characteristics (relatively slow to mature, long span between female reproductive bouts), specialized habitat requirements (Todd et al. 2010), and undeserved reputation that often results in persecution (Burghardt et al. 2009).

While long-term data are lacking for most snake species, some notable exceptions exist and have contributed substantially to our knowledge of snake population biology. A 50+ year study of a snake community in northeastern Kansas has provided invaluable data on life history and population dynamics of several species (Fitch 1999). A small isolated population of European Adders (*Vipera berus*) has been monitored in Sweden since 1981 and has provided insights into population genetics and inbreeding, reproductive biology, and demography (Madsen and Shine 1994; Madsen et al. 1995; Madsen et al. 1999; Madsen et al. 2004). Additionally, a population of the North American Timber Rattlesnake (*Crotalus horridus*) in New York was monitored using capture-make-recapture from 1978-2002 providing important population-level data for assessment of life history variation, population dynamics, and conservation (Brown et al. 2007). These studies highlight both the similarities and differences in viper species that preclude the development of general trends and make species specific studies extremely valuable.

Having long-term data for imperiled species is especially important so that appropriate conservation strategies can be developed (Harris et al. 2012). The Eastern Massasauga (*Sistrurus catenatus*; Rafinesque 1818) is declining throughout its range and was recently proposed for listing as threatened under the federal Endangered Species Act (Szymanski 2015). The largest remaining populations are located in Canada where the species is also listed as threatened (Parks Canada Agency 2013). The Eastern Massasauga is a small (55-80cm adult total body length) rattlesnake distributed from eastern Iowa to Ontario (Ernst and Ernst 2003). Habitat preferences for the species vary throughout its range from marshes and peat bogs to wet prairie and grasslands (Weatherhead and Prior 1992; Johnson and Leopold 1998; Kingsbury 1999; Wilson and Mauger 1999). In Illinois, they prefer open canopy grasslands with abundant terrestrial crayfish burrows to use as over-winter refugia (Dreslik 2005).

The decline of the Eastern Massasauga in Illinois is attributed to conversion of prairie habitat to agriculture and was documented as early as the 1860s (Hay 1887; Atkinson and Netting 1927). The Illinois Natural Areas Inventory estimates as little as 0.01% of original prairie-grassland habitats remain Illinois, scattered throughout the state. Historically, the range of the Eastern Massasauga in Illinois is believed to have tracked the extent of the prairie (i.e. the northern four-fifths of the state) and by the 1960s was reduced to fragmented populations in approximately 18 counties (Smith 1961). By 1999 its distribution was further reduced to five counties (Phillips et al. 1999), and in 2006, only four disjunct populations remained extant; located in Cook, Piatt, Knox, and Clinton counties (Dreslik et al. 2006; Wilson and Simone 2013), and subsequent surveys have determined at present only one population remains extant in the state, located at Carlyle Lake in Clinton County. Carlyle Lake is a human-made reservoir completed in 1967 by damming the Kaskaskia River for flood control. The remaining habitat suitable for Eastern Massasaugas exists around the southern periphery of the lake in parks and recreation areas managed by the Illinois Department of Natural Resources and Army Corps of Engineers. Suitable habitat patches are surrounded by agriculture fields and fragmented from each other by the lake itself, the river channel below the dam, the city of Carlyle, roads, and other human development. Capture-mark-recapture sampling was initiated in 1999 and previous studies done on the Carlyle Lake population have included population genetics (Andre 2003; Chiuchhi and Gibbs 2010), reproductive biology (Jellen 2007; Aldridge et al. 2008), spatial and thermal ecology, growth (Dreslik 2005; Dreslik et al. in press a, b, c), disease (Allender 2006; Allender et al. 2011, 2013; Allender et al. in press), and physiological ecology (Baker et al. in press).

This study builds upon previous work by integrating the full temporal span of data available on the Eastern Massasauga at Carlyle Lake to expand our knowledge of viper life-history and population biology and to devise conservation strategies for the species that may also be used as a surrogate for other species lacking sufficient data (Schtickzelle et al. 2005). Using population genetics, life history theory, and population viability and sensitivity analyses, this work provides the most complete conservation biology study for a rattlesnake species yet conducted.

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CHAPTER 1

TEMPORAL PATTERNS OF GENETIC DIVERSITY IN AN IMPERILED POPULATION OF THE EASTERN MASSASAUGA (*SISTRURUS CATENATUS*)

ABSTRACT

Demographic and environmental stochasticity can lead to a loss of genetic diversity, and are particularly damaging in those species that persist as small isolated populations with elevated probabilities of extirpation. Thus, monitoring genetic diversity is a critical component of adaptive management plans. The Eastern Massasauga (*Sistrurus catenatus*) is declining throughout its native range, and is thus a species of high conservation priority. In Illinois, only a single population remains of a once widespread distribution. I documented genetic diversity in this population at three time periods (2002, 2007, and 2012), and assessed levels of heterozygosity, allelic diversity, inbreeding (F_{IS}), and effective population size (N_e). Neither heterozygosity nor levels of inbreeding differed significantly among time periods. I identified 21 private alleles (of 144 total alleles), some of which may have been lost from the population given our estimated detection probability of 88%. Effective population sizes (N_e) were numerically small, but high relative to the census population size (N_c). While the small population size and a lack of connectivity are troubling, there was no significant decline in genetic diversity over the 10-year study. Multiple paternity of litters and possible kin recognition, coupled with a preference for an historically patchy habitat, may mitigate the loss of genetic diversity in the species, and promote their persistence in the fragmented habitats of the Anthropocene. Nevertheless, $N_e < 50$ is predicted to increase inbreeding and elevate the extinction risk of the population. Therefore, continued genetic monitoring is recommended, and recovery measures

should be implemented as soon as possible to mitigate the deleterious effects of small population size. These recovery actions should focus on road closures, predator control, and assisted migration.

INTRODUCTION

Global biodiversity is being lost at a staggering rate [1, 2], with primary drivers being habitat loss and fragmentation acting together to isolate populations [3] and limit gene flow [4]. Threatened and endangered (T&E) species are at increased risk of genetic diversity loss, as they typically exist in numerically small and spatially isolated populations susceptible to stochastic events [5, 6], and a concomitant loss of genetic diversity. Genetic diversity loss is most often manifested by genetic drift, and fixation of deleterious alleles and elevated inbreeding, the consequences being a reduction in fitness and reduced survival [5, 7, 8, 9]. These factors also impinge upon the long-term persistence of T&E species in that they steadily erode evolutionary and adaptive potential [7, 10], and drive T&E species into an extinction vortex [11]. Thus, measuring genetic parameters to assess risks to population persistence can provide invaluable information to conservation plans for T&E species.

Reptiles are declining worldwide [12] and venomous snakes are of particular conservation concern as their slow life history and habitat requirements can exacerbate their vulnerability to anthropogenic activities [13, 14, 15, 16, 17]. The Eastern Massasauga (*Sistrurus catenatus*) is one such species declining throughout its range [18] and it persists in geographically isolated and numerically small populations that are susceptible to inbreeding depression and loss of adaptive capacity [7, 19]. Previous studies documented moderate levels of genetic diversity, low levels of inbreeding, and little evidence of historic bottlenecks [20, 21], but were limited by brief temporal spans, small sample sizes, or low numbers of molecular markers. To maximize value, genetic monitoring should be of a longer temporal span (≥5 years), and employ robust sample sizes and sufficient loci appropriate for the study system and research questions [22].

Here I assess genetic diversity in a relictual population of Eastern Massasauga in Illinois using samples collected over a 10-year span to examine allelic diversity, inbreeding coefficients, and genetic bottlenecks. Finally, I estimate effective population size (N_e) relative to census size (N_c) and use this information to inform adaptive management of this population, with applicability to similarly imperiled species.

MATERIALS & METHODS

Study species and site

The Eastern Massasauga is a prairie and bog species endemic to the eastern United States and Canada [23]. It is a candidate for federal listing [24] and endangered in Illinois [25], where its distribution has been reduced to sparse, fragmented habitat around the southern perimeter of Carlyle Lake, Clinton County. The lake was constructed as an impoundment of the Kaskaskia River in 1967, and approximately 10,500 ha of habitat was lost as a consequence. Remaining individuals are now clustered in 10-15 wet prairie areas of less than 5 ha each containing crayfish burrow complexes that serve as hibernacula and represent the only remaining suitable habitat patches [26, 27]. Genetic variation was assessed at the largest hibernaculum (approximately 3.5 ha) located in South Shore State Park (SSSP).

Molecular genetic methodology

Total genomic DNA was extracted from blood samples collected at three time periods (2002, 2007, and 2012) via Qiagen DNeasy Blood & Tissue Kits (Qiagen INC.) using standard protocols. Microsatellite loci were screened for amplification using both species-specific primers and those developed for closely related species (S1 Appendix), with standardized conditions and polymerase chain reaction (PCR) amplifications in 10μl volumes. A total of 24 loci were chosen for fluorescent dye assignment (Applied Biosystems, ABI) and subsequently used in this study. Fragment analysis was carried out on an ABI 3730 GeneAnalyzer, and alleles were scored using GeneMapper 4.1 software (ABI).

Genetic analyses

The presence of null alleles and scoring errors were assessed via Micro-Checker 2.2.3 [28]. Linkage disequilibrium, a departure from Hardy-Weinberg Equilibrium (HWE), and inbreeding coefficients (F_{IS}) were assessed in GenePop 4.0 [29]. For multiple comparisons, alpha levels were adjusted via Bonferroni correction [30]. Allele frequencies and observed heterozygosities (H_{O}) were estimated in GenAlEx 6.41 [31] for all samples and each time interval.

To assess allelic diversity, allelic richness ($\mathrm{A_R}$) and private allelic richness ($\mathrm{P_{AR}}$; alleles unique to one time period), were derived from rarefaction and corrected for sample size in HP-Rare [32]. To test for the presence of a genetic bottleneck, I assessed loss of genetic diversity between time intervals using the two-phase mutation model option of program BOTTLENECK 1.2.02, with 95% single steps and 5% multiple steps and a variance for mutation size set to 12 [33]. The Wilcoxon sign test was used to test for heterozygote excess [34, 35], while the qualitative mode shift test evaluated shifts in allele frequencies stemming from loss of rare alleles [36]. As it can be difficult to determine if rare alleles have truly been lost from the population, or were merely missed due to sampling, I assessed the probability of failing to detect rare alleles using a three-sample binomial probability distribution, with a sample size of 30 (sampling probability assumed equal), and the lowest observed allele frequency (0.014). I calculated this probability in R using the following function: pbinom (0,30,0.014). Effective population size (N_e) was calculated for each time period across three allele frequency exclusion values (P_{crit} = 0.01, 0.02, 0.05) via the linkage disequilibrium method in LDNe [37].

RESULTS

I genotyped 94 Eastern Massasauga samples across 24 microsatellite loci (Table 1.1), and found no evidence of null alleles, scoring errors, or linkage disequilibrium. All loci conformed to HWE expectations at a Bonferroni adjusted probability ($\alpha = 0.002$), with the exception of one locus (Scu200). Two loci (Scu206, Scu209) were monomorphic. Thus, subsequent analyses were conducted using the remaining 21 loci. Observed heterozygosity (H_O) ranged between 0.09-0.87 (Table 1.1), while average H_O was temporally stable and extended from 0.64-0.66 (Table 1.1). Calculation of F_{ST} across time periods (essentially 3 "populations") gives a value of -0.003. An F_{ST} of zero essentially means complete sharing of genetic material, indicating no difference in heterozygosity across time periods.

Allelic richness decreased slightly from 6.3 in 2002 to 5.6 in 2012 (Table 1.2). Of 144 total alleles, 70 (48%) could be considered rare (i.e. those with a frequency < 0.1) [36] and 21 were private alleles occurring in only one time period (Table 1.3). The probability of failing to detect a rare allele over the study period is 4%, assuming equal sampling probabilities, a sample size of 30, and the lowest observed allele frequency (0.014). Hence, the probability of detecting a rare allele in at least one time interval is 96% and the probability of detecting an allele found in one time interval in another interval is 88%. As most private alleles occurred in 2002 (67%) and were undetected in both 2007 and 2012, the high detection probability suggests at least some of these alleles were lost from the population, rather than undetected.

There was no evidence of a significant bottleneck after Bonferroni correction ($\alpha = 0.017$) for 2002 ($p = 0.54$), 2007 ($p = 0.08$), or 2012 ($p= 0.30$). Further, a qualitative examination of allele frequencies showed that all time periods maintained a normal L-shaped distribution, indicating no genetic bottleneck [36].

Effective population size was small (19-30) with minor temporal fluctuations (Fig. 1.1). In 2002, N_e ranged from 19-26 (15-35 95% CI), in 2007 24-25 (19-32 95% CI), and in 2012 20-30 (15-40 95% CI). Although effective population size estimates (N_e) were low at SSSP, they approximated the calculated census size (N_c) of 26-69 [27].

DISCUSSION

The greatest genetic threats to small populations are genetic drift and inbreeding [38]. In small populations, the importance of genetic drift is elevated relative to selection, resulting in a loss of some potentially beneficial alleles and a concomitant fixation of deleterious alleles [39]. Inbreeding can result in decreased fitness and, along with drift, can interact with demographic and environmental factors creating a positive feedback loop termed the extinction vortex [11], hastening the extirpation of isolated populations [7, 40]. Threatened and endangered species typically persist in small populations that are also fragmented and isolated; thus migration may be disrupted or non-existent. While the literature is replete with studies documenting the deleterious effects of fragmentation and isolation on genetic diversity, not all taxa are equally susceptible, and some can maintain moderate genetic diversity despite isolation. Recently documented examples include marine angelfish (*Centropyge spp.*) inhabiting patchy coral reef ecosystems [41], an introduced population of white-tailed deer (*Odocoileus virginianus*) founded by only four individuals [42], a remnant population of the endangered hihi bird (*Notiomystis cincta*) in New Zealand [43], as well as the Eastern Massasauga [21, this study].

Genetic diversity, as assessed via heterozygosity, remained stable over the 10-year span and was moderate in the SSSP population, and consistent with values documented throughout the range, including larger populations in Canada [21, 44, 45]. These cumulative results indicate moderate levels of genetic diversity are maintained in the species, despite the isolation of populations and attenuated gene flow [21]. An assessment of two salamander species (*Ambystoma opacum* and *A. talpoideum*) over 20 years similarly found stable levels of heterozygosity despite a documented population decline in *A. talpoideum* [46]. Numerous life history characteristics, including high philopatry and low dispersal ability, have likely predisposed the Eastern Massasauga to a patchy distribution and low gene flow, even before European settlement [21]. However, we note that heterozygosity is less sensitive to population declines, particularly when compared to other metrics such as allelic richness [47].

While genetic diversity was maintained throughout the study, 67% of the private alleles detected occurred in 2002 and at least some of these were likely lost from the population. Declines in allelic richness may be indicators of recent population declines, as has been observed in Timber rattlesnakes [17]. Theory predicts a gradual loss of rare alleles via genetic drift will precede a loss of heterozygosity in small populations [48], thereby increasing extinction risk via fixation of deleterious alleles [7]. Consistent with theoretical predictions, common frog (*Rana temporaria*) [49] and Greater Prairie Chicken (*Tympanuchus cupido pinnatus*) [50, 51] populations experienced dramatic declines, followed by a loss of rare alleles in the short term, with heterozygosity declines in the long term. The Illinois Eastern Massasauga shares many similarities with the Greater Prairie Chicken, including moderate heterozygosity, (likely) loss of some rare alleles, and low N_e.

Significant inbreeding was not detected, despite expectations, as the SSSP hibernaculum is isolated from nearby hibernacula by approximately 4.5 km of open water to the west, 5 km of highly developed recreation areas to the south, and 5 km of agriculture fields and roads to the north. Historically, the conversion of the Illinois landscape from prairie to agriculture was gradual, and thus the decline of the Eastern Massasauga was assumed also to be gradual. Such population declines attenuate genetic diversity loss, particularly in relatively long-lived organisms and those with overlapping generations [52, 53, 54] such as the Eastern Massasauga, because the probability of loss of alleles is lower. Heterozygosity estimates have been criticized as being ill suited to detect inbreeding [55]; however, pedigree analyses have also failed to detect inbreeding in SSSP Eastern Massasaugas [56]. Further, although SSSP is isolated from other hibernacula, individual Eastern Massasaugas are occasionally found dead on roads up to 1 km away from SSSP, especially when male movement increases during the mating season. Thus occasional long-distance migrations between sub-populations are possible. Infrequently, transient individuals have been identified in otherwise sedentary populations of box turtles (*Terrapene carolina triunguis*) in Missouri [57]. These individuals maintain no home range and exhibit nomadic behavior, thus potentially enhancing gene flow in the populations they traverse [57]. It is unlikely transient individuals represent a substantial source of gene flow for the Eastern Massasauga, as roads are significant barriers to rattlesnake movement [17, 58, 59]. This is undoubtedly a challenge for rattlesnake conservation, in that roads are often a common landscape feature.

At Carlyle Lake, SSSP is bordered on three sides by roads, with a paved bike path and subdivision on the fourth. Open water, which separates SSSP from the next largest hibernacula complex, significantly inhibits dispersal and has contributed to genetic clustering of Canadian populations [59]. Also, no direct evidence of long-distance migration has been detected in 15 years of mark-recapture at Carlyle Lake (unpubl. data). However, even a low level of immigration can have fitness benefits greater than predicted by theoretical models [60], and even occasional migrants may serve to attenuate inbreeding and maintain heterozygosity in the population.

Though slightly variable over time, N_e estimates (24-45) overlap census population sizes (N_c = ~26—69) [27] at SSSP, and are small in comparison to those reported for other rattlesnakes [61, 62]. Similarly, a small declining population of mole salamanders (*A. talpoideum*) had an N_e that was equal to or exceeded N_c when monitored over 20 years, while a large stable population of marbled salamanders (*A. opacum*) had an N_e only 1-6% of N_c [46]. For over 30 years, the "50/500" rule" was widely accepted in conservation genetics, suggesting that an N_e of 50 was needed for short-term maintenance of genetic diversity, whereas to avoid long-term loss of evolutionary potential, an N_e of 500 was required [63]. However, these guidelines were formed based largely on opinions of animal breeders and limited laboratory studies. Given advances in theory and technological ability, it has recently been argued an N_e > 100 is necessary for the short-term, and N_e >1000 is required for long-term conservation [64]. The SSSP population falls short of both recommendations, thus raising concerns for population viability. Our N_e estimates for SSSP are less than (or nearly equal) to those for the endangered New Mexico Ridge-nosed rattlesnake (*Crotalus willardi obscurus*) for which population viability analysis indicates a 100% probability of extinction in 100 years, with a mean time to extinction of 17 years [65]. Although N_e is only one of many factors impacting population viability, the low value for SSSP remains disconcerting.

The ratio of N_e/N_c is variable among taxa and averages 0.19 (±0.11 SD) for 102 species of plants and animals [66]. The long-term mark-recapture data for Eastern Massasaugas at SSSP produced N_c estimates between 26 and 69 [27], resulting in an N_e/N_c ratio of 0.83—0.90. This higher ratio may be masking deleterious genetic consequences [39], as the SSSP population has remained small yet stable over the last 15 years [27]. A small isolated population of fire salamanders (*Salamandra salamandra*) also exhibited a high N_e/N_c of 0.50-0.84 that has been attributed to low reproductive variance and multiple paternity [67] A relatively high N_e/N_c ratio may also indicate a historically large population size with recent decline [54, 68, 69], which would be consistent with the construction of Carlyle Lake and the concomitant inundation of suitable habitat.

I detected no contemporary genetic bottlenecks related to the construction of Carlyle Lake 50 years ago. Studies of long-lived sea turtles were able to detect bottlenecks resulting from fishing activities that occurred 25-50 years ago [70], suggesting the time scale should be appropriate. Our results compliment a previous study showing a lack of historical bottlenecks in the population [21]. Lack of both contemporary and historical bottlenecks are unexpected given the magnitude of habitat loss and fragmentation that stemmed from conversion to agriculture and the construction of Carlyle Lake. However, genetic bottlenecks can remain undetected despite the presence of demographic data that confirm dramatic population decreases [71]. In this sense, the analytical methods used herein (i.e. program BOTTLENECK) are ideal for the detection of very recent or less severe bottlenecks [72], our data conformed to its sample size recommendations, and further, met all assumptions for the detection of a recent bottleneck [35]. However, shifts in allele frequency that stem from a bottleneck are not manifested until 5-10 generations post-event [35]. Because the generation time for the Eastern Massasauga is 3-5 years [73], the possibility remains that sufficient time has not passed since the building of the dam and subsequent fragmentation of habitat to allow detection of a bottleneck. A similar study of copperhead (*Agkistrodon contortrix;* similar generation time to Eastern Massasauga) in Connecticut also failed to detect a bottleneck following the construction of a reservoir that fragmented populations [74].

Concluding remarks

Results of this study are encouraging for the conservation of the Eastern Massasauga at Carlyle Lake. A lack of significant inbreeding and no detectable bottlenecks indicate that genetic considerations should not impede conservation efforts, particularly if other management actions to increase population size (e.g. habitat restoration, predator control, road closures) are carried out before additional population reductions occur. Priority should be given to projects that close roads or restore habitat adjacent to currently occupied patches to maximize genetic benefits. Ongoing genetic monitoring should continue, as these data provide a baseline for conservation, and will provide additional insights as new techniques and analyses are developed [22].

Our data support the conclusions drawn from previous studies that Eastern Massasaugas have historically existed in fragmented populations, likely due to the patchy occurrence of wet prairie habitat [21]. Similar results have been found in the prickly skink (*Gnypetoscincus queenslandiae*) that uses naturally patchy rainforest habitat and whose population genetic structure was found to be dependent upon natural rather than anthropogenic fragmentation [75]. Additionally, rattlesnakes may display a capacity for kin recognition [76] that could prevent matings with closely related individuals, and thus minimize inbreeding. Our results suggest that the life history of Eastern Massasaugas, as well as those of other species evolutionarily adapted to naturally patchy habitats, may thus promote the capacity for persistence in anthropogenically fragmented landscapes.

The dynamics of small populations are governed by stochasticity from demographic, environmental, and genetic sources, all of which can interact to produce rapid declines and extinction [82]. Despite encouraging genetic results, the Eastern Massasauga at Carlyle Lake still faces demographic challenges, and stochastic events could endanger the persistence of the Eastern Massasauga and other species with patchy distributions. Contemporary populations are separated by greater distances and encounter a greater number of barriers to movement than prior to anthropogenic landscape modifications, and thus are less likely to repatriate without human facilitation. Particular attention should be given to roads during conservation planning as these can play a significant role in genetic isolation of rattlesnake populations [77]. While there is no universal solution to species conservation, studies such as this one provide valuable insights into genetic functionality of populations in anthropogenically altered landscapes. Most notably, not all small populations are genetically impoverished and still hold substantial conservation value.

Whenever possible, population genetic data should be integrated with spatial and demographic data to inform recovery efforts and optimize the probability of success.

Chapter 1 meets the formatting requirements of *PLoS One*

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Tables and Figures

Table 1.1: Estimates for number of alleles ($^{\#}$A), observed heterozygosity (H_{O}), probability of Hardy-Weinberg deviation (P_{HWE}), and inbreeding coefficients (F_{IS}) for 94 Eastern Massasauga Rattlesnake sampled from South Shore State Park at Carlyle, Illinois. Data were derived from 24 microsatellite loci developed for the Eastern Massasauga (*Sistrurus catenatus*; 78), Timber Rattlesnake (*Crotalus horridus*; 79), Ridge-nosed Rattlesnake (*C. willardi obscurus*; 80), and Copperhead (*Agkistrodon contortrix*; 81). All loci conformed to the assumptions of HWE after Bonferroni correction (adjusted α = 0.002), with the exception of one locus (Scu200). Two loci (Scu206 and Scu209) were monomorphic.

Locus	**N**	**$^{\#}$A**	**H_{O}**	**P_{HWE}**	**F_{IS}**
AGK12	94	3	0.52	0.07	0.059
AGK16	94	9	0.79	0.56	-0.006
AGK21	94	6	0.79	0.47	-0.007
AGK24	94	6	0.70	0.31	0.047
AGK31	94	4	0.68	0.21	0.061
AGK38	94	9	0.87	0.56	-0.009
AGK39	94	2	0.14	1.00	-0.069
AGK44	94	4	0.61	0.01	0.155
CH05	94	4	0.52	0.61	-0.017
CH144	94	7	0.70	0.25	0.052
CW06	94	2	0.09	1.00	-0.039
Scu200	93	13	0.33	**0.00**	0.602
Scu201	92	9	0.73	0.08	0.089
Scu202	91	8	0.65	0.44	-0.110
Scu203	92	6	0.66	0.04	0.058
Scu204	92	5	0.60	0.16	0.098
Scu205	93	8	0.77	0.27	0.057
Scu206	91	1	--	--	--
Scu209	93	1	--	--	--
Scu210	93	11	0.72	0.37	0.071
Scu212	92	8	0.80	0.80	-0.039
Scu213	92	9	0.78	0.25	0.013
Scu215	93	10	0.63	0.21	0.040
Scu216	93	14	0.83	0.05	0.037
Average	**93.1**	**6.6**	**0.63**	**0.35**	**0.052**
SE	0.2	0.7	0.04	0.06	0.029

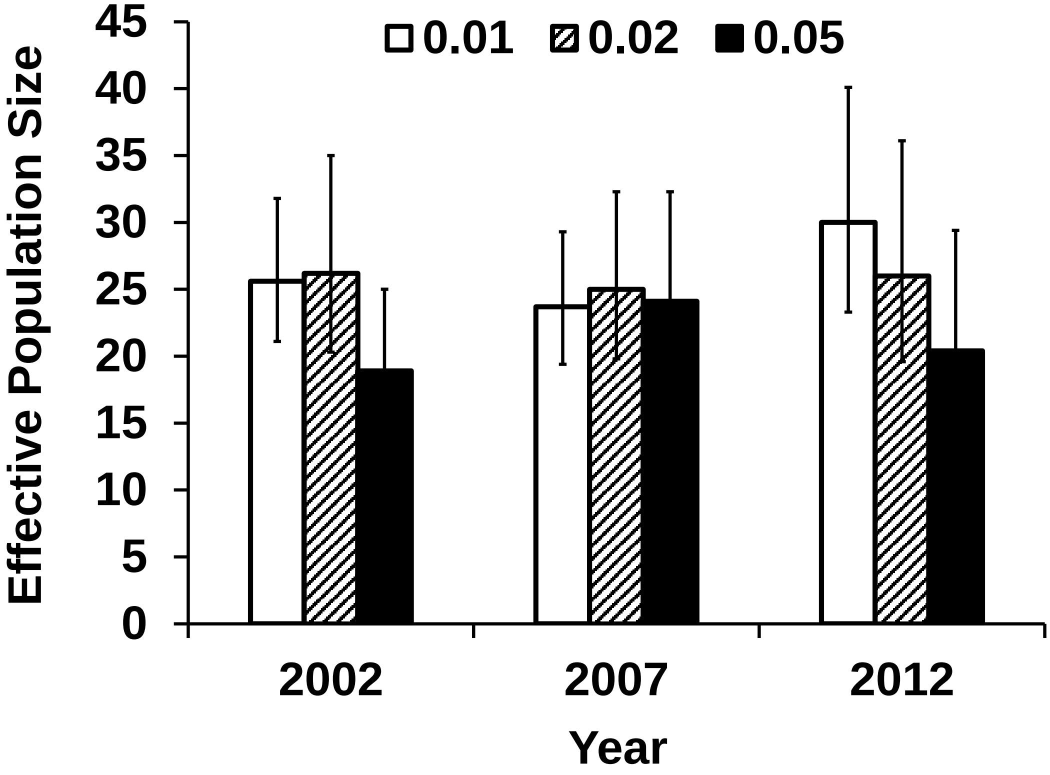
Table 1.2 Standard genetic diversity indices for mean estimates for number of alleles ($^{\#}A$), allelic richness (A_R), private allele richness (PA_R), observed heterozygosity (H_O), probability of Hardy-Weinberg deviation (P_{HWE}), and inbreeding coefficients (F_{IS}) for Eastern Massasauga Rattlesnake sampled over three time periods from South Shore State Park at Carlyle, Illinois. Data were derived from 21 microsatellite loci. All loci conformed to the assumptions of HWE after Bonferroni correction (adjusted $\alpha = 0.002$).

Year	**N**	**$^{\#}A$**	**A_R**	**PA_R**	**H_O**	**P_{HWE}**	**F_{IS}**
2002	30	6.4	6.3	0.66	0.65	0.04	0.027
2007	33	5.9	5.8	0.22	0.66	0.06	-0.003
2012	31	5.6	5.6	0.10	0.64	0.005	0.001

Table 1.3. Private alleles detected in an analysis of 21 microsatellite loci in 94 Eastern Massasaugas from 2002-2012 at South Shore State Park at Carlyle Lake, Clinton County, Illinois.

Year	Locus	Allele	Frequency
2002	CH05	152	0.016
2002	AGK38	138	0.016
2002	AGK16	246	0.094
2002	AGK16	266	0.016
2002	AGK24	189	0.016
2002	Scu215	158	0.016
2002	Scu216	236	0.016
2002	Scu216	243	0.016
2002	Scu216	251	0.016
2002	Scu202	209	0.017
2002	Scu210	191	0.016
2002	Scu210	194	0.016
2002	Scu210	206	0.097
2002	Scu201	230	0.017
2007	AGK16	242	0.014
2007	Scu212	357	0.029
2007	Scu205	202	0.014
2007	Scu203	257	0.014
2007	Scu210	173	0.014
2012	Scu216	219	0.016
2012	Scu213	225	0.016

Figure 1.1 Estimates of effective population size (N_e) and 95% CIs evaluated at three allele frequency exclusion values (P_{crit} = 0.01, 0.02, 0.05). Estimates were derived from 21 microsatellite loci across 94 Eastern Massasauga sampled over three time periods at South Shore State Park at Carlyle, Illinois.



CHAPTER 2

REPRODUCTIVE LIFE HISTORY, CONSTRAINTS, AND TRADE-OFFS IN THE EASTERN MASSASAUGA

ABSTRACT

Reptile life history strategies are extremely diverse, and can vary drastically even among species living in the same geographic area. Constraints, trade-offs, and selective pressures on life history traits are important to identify in light of the declining population paradigm and their importance in population dynamics. The Eastern Massasauga is a small endangered rattlesnake declining throughout its range and the subject of active conservation measures. Using data from long-term monitoring I examined the reproductive life history characteristics of litter size, neonate snout-to-vent length (SVL) and mass, and litter sex ratios to identify constraints, trade-offs, and selective pressures. I found litter size at the southern range limit in Illinois is consistent with the range-wide mean but lower than the northern range limit in Ontario, Canada. Neonate SVL is larger than the range-wide mean, and neonate mass is consistent with the range-wide mean. I did not find a significant effect of maternal SVL on litter size, nor a trade-off between offspring size and number, possibly indicating both are determined by the acquisition of resources before folliculogensis. Understanding variation in life history traits of threatened and endangered species is necessary for conservation as these traits play a significant role in population dynamics. Results of this study are valuable to inform conservation tools such as population viability models for the Eastern Massasauga, and to expand knowledge of viper life history.

INTRODUCTION

Life history traits among reptile species are diverse, and even species occupying the same geographic area can utilize markedly different reproductive strategies (Shine 2003). Describing reproductive characteristics such as number and size of offspring is straight-forward and informative, but it is also important to identify constraints, selective pressures, and trade-offs, especially to increase understanding of small population dynamics. Constraints to life history traits include those that are genetic, phylogenetic, physiological, mechanical, and ecological (Roff 1992). In ectotherms, the mechanical constraints on offspring number and size imposed by female body size are often of particular interest (Roff 1992). Trade-offs exist between linked life history traits and thus constrain their simultaneous evolution (Stearns 1992). Life history tradeoffs can take many forms; the best studied include those between reproduction and survival, current and future reproduction, reproduction and growth, reproduction and body condition, and number and quality of offspring (Stearns 1992). Life history is important for conservation and management of endangered species in light of the declining population paradigm (Caughley 1994; Norris 2004). Because most endangered species currently exist as small isolated populations, it is critical to determine the factors that contributed to their decline and if management actions can be applied to assist in population recovery (Norris 2004). In endangered species populations, typically governed by the stochastic forces of small population dynamics, conservation actions are more likely to be successful when as much as possible is known about the species and population characteristics. Life history, especially reproductive traits and how they vary, is therefore an important component of conservation.

Within life history framework, traits related to reproduction include size at birth; number, size, and sex ratio of offspring; and age and size-specific reproductive investment (Stearns, 1992). Offspring number is an important factor in population dynamics because, coupled with survival rates, it has the potential to impact population growth rate (Beckerman et al. 2002), and its maximum is constrained by female body size (Shine 1988). In organisms that exhibit indeterminate growth, such as reptiles, maximum offspring number is not fixed and can potentially increase throughout an individual's lifetime (Olsson and Shine 1996), possibly conferring a fecundity advantage to larger females. However, a common life history trade-off is size versus the number of offspring, manifesting as fewer large offspring or more small offspring, which may or may not be correlated with female size (Stearns 1992). Larger offspring are theoretically of higher quality and thus have a greater probability of survival (Sinervo et al. 1992), but having more offspring can increase the chance at least some will survive to reproduce (Smith and Fretwell 1974). Snakes are gape-limited predators and thus may have a minimum offspring size below which the neonates would have difficulty foraging successfully. Many pit-vipers feed primarily on mammals (Weatherhead et al. 2009); thus optimal neonate size may be influenced by the size of available prey (King 2002). In addition to manipulating offspring size or number, the sex ratio of offspring produced may also confer benefits to the mother if one sex is likely to contribute disproportionately to overall fitness.

Life history traits are germane to pit viper conservation because female pit vipers experience high costs of reproduction, often losing ≥50% of their body mass post-partum (Shine 2003). As such, many individuals are only able to produce one litter in their lifetime (Shine 2003; Bonnet 2011). This trend toward semelparity makes maximizing fitness through the optimization of offspring number, size, or sex ratios especially important (Shine 1980, 2003;

Bonnet 2011). In endangered species populations, typically governed by the stochastic forces of small population dynamics, conservation actions are more likely to be successful when as much as possible is known about the species and population characteristics. Life history, especially reproductive traits and how they vary, is therefore an important component of conservation.

The Eastern Massasauga (*Sistrurus catenatus*) is an imperiled rattlesnake species. The reproductive cycle begins the year before parturition with vitellogenesis occurring in the summer/fall, follicles overwinter in the ovaries, and ovulation occurs in the spring (Aldridge et al. 2008). Thus, the number of possible offspring is determined prior to ingress. Previous investigations into its reproductive biology have documented litter sizes, ovulation and spermatogenesis, and seasonal mating movements (Jellen et al. 2007; Aldridge et al. 2008; Davis et al. *in review*). However, previous work relied on small sample sizes collected over a short time frame and may not account for temporal variation. Thus with the integration of additional data the objectives of this research were to better determine the reproductive characteristics of the Eastern Massasauga at its southern range limit. Using data from litters born in captivity I investigated bias in offspring sex ratios, constraints on litter characteristics based on female size, and trade-offs between offspring size and number. Given that previous work dealt with a small sample size, I also assessed sampling bias in the data set. Using the larger data set, I was also able to infer selection in offspring characteristics through examination of the size frequency distributions. The results of this study will not only expand knowledge of pit viper life history, but can also be used to inform conservation tools such as population viability analysis.

MATERIALS AND METHODS

Study Species

The Eastern Massasauga is a prairie and bog species endemic to the eastern United States and Canada (Ernst and Ernst 2003). It is proposed for listing as threatened under the Endangered Species Act (USFWS 2015) and as endangered in Illinois (Herkert, 1994), where only one population remains extant, located at Carlyle Lake, Clinton County. Neonate Eastern Massasaugas are extremely difficult to detect in the field following parturition. Thus, all neonates in this study were the result of females that were either implanted with radio-transmitters (2001-2002) or captured via visual encounter survey (VES) 2-8 weeks before parturition (2009-2012) and allowed to give birth in captivity. Captive snakes were housed on newspaper substrate and maintained at a temperature and photoperiod similar to that which they would experience in the field. Following parturition, female snakes and their offspring were released at the female's original capture location.

Statistical Analysis

I performed χ^2 goodness-of-fit tests to determine if there were any significant biases in the sex ratio of offspring by year and overall. In addition, I extended the methodologies outlined in Akçakaya (2002) to partition the variance in offspring sex ratios due to environmental and demographic stochasticity. This is done by calculating the variance due to demographic stochasticity, based on the variance of the binomial distribution, and subtracting it from the total variance to obtain the environmental variance (Akçakaya 2002). To asses if there is selection on offspring size, I used the D'Agostino test for skewness and Anscombe test for kurtosis provided in the R package moments (Komsta and Novometsky, 2015) and the Shapiro-Francia test in the package nortest (Gross and Ligges, 2015) to determine if offspring SVL and mass followed a normal distribution or exhibited significant skewness or kurtosis. I then estimated the probability density function for offspring SVL and mass using the density function in R (R Core Team, 2014) and plotted them.

Next, to evaluate reproductive constraints and trade-offs, I began by summarizing the data by mother to obtain four new litter-specific variables, mean offspring SVL, mean offspring mass, total litter mass, and number of offspring. To assess the potential for selection on offspring characteristics, I used the Shapiro-Francia test for normality. I standardized all litter specific variables using a z-transformation. A relationship between mother SVL and offspring size (SVL or mass) that doesn't increase linearly to infinity would indicate a constraint. Therefore, I used a series of linear regressions to determine if there were constraints on mean offspring SVL, mean offspring mass, total litter mass, and number of offspring related to mother SVL and to identify any trade-offs with litter characteristics. Regressions were conducted for both the overall and sex partitioned data sets. All regressions were conducted in R statistical software, and regression slopes showed little to no bias when compared to bootstrap estimates, suggesting sample sizes were sufficient (Boot function in car package; Fox and Weisberg, 2011). All nominal alpha levels were set at 0.05 unless a Bonferroni penalty was assessed to correct for multiple analyses on the same data set.

RESULTS

General Female and Litter Characteristics

Twenty-seven litters were born in captivity between 2001 and 2012. Total litter size (viable offspring plus non-viable material) ranged from 2-15 with a mean of 7.74 ± 3.27. Viable offspring per litter ranged from 1-15 with a mean of 6.67 ±3.94 (Table 2.1). Individual females produced from 0-8 non-viable embryos per litter. Total reproductive failure occurred in one litter, and non-viable embryos composed 18-80% of litters in which they occurred. Overall, 15% (32/209) embryos were non-viable (Table 2.1). Maternal SVL ranged from 47.4-67.2 with a mean of 57.4 (Table 2.2).

Offspring Sex Ratios

I found offspring sex ratios were biased in some years (Table 2.3). In 2001, 2002, and 2012 more male offspring were born compared to 2009 – 2011 which showed equal offspring sex ratios (Table 2.3). The male bias observed in some years did not result in an overall bias in offspring sex ratios during the study, although it was approaching statistical significance (Table 2.3). My estimates show demographic and environmental stochasticity contribute equally to produce the variation observed in offspring sex ratio (Table 2.3).

Offspring Sizes

Overall, average offspring SVL was 20.5 cm and mass 10.1g when pooling the data from all years (Table 2.4). Female offspring were slightly longer and weighed slightly less than male offspring (Table 2.4). Male mass was skewed with the majority of individuals ranging from 8.9 – 9.5g, and a long tail up to ~14.5g (Table 2.4; Figure 2.1). This skew was not present for female offspring mass but did carry over to the overall offspring data (Table 2.4: Figure 2.1). Female SVL has a significantly peaked distribution exemplified in the range from ~ 20.4 – 22.0cm SVL whereas male SVL and the overall data do not (Table 2.4: Figure 2.1). Considering this, the distributions of overall SVL and mass, female SVL and mass, and male mass were not normally distributed and may be under stabilizing (SVL) or directional (mass) selection (Table 2.4; Figure 2.1).

Assessment of Reproductive Constraints

The litter variables of mother SVL, mean offspring SVL, and mean offspring mass fit a normal distribution for the overall litter data set and when partitioned by offspring sex (Table 2.5). Total litter mass and number of offspring fit a normal distribution for the overall data and for male offspring, but were not normally distributed for female offspring (Table 2.5). There was little to no bias present in regression slopes when compared to the bootstrap estimates suggesting samples sizes were sufficient (Table 2.6). After Bonferroni penalty accounting for multiple tests, I found offspring mass, offspring SVL, litter mass, and litter size are not associated with mother SVL (Table 2.6; Figure 2.2).

Assessment of Reproductive Trade-offs

I did not find any evidence of trade-offs in litter characteristics but in some cases found signals of positive allometry (Table 2.7; Figure 2.3). When correcting for multiple tests, on average, longer offspring were heavier for the overall data set and for male offspring (Table 2.7; Figure 2.3). The only consistent association among the overall and sex partitioned data sets was litters having more offspring had higher litter masses (Table 2.7; Figure 2.3). This suggests that smaller litters were not composed of heavier offspring.

DISCUSSION

Reproductive Life History Traits

The Eastern Massasauga is a conservation priority throughout its range, and as such, documenting range-wide variation in reproductive traits is informative for conservation tools such as population viability analysis (PVA). Tools such as PVA require estimates of litter size and offspring sex ratios, and their accuracy could be reduced if the full range of variation in reproductive traits is unknown. Estimates of litter size in the Eastern Massasauga show a positive relationship with latitude, ranging from 7.04 at Carlyle Lake (the southern range limit) to 13.33 at the northern range limit in Ontario (Parent and Weatherhead 2000). Offspring sex ratios do not significantly differ from 1:1 throughout the range (Ernst and Ernst 2011).

Examining the frequency distribution of neonate SVL shows a peaked distribution with narrow tails which could indicate neonate SVL is under stabilizing selection (Estes and Arnold 2007). The frequency distribution for mass appears skewed possibly suggesting directional selection toward lighter offspring (Estes and Arnold 2007). This could indicate less of an investment by the female in yolk size of follicles. Artificial removal of yolk from fence lizard (*Sceloporus occidentalis*) eggs resulted in significant decrease of up to two-fold in hatchling weight (Sinervo 1990). Since energy available to females to invest in reproduction is finite and must be distributed among all life processes (Congdon et al. 1982), having longer, lighter offspring may represent the optimal compromise between offspring size and female investment.

Size Constraints

Maternal SVL does not appear to influence litter size, mean offspring SVL or mean offspring mass. This result was surprising as larger females are presumed to have the capacity to have larger litters (Shine 1980). However, maternal SVL was found to be significantly and positively correlated with litter size at the Eastern Massasauga's northern range limit in Canada (Parent and Weatherhead 2000), in populations of Western Massasaugas (*S. c. tergeminus*; formerly considered Eastern) in Missouri (Seigel 1986), and in the closely related Pygmy Rattlesnake (*S. miliarius barbouri*) in Florida (Farrell et al. 1995). Previous data collected for the

Carlyle Lake population of Eastern Massasuagas similarly found no significant correlation between maternal SVL and litter size (Aldridge et al. 2008). Litter size has likewise been reported to increase with female size in the Ridge-nosed Rattlesnake (*C. willardi*; Holycross and Goldberg 2001) and the Northern Pacific Rattlesnake (*C. oreganus*; Diller and Wallace 1984). In the similarly sized *V. aspis,* a positive correlation between maternal SVL and litter size was reported in Italy (Luiselli and Zuffi 2002) but litter size in a French population was driven by prey availability in nearly all years (Lourdais et al. 2002). As reproduction in snakes is highly dependent upon resource acquisition (Naulleau and Bonnet 1996), female size need only constrain maximum litter size if females delay reproduction until they have enough energy stores to produce the maximum number of offspring (Shine 2003). It therefore appears at the southern range limit of the Eastern Massasauga, larger body size does not confer a fecundity advantage, and the system may instead be driven by prey availability.

Because gravid females were either part of a telemetry study or housed in captivity for a period of time, it is possible that capture and captivity stress coupled with exposure to artificial conditions impacted the size of their offspring (Farr and Gregory 1991; Cree et al. 2003), obscuring the litter size/SVL relationship. Indeed, Seigel (1986) found neonate Massasaugas from Missouri born in captivity were smaller than those captured in the wild, but could not determine if wild individuals had grown prior to capture. Mother's time in captivity appeared to have no effect on neonate size of Eastern Massasaugas from Wisconsin (Keenlyne and Beer 1973).

When examining the range of maternal SVLs (47.4-67.2), only 20cm separates the smallest female from the largest, and most females fall within 10cm of each other. The largest female ever captured at Carlyle Lake measured 73.1cm, and 47.4cm represents the smallest female known to have given birth. Therefore, we are confident that this study captures the range of adult female sizes in the population. Although growth in snakes is theoretically indeterminate (Kozlowski 1996), given limited energetic resources, once a female reaches reproductive size more energy is allocated to reproduction and diverted away from growth (Zuo et al. 2011) potentially leading to a narrow range of female body sizes. Female growth rates in the Carlyle Lake population have been shown to be rapid until the approximate age of sexual maturity (Dreslik et al. *in press*). Additionally, experimental reduction of lizard body volume resulted in only a minute reduction in litter size, showing the determination of litter size is complicated and dependent upon many factors besides maternal size (Du et al. 2005). As Eastern Massasaugas display considerable variation in offspring number (2-15), this is further evidence that reproductive output is dependent more on individual females' ability to gather sufficient resources than space available to hold offspring.

Any additional energetic demands placed on a female could decrease the amount of energy she can allocate to reproduction, possibly decoupling the maternal size-offspring number relationship. Upregulation of the immune system is necessary in the presence of pathogens and could be influencing female reproductive investment. Polyandrous female *Zootoca vivipara* lizards were found to produce lower quality litters in favor of higher investment in immune function to compensate for greater exposure to pathogens than monandrous individuals (Richard et al. 2012). Female Eastern Massasaugas often mate with multiple males (Jellen et al. 2007), thereby increasing pathogen exposure in the form of an emerging infectious disease (*Ophidiomyces ophidiicola*; snake fungal disease) that has been present in the population at least since 2000 (Allender et al. 2016). If pathogen exposure is increasing energetic demands of the immune system in Eastern Massasaugas, this could be reflected as a decrease in number or quality of offspring. Correspondingly, stochastic disruptions in prey availability reduce the total amount of energy available to females which also constrains them from reproducing at the highest possible capacity.

The metabolic costs of vitellogenesis in viviparous snakes have been found to be significant, resulting in a 30% increase in female metabolic rate across five species (Van Dyke and Beaupre 2011). Therefore, as offspring size is determined by the amount of energy obtained from yolk and energy content of yolk is determined during vitellogenesis (Van Dyke and Beaupre 2011), energy available to the female immediately before vitellogenesis is likely the best predictor of offspring number and size. In systems where the decision to reproduce or not is determined by a minimal body condition threshold (Naulleau and Bonnet 1996) rather than delayed until females have acquired enough energy for maximum reproductive output (Shine 2003), little opportunity for a fecundity advantage of larger body size remains. This scenario applies to viviparous lizards (Doughty and Shine 1998), and the Aspic viper where reproductive output is determined by resources availability at different times during the reproductive cycle with little influence of female body size (Bonnet et al. 2001).

Offspring Size Trade-Offs

I found no trade-offs within litters between offspring size and mass, indicating females that have smaller litters do not produce larger offspring. No offspring size/number trade-offs were found for the closely related Pygmy Rattlesnake (*S. miliarius barbouri*) in Florida (Farrell et al. 1995), or the similarly sized viviparous smooth snake (*Coronella austriaca*; Luiselli et al. 1996). However, trade-offs were detected in the Brown Snake (*Storeria dekayi;* King 1993) and have been consistently observed in the viviparous lizard *Eulamprus tympanum* (Doughty and Shine 1998). A clear trade-off is present in an island population of the Shedao Pit-viper (*Gloydius shedaoensis*) where there is strong selection for large offspring size because of the large size of available prey (Sun et al. 2002). However, that population is also unusual in that the snakes have no predators on the island (Sun et al. 2002). Maternal stress levels (measured as plasma corticosterone levels) during gestation can influence offspring size at birth in skinks (*Pseudemoia entrecasteauxii*; Itonaga et al. 2012), which may serve to decouple any relationship between offspring number and size within a litter. Therefore, factors that contribute to the presence of offspring size-number trade-offs are likely to be species and system specific, influenced by life history and selective pressures.

In addition to maternal constraints, offspring size may be further influenced by the size of available prey, as snakes are gape-limited predators. Other members of *Sistrurus* consume large proportions of lizards and centipedes, but these items are not available to Eastern Massasaugas at Carlyle Lake where small mammals are the most abundant prey item. Although neonate Eastern Massasaugas will readily consume other snakes in captivity, wild individuals prefer to prey on small rodents at Carlyle Lake (Shepard et al. 2004) as well as elsewhere in their range (Holycross and Mackessy 2002; Weatherhead et al. 2009). Unlike farther north in the Eastern Massasauga’s range, southern short-tailed shrews (*Blarina carolinensis*) are available at Carlyle Lake and are the preferred mammalian prey of neonates as they are smaller overall with narrower heads than sympatric mouse (*Peromyscus*) species (Shepard et al. 2004). Given that small mammals are generally larger than the juvenile *Thamnophis* or *Storeria* that are available to Eastern Massasaugas and readily eaten in captivity (Shepard et al. 2004), the ability for neonates to eat fewer, larger mammals rather than many smaller snakes could influence juvenile size (Hamilton et al. 2012). Available prey can impact morphology, as documented in European adders (*Vipera berus*; ecologically similar to Eastern Massasaugas), where the size of available prey creates inter-populational differences in relative head size (Forsman 1991). Thus, the size of neonate Eastern Massasaugas in Illinois may be optimized for successful foraging on mammals thus constraining their minimum size. Although other members of the genus *Sistrurus* do have smaller neonates than the Eastern Massasauga, these species live in habitats where invertebrate or lizard prey is abundant (Ernst and Ernst 2011). As I also found neonate mass to be skewed left toward lighter offspring, the availability of mammalian prey early in life may allow females to reduce their investment in follicle yolk resources. Thus, longer, lighter neonate Eastern Massasaugas may represent optimality, requiring less energetic input by females while still having heads large enough to consume mammalian prey.

Predation risk to neonates could also be a contributing factor to the lack of a trade-off in offspring size and number. In Illinois, survival is highly variable between years with a mean survival probability of neonates to adulthood of 0.503 for females and 0.471 for males (Davis et al. *in review*). Predation is the second most common cause of mortality in Eastern Massasaugas at Carlyle Lake (Chapter 3). Having fewer, larger offspring may not be beneficial as Eastern Massasaugas co-exist throughout their range with other grassland species that are known to be ophiophagus. In Illinois, Prairie Kingsnakes (*Lampropeltis calligaster*) and Blue Racers

(*Coluber constrictor*), have been documented to consume neonate Eastern Massasaugas (*Pers. obs.*), and avian and mammalian predators also occur in grassland habitats. Therefore, given that only about 50% of neonates are expected to make it to adulthood, Eastern Massasaugas may benefit from producing the maximum number of offspring that can still successfully prey on mammals, rather than fewer larger offspring as they would not be large enough to avoid predation risk.

Conclusions

A solid understanding of variation in life history traits of threatened and endangered species is necessary for conservation as these traits play a significant role in population dynamics. Current rates of habitat fragmentation and species loss have highlighted the importance of identifying those life history traits that most influence extinction risk for use in conservation planning (Foufopoulos and Ives 1999). Knowing that litter size is not constrained by female size, but rather potentially dependent upon prey availability and acquisition means conservation plans that include steps to maintain an abundant small mammal population could increase number of offspring born annually. As no trade-offs were present, females with more energy reserves to allocate to reproduction are unlikely to just make larger neonates as opposed to more. As offspring size at Carlyle Lake might be related to consumption of mammalian prey, managing to ensure healthy populations of southern short tailed shrews could be beneficial for neonates. Habitat restorations should account for prey base. Any potential reintroduction programs should include small mammal population surveys of the sites to ensure adequate suitable prey for both reproductively mature females and neonates.

Data from studies such as this one are invaluable to obtain the most accurate results from conservation tools such as population viability and sensitivity analyses. If reproductive traits are found to play a significant role in population viability, understanding constraints and trade-offs operating in the population will serve to assist managers in determining feasible conservation actions. For example, supplemental feeding to increase litter size or more focused management of prey sources for neonates. Given the synergistic nature of the processes contributing to small population persistence (Brook et al. 2008), conservation will best be achieved with a thorough understanding of population parameters, including life history.

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Tables and Figures

Table 2.1: Litter size estimates and offspring sex ratios for 27 adult female Eastern Massasaugas (*Sistrurus catenatus*) by year and including all vitlellogenic follicles (Vit.), later stage development terminated offspring (Part. Dev.), stillborn, and live offspring from Carlyle Lake, Clinton County, Illinois.

Year/	**Live Offspring**			**Sex Ratio**		**Total Patriated**				
Snake	**♀♀**	**♂♂**	**Unk.**	**Prop. ♀**	**Prop. ♂**	**Vit.**	**Part. Dev.**	**Stillborn**	**Live**	**Total**
2001										
35	---	9	---	0.00	1.00	2	---	---	9	11
131	---	1	---	0.00	1.00	---	---	1	1	2
132	2	6	---	0.25	0.75	---	---	---	8	8
162	8	4	---	0.67	0.33	---	---	---	12	12
174	2	3	---	0.40	0.60	---	---	---	5	5
192	1	6	---	0.14	0.86	2	---	1	7	10
2002										
113	3	6	---	0.33	0.67	---	---	---	9	9
164	1	1	---	0.50	0.50	1	---	---	2	3
306	---	3	---	0.00	1.00	1	---	---	3	4
335	---	2	---	0.00	1.00	3	3	2	2	10
2009										
458	4	---	---	1.00	0.00	---	---	---	4	4
617	3	5	---	0.38	0.63	---	---	---	8	8
624	4	6	---	0.40	0.60	---	---	---	10	10
687	2	---	1	1.00	0.00	3	---	---	2	5
2010										
540	6	3	---	0.67	0.33	---	---	---	9	9
554	4	10	---	0.29	0.71	---	---	---	14	14
588	8	7	---	0.53	0.47	---	---	---	15	15
615	6	3	---	0.67	0.33	---	---	---	9	9
703	---	---	3	-----	-----	4	---	1	---	5
2011										
639	4	5	---	0.44	0.56	---	---	---	9	9
701	3	3	---	0.50	0.50	---	---	---	6	6
772	5	1	---	0.83	0.17	---	---	---	6	6
791	5	6	---	0.45	0.55	---	---	---	11	11
2012										
837	1	3	---	0.25	0.75	---	---	---	4	4
765	1	4	---	0.20	0.80	2	---	---	5	7
883	---	7	---	0.00	1.00	2	---	---	7	9
826	1	2	---	0.33	0.67	2	---	2	3	7
Total	**74**	**106**	**4**	**0.41**	**0.59**	**22**	**3**	**7**	**180**	**209**
Mean	**2.74**	**3.93**	**0.15**	**-----**	**-----**	**0.81**	**0.11**	**0.26**	**6.67**	**7.74**
Stdev	**2.44**	**2.70**	**0.60**	**0.29**	**0.29**	**1.21**	**0.58**	**0.58**	**3.93**	**3.27**

Table 2.2: Mean offspring sizes (snout-vent length-SVL: mm, mass: g), total litter mass (g), and number of offspring, associated with mother SVL for female Eastern Massasaugas (*Sistrurus catenatus*) from Carlyle Lake, Clinton County, Illinois.

Mother		Offspring SVL		Offspring Mass			
ID	SVL	Mean	Std. Dev.	Mean	Std. Dev.	Total Litter Mass	# Offspring
35	57.3	18.7	0.63	9.9	0.96	89.2	9
113	59.5	18.6	0.40	9.1	0.29	82.0	9
131	53.6	18.6	2.83	8.8	0.35	17.5	2
132	62.3	21.1	0.56	9.7	0.50	77.6	8
162	59.2	20.7	0.50	10.0	0.43	109.9	11
164	61.7	19.9	0.00	6.7	9.40	13.3	2
174	58.8	22.5	0.52	11.3	0.25	56.3	5
192	55.5	19.4	0.62	10.6	0.53	73.9	7
306	57.5	19.8	0.42	8.0	6.95	24.0	3
335	56.3	19.4	0.28	9.1	0.35	18.1	2
458	67.2	18.4	0.71	8.9	1.47	35.5	4
540	62.7	22.3	0.67	11.6	0.96	104.4	9
554	61.7	20.6	0.52	8.6	0.74	121.0	14
588	60.8	20.9	0.64	9.3	0.48	138.9	15
615	54.7	20.9	0.36	8.6	0.17	77.4	9
617	57.5	21.5	1.14	11.9	1.57	71.4	6
624	59.2	20.3	0.53	11.0	0.87	109.8	10
639	60.0	22.6	0.41	12.3	0.50	111.0	9
701	57.6	20.2	0.34	9.5	0.55	57.0	6
765	52.7	20.5	0.50	11.2	0.84	56.0	5
772	57.2	20.9	0.79	10.5	0.84	63.0	6
791	54.9	21.8	1.38	10.0	0.94	100.0	10
826	50.5	17.2	0.15	8.7	0.58	26.0	3
837	50.1	19.6	0.40	11.3	0.50	45.0	5
883	47.4	20.0	0.92	10.7	0.49	75.0	7
Grand Mean	**57.4**	**20.2**	**1.3**	**9.9**	**1.4**	**70.1**	**7.04**

Table 2.3: Overall offspring sex ratios by year, χ^2 goodness-of-fit tests, and estimations of the percent environmental and demographic variance for 27 litters of Eastern Massasaugas (*Sistrurus catenatus*) from Carlyle Lake, Clinton County, Illinois.

Year	**♀♀**	**♂♂**	**Unk.**	**Total**	**p ♀**	**p(1-p)**	**p*Total**	χ^2	***p***
2001	**13**	**29**	**---**	**42**	**0.310**	**0.214**	**0.28**	**6.10**	**0.014**
2002	**4**	**12**	**---**	**16**	**0.250**	**0.188**	**0.32**	**4.00**	**0.046**
2009	13	11	1	25	0.520	0.250	0.41	0.17	0.683
2010	24	23	3	47	0.511	0.250	0.67	0.02	0.884
2011	17	15	---	32	0.531	0.250	0.62	0.13	0.724
2012	**3**	**16**	**---**	**27**	**0.111**	**0.099**	**2.12**	**8.89**	**0.003**
Total	**74**	**106**	**4**	**189**	**0.392**	**0.901**	**1.68**	**3.42**	**0.064**

Average Proportion of Females(Weighted)	0.392
Total Variance of Proportion of Females (Weighted)	0.009
Demographic Variance (Weighted)	0.005
Environmental Variance	0.004
% Demographic	53.5%
% Environmental	46.5%

Table 2.4: Distributional statistics for offspring snout-vent length (SVL) in cm and mass in grams partitioned by sex and for the overall data set for Eastern Massasauga (*Sistrurus catenatus*) litters from Carlyle Lake, Clinton County, Illinois. Data includes the mean standard error, 95% confidence intervals, skewness, kurtosis, and sample sizes. Skewness was tested using D'Agostino test, kurtosis using the Anscombe-Glynn test, and normality using the Shapiro-Francia test in the R packages moments and nortest. Significant results are bolded.

	Mean	**SE**	**95% C.I.**	**Skew**	***Z***	***p***	**Kurt**	***Z***	***p***	**Norm**	***p***	***n***
Overall												
SVL	20.52	0.104	20.32, 20.73	-0.06	-0.31	0.753	3.68	1.76	0.079	**0.984**	**0.039**	**175**
Mass	10.13	0.101	9.93, 10.33	**0.44**	**2.38**	**0.017**	3.09	0.51	0.610	**0.974**	**0.004**	**173**
Females												
SVL	20.77	0.194	20.38, 21.16	-0.09	-0.31	0.759	**4.50**	**2.14**	**0.033**	**0.959**	**0.040**	**60**
Mass	10.03	0.169	9.69, 10.37	0.48	1.61	0.108	2.17	-1.82	0.069	**0.943**	**0.009**	**60**
Males												
SVL	20.31	0.123	20.07, 20.55	-0.05	-0.21	0.837	2.87	0.00	0.998	0.990	0.565	104
Mass	10.21	0.125	9.96, 10.46	**0.50**	**2.08**	**0.038**	3.61	1.42	0.156	**0.964**	**0.008**	**102**

Table 2.5: Normality tests for the litter characteristics of mother snout-vent length (SVL), mean offspring SVL, mean offspring mass, total litter mass, and number of offspring for Eastern Massasauga (*Sistrurus catenatus*) litters from Carlyle Lake, Clinton County, Illinois. Results include the overall data set and partitioned by offspring sex. Normality was tested using the Shapiro-Francia test in the R package nortest. Significant results are bolded.

	Mother SVL	**Mean Offspring SVL**	**Mean Offspring Mass**	**Total Litter Mass**	**Number of Offspring**
Overall					
Test Stat	0.975	0.983	0.978	0.975	0.958
p	0.692	0.871	0.754	0.669	0.326
Females					
Test Stat	0.977	0.937	0.956	**0.867**	**0.848**
p	0.805	0.169	0.374	**0.010**	**0.005**
Males					
Test Stat	0.954	0.952	0.984	0.975	0.928
p	0.280	0.254	0.908	0.723	0.084

Table 2.6: Regression results for mother to offspring relationships in, mean offspring snout-vent length (mm), mean offspring mass, total litter mass, and number of offspring for Eastern Massasauga (*Sistrurus catenatus*) litters from Carlyle Lake, Clinton County, Illinois. Mean litter SVL, mass and total litter mass are also partitioned by offspring sex. All variables were z-transformed and when using a Bonferroni correction the nominal α = 0.05/12 = 0.004.

			Overall			
Dependent	r^2	β	**95% C.I.**	***Bias***	p	n
Mean Offspring SVL	0.022	0.251	-0.166, 0.669	0.026	0.226	25
Mean Offspring Mass	-0.015	-0.165	-0.591, 0.260	0.014	0.430	25
Total Litter Mass	0.029	0.264	-0.152, 0.680	0.032	0.202	25
Litter Size	0.036	0.276	-0.139, 0.690	0.033	0.182	25
			Females			
Mean Offspring SVL	0.053	0.317	-0.139, 0.772	0.014	0.162	21
Mean Offspring Mass	-0.044	0.093	-0.385, 0.571	0.016	0.688	21
Total Litter Mass	0.027	0.276	-0.186, 0.737	0.008	0.226	21
Litter Size	0.019	0.260	-0.203, 0.724	-0.001	0.255	21
			Males			
Mean Offspring SVL	0.135	0.416	0.014, 0.818	0.000	0.043	24
Mean Offspring Mass	-0.047	0.027	-0.431, 0.485	0.015	0.903	23
Total Litter Mass	-0.002	0.210	-0.238, 0.658	0.004	0.342	23
Litter Size	-0.023	0.146	-0.291, 0.584	0.029	0.495	24

Table 2.7: Interrelationships of litter characteristics for Eastern Massasauga (*Sistrurus catenatus*) litters from Carlyle Lake, Illinois. When using a Bonferroni correction the nominal α = 0.05/6 = 0.008, significant relationships are bolded.

Dependent	Independent	r^2	*β*	95% C.I.	*Bias*	*p*	*n*
			Overall				
Mean Offspring SVL	**Mean Offspring Mass**	**0.257**	**0.536**	**0.172, 0.900**	**0.014**	**0.006**	**25**
	Total Litter Mass	**0.243**	**0.524**	**0.156, 0.891**	**0.001**	**0.007**	**25**
	Number of Offspring	0.132	0.410	0.017, 0.804	0.009	0.042	25
Mean Offspring Mass	Total Litter Mass	0.134	0.412	0.019, 0.805	0.000	0.041	25
	Number of Offspring	0.004	0.214	-0.208, 0.635	0.015	0.305	25
Total Litter Mass	**Number of Offspring**	**0.936**	**0.969**	**0.862, 1.076**	**0.009**	**<0.001**	**25**
			Females				
Mean Offspring SVL	Mean Offspring Mass	0.170	0.460	0.034, 0.887	0.015	0.036	21
	Total Litter Mass	0.054	0.318	-0.137, 0.774	0.019	0.159	21
	Number of Offspring	0.017	0.257	-0.207, 0.721	0.011	0.261	21
Mean Offspring Mass	Total Litter Mass	-0.047	-0.074	-0.553, 0.405	-0.007	0.749	21
	Number of Offspring	-0.003	-0.217	-0.686, 0.252	-0.006	0.345	21
Total Litter Mass	**Number of Offspring**	**0.966**	**0.984**	**0.898, 1.070**	**0.002**	**<0.001**	**21**
			Males				
Mean Offspring SVL	**Mean Offspring Mass**	**0.263**	**0.555**	**0.167, 0.943**	**-0.006**	**0.007**	**23**
	Total Litter Mass	-0.047	0.024	-0.438, 0.487	0.029	0.914	23
	Number of Offspring	-0.042	-0.054	-0.495, 0.388	0.013	0.804	24
Mean Offspring Mass	Total Litter Mass	-0.001	0.212	-0.232, 0.655	0.028	0.332	23
	Number of Offspring	-0.047	-0.012	-0.479, 0.455	0.038	0.958	23
Total Litter Mass	**Number of Offspring**	**0.940**	**1.000**	**0.888, 1.111**	**0.015**	**<0.001**	**23**

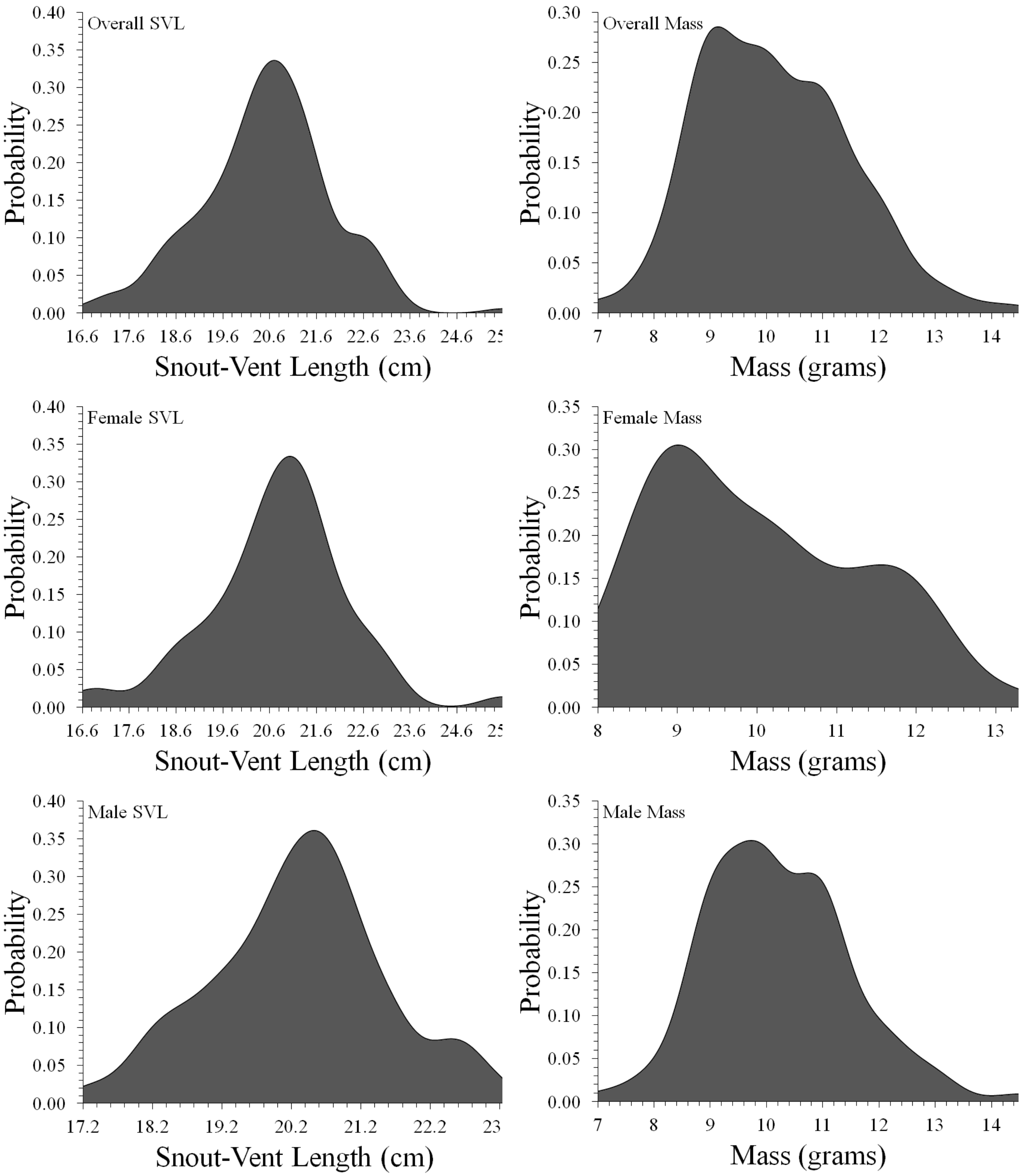

Figure 2.1: Distributions of overall, male, and female snout-vent length (SVL) and Mass of neonate Eastern Massasaugas from the Carlyle Lake, Illinois population from 27 litters born in captivity between 2001-2012.

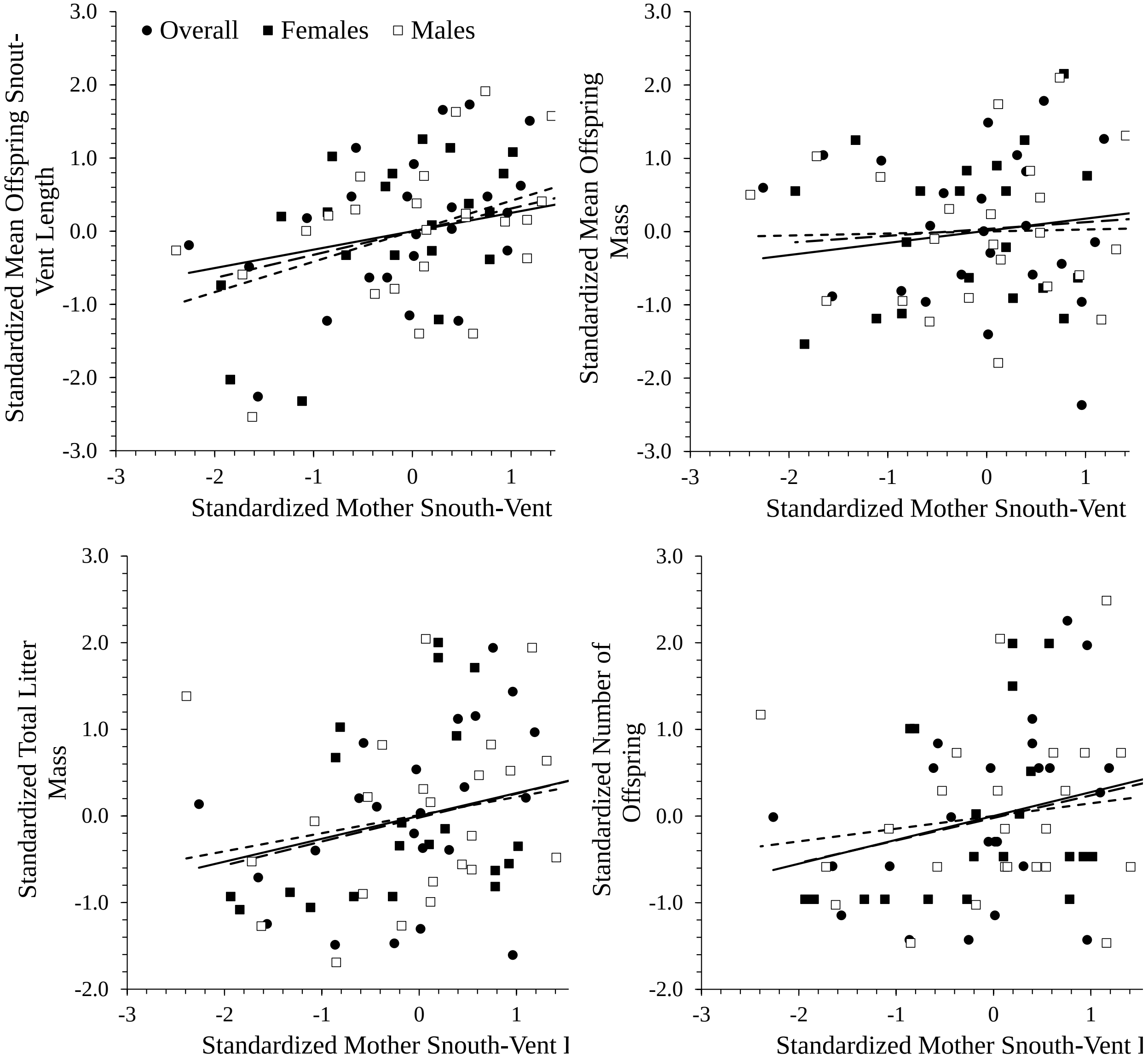

Figure 2.2: Regressions of standardized mean offspring snout-vent length, mean offspring mass, total litter mass, and number of offspring against standardized mother snout-vent length from 27 litters of Eastern Massasaugas from Carlyle Lake, Illinois

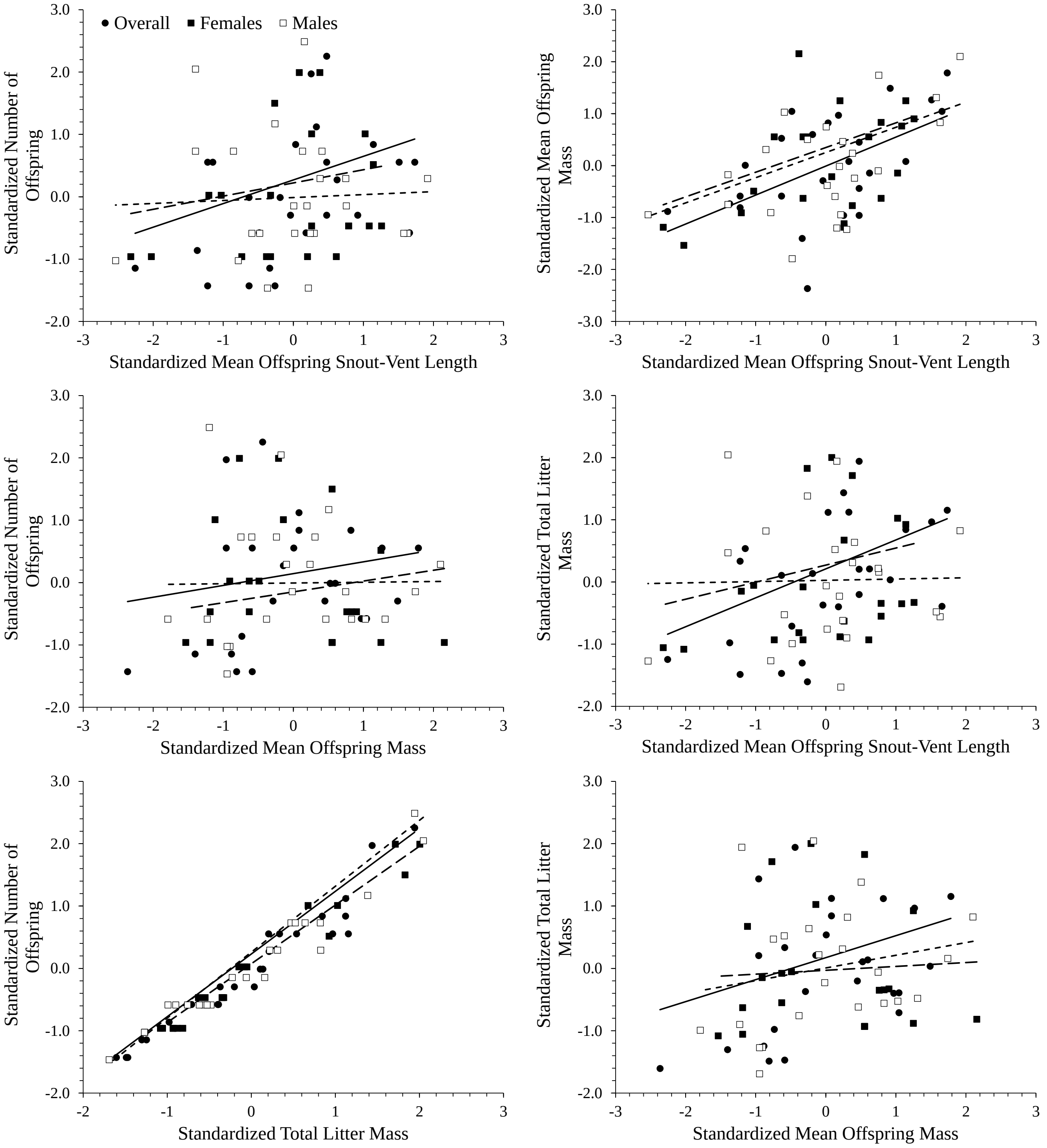

Figure 2.3: Regressions of offspring characteristics collected from 27 captive born litters of Eastern Massasaugas from Carlyle Lake, Illinois.

CHAPTER THREE

SOURCES OF MORTALITY IN THE ENDANGERED EASTERN MASSASAUGA (*SISTRURUS CATENATUS*) IN ILLINOIS

ABSTRACT

The identification and management of threats to endangered species are imperative for conservation. Reptile populations are declining globally, yet their secretive nature and a lack of demographic data often make the implementation of conservation strategies difficult. The Eastern Massasauga (*Sistrurus catenatus*) has been recommended for listing as threatened under the federal endangered species act and listed in Illinois as endangered since 1994. As part of a long-term population monitoring project, we quantified sources of mortality in a population of the Eastern Massasauga using data collected from 2000–2011. Using mortality data amassed during both observational and radio-telemetry studies (2001–2003 and 2009–2011), we classified each mortality event into one of eleven categories. We used Poisson regression to evaluate the impacts of sex, stage-class, season, and study type on mortality. Our results indicate automobiles and predation are the leading sources of mortality, together accounting for over 50% of observed mortalities. We found season and mortality category significantly affected the number of observed mortalities. Most mortalities occur in the summer and fall when snakes are most active. Observational studies detected nearly all the automobile mortality while radio-telemetric studies detected nearly all the predation events. Conservation and management recommendations are offered to reduce Eastern Massasauga mortality at Carlyle Lake and are applicable throughout the range.

INTRODUCTION

Conservation of endangered species relies heavily on our ability to identify causes of population decline, past, present, and future, and effectively manage threats (Norris 2004). In the case of worldwide reptile populations, six significant threats have been identified: habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change (Gibbons et al. 2000). For snakes, habitat loss and fragmentation have been cited as the most significant population threats (Dodd 2001). Human-induced changes in the landscape can impact mortality rates directly but may also have other effects. Rattlesnakes living in disturbed habitats (such as those altered for human use) have been found to display poorer body condition than those living in less disturbed areas (Jenkins et al. 2009). Poor body condition can lead to decreased fecundity, increased susceptibility to disease, and mortality (Naulleau and Bonnet 1996; Metcalfe and Monaghan 2001; Beldomenico et al. 2008). Thus, when managing individual populations of a single species, it is necessary to determine the exact mechanisms by which mortality is occurring to combat significant threats most effectively.

Mortality risk may differ between sexes, may change ontogenetically, or may vary seasonally (Plummer 1990; Mushinsky and Miller 1993). If mortality is biased toward one sex or age class it could reduce recruitment and impact population dynamics (Mushinsky and Miller 1993). Seasonal mortality differences in temperate reptiles may be expected because of differing threats during the active/inactive seasons (Sperry et al. 2010). Venomous snakes may be subject to further anthropogenic mortality sources given the high degree of ophiophobia in the general public (Burghardt et al. 2009).

The Eastern Massasauga (*Sistrurus catenatus*) is a small rattlesnake which ranges from Ontario, Canada, to southern Illinois, USA, and extends east to northwestern Pennsylvania and

New York, USA. It occurs primarily in wet prairie or marsh/bog habitat (Ernst and Ernst 2003). The Eastern Massasauga was added to the Illinois state endangered species list in 1994 (Herkert 1994) and has been recommended for listing as threatened under the federal Endangered Species Act (U.S. Fish and Wildlife Service [USFWS] 2015). The largest remaining Illinois population occurs on a small area of highly fragmented habitat surrounding Carlyle Lake, Clinton County (see Dreslik 2005 for a detailed description of study site). We have been monitoring the population continuously since 1999, and using the resulting long-term data set, we identified the most significant sources of mortality and tested for significant differences due to sex, stage-class, season, and study design. This information can be used when making management decisions to allocate limited time and money toward reducing the most significant threats.

MATERIALS AND METHODS

We collected dead snakes from the Carlyle Lake population in Clinton County, Illinois, from 2000–2011 while conducting visual encounter surveys (VES), radio-telemetry (2001–2003 and 2009–2011), and while driving on roads. We classified study type as telemetry for mortality of individuals implanted with radio-transmitters and observational for mortalities encountered during VES and while driving on roads. For each dead individual, we recorded date, GPS coordinates, stage (adult or juvenile), sex (when possible), and determined if it was an individual we had captured previously. We determined sex by subcaudal scale count, cloacal probing, or by the presence of visible everted hemipenes. Stage-class was categorized as either adult or juvenile based on the minimum size of reproduction for males (49.7cm; Jellen et al. 2007) and females (48.4cm; Dreslik et al., In press). We classified season by periods of biological significance rather than calendar seasons. Thus, we considered the spring egress period (March to April) as Spring; end of egress to end of birthing season as Summer (May to August); post birthing season to ingress as Fall (September to November); and Winter we considered as December to February, the months most snakes are typically hibernating. We classified each mortality as one of 11 types (Table 1). All salvaged individuals were deposited in the Illinois Natural History Survey Amphibian and Reptile Collection.

To determine what factors influenced the number of observed mortalities, we collapsed our original 11 categories into five based on similarity. The five simplified categories are Automobile, Predation, Illness (combined died in captivity, euthanized, illness), Human (combined management and persecution), and Other (combined other and unknown). We omitted stillborn and surgical complications from analysis because they are unique to specific stage classes. We used Poisson regression in R (R Core Team 2015) to determine if sex, stage-class, season, or study type (telemetry or observational) significantly impacted observed mortality. We evaluated candidate models using AIC (Burnham and Anderson 2002) and included a null model (intercept only), a global model (all main effects), all single effects models, and all two effects models.

RESULTS

We recorded 156 instances of mortality over 11 years. Of these, 68 could be positively identified as males and 65 as females. We were able to classify 79 individuals as adults and 70 as juveniles. We encountered 70 mortalities during radio-telemetric studies and 85 during observational studies. There were 27 mortalities observed in the Spring, 64 during the Summer, 54 during the Fall, and nine during the Winter (Table 3.1). Overall, automobiles were the leading cause of mortality, accounting for 32% of all observed mortalities. Predation was the second greatest threat, accounting for 22% of observed mortality. All other categories each contributed < 15% to overall mortality (Table 3.2; Fig. 3.1). Although observed mortalities from automobiles and predation were overall numerically similar, they were detected disproportionally depending on study type. Automobile mortality accounted for only 1% observed during telemetry studies, and predation accounted for only 1% of mortalities observed during observational studies (Fig. 3.1). Of the 17 candidate models evaluated, the top model included terms for season and mortality type and carried an Akaike weight of 0.77 (Table 3.3; model parameter estimates Table 3.4). Seasonally, mortality is more likely to occur in the summer and fall, and automobiles cause the highest number of mortalities (Table 3.2). However, adults and juveniles die in equal numbers, as do males and females. Type of study being conducted does not change the number of mortalities detected, but it does change the type of mortality most likely to be observed (Fig. 3.1).

DISCUSSION

Sources of mortality

Our study shows automobiles are a significant threat to the Eastern Massasauga population at Carlyle Lake. Snakes may be found on roads either because they are using them to thermoregulate or crossing them to move between habitat areas. Roads may be tempting basking sites for snakes because the pavement absorbs heat quickly and retains it longer than the surrounding environment (Shine et al. 2004). Experiments using Timber Rattlesnakes (*Crotalus horridus*) have found venomous snakes cross roads more slowly than non-venomous species and also become immobile when vehicles approach (Andrews and Gibbons 2005). Additionally, the number of roads present at Carlyle Lake and the high traffic volume (the area receives 700,000 to 1,000,000 visitors annually) likely contribute to the number of automobile deaths. Snakes are abhorred by much of the general public, and a study using model snakes placed on roads found most people will go out of their way to purposely run over snakes when they encounter them (Langley et al. 1989; Ashley et al. 2007).

The high degree of habitat fragmentation created by roads around Carlyle Lake means snakes are either forced to cross roads when searching for mates, suitable foraging, and hibernation sites or remain restricted to one habitat patch. Crossing roads could result in mortality while staying in one habitat patch restricts gene flow between patches, which may reduce population viability (Epps et al. 2005; Shepard et al. 2008b). Studies of reptile road mortality around Carlyle Lake found road mortality of Eastern Massasaugas was biased towards males, the highest mortality rates coincided with the mating season (Aldridge et al. 2008; Shepard et al. 2008a), and Eastern Massasaugas crossed roads less frequently than expected by chance (Shepard et al. 2008b). Roads also increase the amount of edge habitat present at a site. The density of mesopredators, such as Raccoons (*Procyon lotor*) and Long-tailed Weasels (*Mustela frenata*), has been shown to be positively correlated with the amount of edge habitat (Gehring and Swihart 2003), possibly increasing predation risk.

Predation was the second most common cause of mortality in all groups, although predation is likely underrepresented in our data. In the case of radio-implanted individuals, if the predator eats the entire snake and moves out of range, we cannot confirm the fate, and for non-telemetered animals, evidence of predation is not available. It is also difficult to locate partially consumed animals in grassland habitat during VES surveys, and clearly, we do not find the majority of carcasses. Potential predators present at Carlyle Lake include small mammals, birds of prey, and other snakes. Mammals, including Coyotes (*Canis latrans*) and feral House Cats (*Felis catus*), have been positively identified as predators of snakes (Whitaker and Shine 2000;

Kapfer et al. 2008), and while other small carnivores also likely consume snakes, they could be difficult to identify unless the predation event is witnessed. Four confirmed cases of predation of Eastern Massasaugas by other snake species have been observed at Carlyle Lake, indicating other snakes present at the site may represent a significant threat to Eastern Massasaugas (Sarah Baker and Daniel Wylie, pers. obs.). Predation by other snakes has also been observed in Green Snakes (*Opheodrys aestivus*; Plummer 1990), and was suggested as a possible factor in the documented decline of the Eastern Kingsnake (*Lampropeltis getula*) at a protected site in South Carolina (Winne et al. 2007), indicating intra-guild predation may play a more significant role in population dynamics than previously known. We have one confirmed case of avian predation (Eastern Screech Owl; *Megascops asio*) at Carlyle Lake, although many other avian species capable of killing Eastern Massasaugas inhabit the site. Predation by American Crows (*Corvus brachyrhynchos*) has been identified as the largest source of mortality in a population of Red-sided Gartersnakes (*Thamnophis sirtalis parietalis*) in Manitoba, and predation by hawks has also been observed at the same site (Shine et al. 2001).

Management related activities around Carlyle Lake include mowing along roadsides and other man-made structures, prescribed burns for woody vegetation control, and chemical and mechanical treatments of invasive plant species. These activities are necessary to maintain habitat suitable for the Eastern Massasauga but can also pose significant risks. Direct mortality from exposure to fire, contact with mower blades, and crushing under implement tires have been documented in an Eastern Massasauga population in Missouri (Durbian 2006). These activities have also been found to increase predation risk, specifically by reducing cover sites and attracting avian predators (Durbian 2006). It has been suggested Eastern Massasaugas inhabiting prairie/grassland habitats, such as that found at Carlyle Lake, are more vulnerable to management mortality (especially from fire) than those in wetland/bog habitats further north in their range (Cross et al. 2015).

Disease documented in Carlyle Lake Eastern Massasaugas is primarily attributed to infection by the fungus *Ophidiomyces ophidiicola* (snake fungal disease; SFD). Snake fungal disease was first recognized in the population in 2008, but retrospective investigation of museum specimens has indicated its presence in the population at least since 2000 (Allender et al. 2011; Allender et al., 2016). Snake fungal disease has been confirmed in both venomous and non-venomous snake species in the eastern United States (Guthrie et al., 2016) and causes primary mortality in the Eastern Massasauga (Allender et al. 2011). Other less frequently encountered diseases in the population include tumors and bacterial infections.

Factors explaining mortality

Season and study type were both included in the top model. Fewer mortalities are observed in the spring and winter when snakes are less active due to colder temperatures. Minimum active body temperatures recorded for Eastern Massasaugas at Carlyle Lake range from 4.1–8.8° C (Dreslik 2005). Movement is restricted at these temperatures and individuals are usually associated with refugia (Dreslik 2005), making them less vulnerable to predators. However, we have recorded cases of neonate Eastern Massasaugas failing to enter burrows for hibernation and succumbing to the elements, as well as failing to emerge from hibernation during egress.

Type of study also influenced recorded sources of mortality. The majority of our observational automobile mortalities were encountered concurrently with a radio-telemetry study (see Shepard et al. 2008a for methods). Although only one radio-implanted individual was killed crossing a road, 48 non-radioed individuals were killed on roads during the study. In contrast, only a single predation mortality was reported from observational data, the remaining 33 coming from radio-telemetry. These data suggest a potential bias in which observational studies under-detect cases of predation and radio-telemetric studies under-detect automobile caused mortality. Road mortality could also be impacted by unknown biases in the individuals chosen for transmitter implantation as some are behaviorally more inclined to move or cross roads (Fraser et al. 2001; Sih et al. 2004). Alternatively, it could indicate an aversion to roads in which the low proportion of radio-implanted snakes killed crossing roads is representative of the population (Shepard et al. 2008b). Similar results were reported for Eastern Massasaugas in Michigan in which radio-telemetry recorded only one mortality (depredation), but three automobile deaths of non-telemetered snakes were reported to researchers over a short time frame (Bailey et al. 2011). Thus, both methods of study are required to obtain the most accurate mortality data for a population. This is especially important for species in need of conservation.

Conservation/management recommendations

It is important to quantify mortality in species in need of conservation so significant threats can be identified and managed. We suggest several management actions to reduce mortality, applicable not just at Carlyle Lake but throughout the range of the species.

Automobiles: To manage these threats, we echo the conservation recommendations made by Shepard et al. (2008a) to create unfragmented habitat and use seasonal closure of non-essential roads to reduce the impact of vehicle traffic. The precedent for road closures exists as seasonal road closures are used in Southern Illinois to protect snakes moving between hibernation sites and foraging areas in the La-Rue/Pine Hills Ecological Area (Ballard 1994). For essential roads that cannot be closed, road mortality hotspots can be identified (Langen et al. 2009). Ecopassages under roadways or informational signage have shown some potential to reduce reptile road mortality in Canada (Baxter-Gilbert 2014), and their evaluation for use elsewhere is warranted.

Predation: While predation will never be eliminated, steps can be taken to reduce the number of predators. Mesocarnivore release has resulted in an increase in populations of predators such as Raccoons (Ritchie and Johnson 2009). Because the Eastern Massasauga population is located mainly on state and federal land, existing furbearer hunting/trapping programs could be expanded to include these areas. Hunting programs for White-tailed deer (*Odocoileus virginianus*), Turkey (*Meleagris gallopavo*), and Ring-necked Pheasant (*Phasianus colchicus*) already occur at Carlyle Lake and have not resulted in any known negative interactions between hunters and rattlesnakes.

Disease: Wildlife disease is becoming an increasingly challenging problem in conservation biology. Previously, Eastern Massasaugas in Illinois have tested negative for West Nile Virus but seropositive for ophidian paramyxovirus (OMPV; Allender et al. 2006). No known mortality has resulted from OPMV (Allender et al. 2006), but SFD has been documented to cause mortality and occurs at a prevalence rate of 14–22% (Allender et al., 2016). Investigations into treatment options for SFD-positive individuals are ongoing (Mathew Allender, pers. comm.), and land managers should report any individual Eastern Massasaugas displaying unusual behaviors (basking on roads, remaining on the surface during typical hibernation times, etc) or with clinical signs so they may be tested for SFD and treated when possible. To limit the potential of human-mediated spread of SFD, individuals involved in land management activities or research should disinfect footwear and equipment when moving between sites. Continued monitoring for SFD and other emerging diseases should be included in conservation planning.

Management: Management related mortalities, while only accounting for a small percentage of overall mortality, are easily preventable. At Carlyle Lake, management activities such as prescribed fire and mowing have resulted in Eastern Massasauga mortalities. To eliminate management mortality, we recommend burns be conducted prior to spring emergence on cold, cloudy, winter mornings when snakes are hibernating (Dreslik 2005; Durbian 2006; Cross et al. 2015). Mowing of roadsides and recreation areas should be done frequently to keep the grass short and discourage snakes from occupying these areas. Traditional mowers can create a slight vacuum effect injuring snakes below the blade height; thus any mowing that must be done directly through Eastern Massasauga habitat should use a sickle bar or disk mower which does not create a vacuum (Durbian 2006). Additionally, mowers with a wide wheel base will require fewer passes and reduce the probability of crushing snakes under implement tires (Durbian 2006).

Persecution: Snakes, especially venomous species, are typically despised and feared by the general public (Burghardt et al. 2009), and while we have documented only one known mortality resulting from human persecution, this category is likely under-reported. It is unknown how many people find and kill Eastern Massasaugas on private property, but local residents often boast about these activities. Education and outreach programs are the most effective means of changing attitudes to reduce persecution related mortality (Burghardt et al. 2009).

Surgical complications: This category is caused solely by research activities associated with radio-transmitter implantation. At Carlyle Lake, we have discontinued all research involving surgical implantation of radio-transmitters to eliminate such mortalities. Annual survival rates for Illinois Eastern Massasaugas calculated using radio-telemetry data were low (0.35; Jones et al. 2012) when compared to adult survival calculated with mark/recapture data

(0.809–0.995; Michael Dreslik et al., unpubl. data). We recommend the need for any future radio-telemetry projects on the Eastern Massasauga be carefully weighed against the risk of mortality, or use less invasive methods such as externally attached transmitters. As many threatened and endangered species persist in small isolated populations vulnerable to environmental and demographic stochasticity, the identification and mitigation of threats is critical for their conservation. Long-term data sets for the Eastern Massasauga are invaluable and serve as a guide for targeted conservation actions range-wide.

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TABLES AND FIGURES

TABLE 3.1. Mortality categories used when documenting Eastern Massasauga (*Sistrurus catenatus*) mortality events at Carlyle Lake, Clinton County, Illinois, USA.

Mortality Category	**Description**
Automobile	Individuals found dead on roads with obvious evidence of vehicular trauma
Predation	Individuals found whole or in pieces with signs of predator activity (bite/claw marks, partially eaten, radio transmitter found in scat or inside predator)
Died in Captivity	Individuals that died of natural causes while housed in a captive setting
Euthanized	Sick or injured animals brought into captivity and humanely euthanized by a veterinarian
Illness	Individuals that died of disease (determined by a wildlife veterinarian)
Management	Individuals killed inadvertently during habitat management activities (mowing grass, controlled burns), and found by or reported to the authors
Other	Cause of death was something other than the listed categories
Persecution	Individuals killed purposely by humans
Stillborn	Individuals dead at birth born to females held in captivity
Surgical Complications	Individuals that died post-surgical implantation or removal of a radio-transmitter
Unknown	Cause of death could not be determined

Table 3. 2: All Eastern Massasauga (*Sistrurus catenatus*) mortalities observed from 2000-2011 at Carlyle Lake, Clinton County, Illinois, USA. M = Male, F=Female. Not all classifications (sex, stage) sum to overall total due to difficulty determining sex or stage in some cases.

Type of Mortality	Overall	M	F	Adult	Juvenile	Telemetry	Observational	Spring	Summer	Fall	Winter
Automobile	49	28	17	28	18	1	48	3	26	20	0
Predation	34	20	12	18	15	33	1	5	10	19	0
Died in Captivity	1	0	1	0	1	0	1	0	0	1	0
Euthanized	5	2	3	5	0	3	2	1	2	1	1
Illness	10	3	7	8	2	7	3	3	4	0	2
Management	9	3	3	4	3	2	7	5	2	2	0
Other	19	5	10	5	14	11	8	2	8	4	5
Persecution	1	1	0	0	1	0	1	0	0	1	0
Stillborn	8	0	1	0	8	0	8	0	8	0	0
Surgical Complication	6	2	3	6	0	6	0	2	2	2	0
Unknown	13	4	7	5	8	7	6	6	2	4	1
Total	155	68	64	79	70	70	85	27	64	54	9

TABLE 3.3: Candidate models tested to explain mortality in the Eastern Massasauga (*Sistrurus catenatus*) at Carlyle Lake, Clinton County, Illinois, USA from 2000-2011.

Model	**K**	**AICc**	**ΔAICc**	w_i
Season+MortType	**8**	**413.83**	**0**	**0.77**
Global	11	416.26	2.42	0.23
Stage+Season	5	443.22	29.38	0.00
Season	4	443.75	29.92	0.00
Season+StudyType	5	444.27	30.44	0.00
Sex+Season	5	445.75	31.92	0.00
Stage+MortType	6	458.49	44.65	0.00
MortType	5	458.99	45.16	0.00
StudyType+MortType	6	459.54	45.71	0.00
Sex+MortType	6	461.02	47.19	0.00
Stage	2	488.63	74.79	0.00
Stage+StudyType	3	489.09	75.26	0.00
Null	1	489.24	75.41	0.00
StudyType	2	489.68	75.85	0.00
Sex+Stage	3	490.57	76.74	0.00
Sex	2	491.16	77.33	0.00
Sex+StudyType	3	491.63	77.79	0.00

TABLE 3.4: Parameter estimates for the top model explaining mortality in the Eastern Massasauga (*Sistrurus catenatus*) at Carlyle Lake, Clinton County, Illinois, USA.

	Estimate	**Std Error**	**z-value**	**p**	**Lower confidence limit**	**Upper confidence limit**
Intercept	0.77	0.19	4.12	0.00	0.39	1.13
Season:Spring	-0.76	0.26	-2.94	0.00	-1.28	-0.27
Season:Summer	0.02	0.21	0.10	0.92	-0.38	0.42
Season:Winter	-2.24	0.47	-4.76	0.00	-3.30	-1.42
Mort type:Predation	-0.34	0.23	-1.47	0.14	-0.80	0.11
Mort type:Human	-1.86	0.41	-4.58	0.00	-2.75	-1.13
Mort type:Illness	-1.17	0.31	-3.82	0.00	-1.80	-0.59
Mort type:Other	-0.63	0.25	-2.49	0.01	-1.14	-0.14

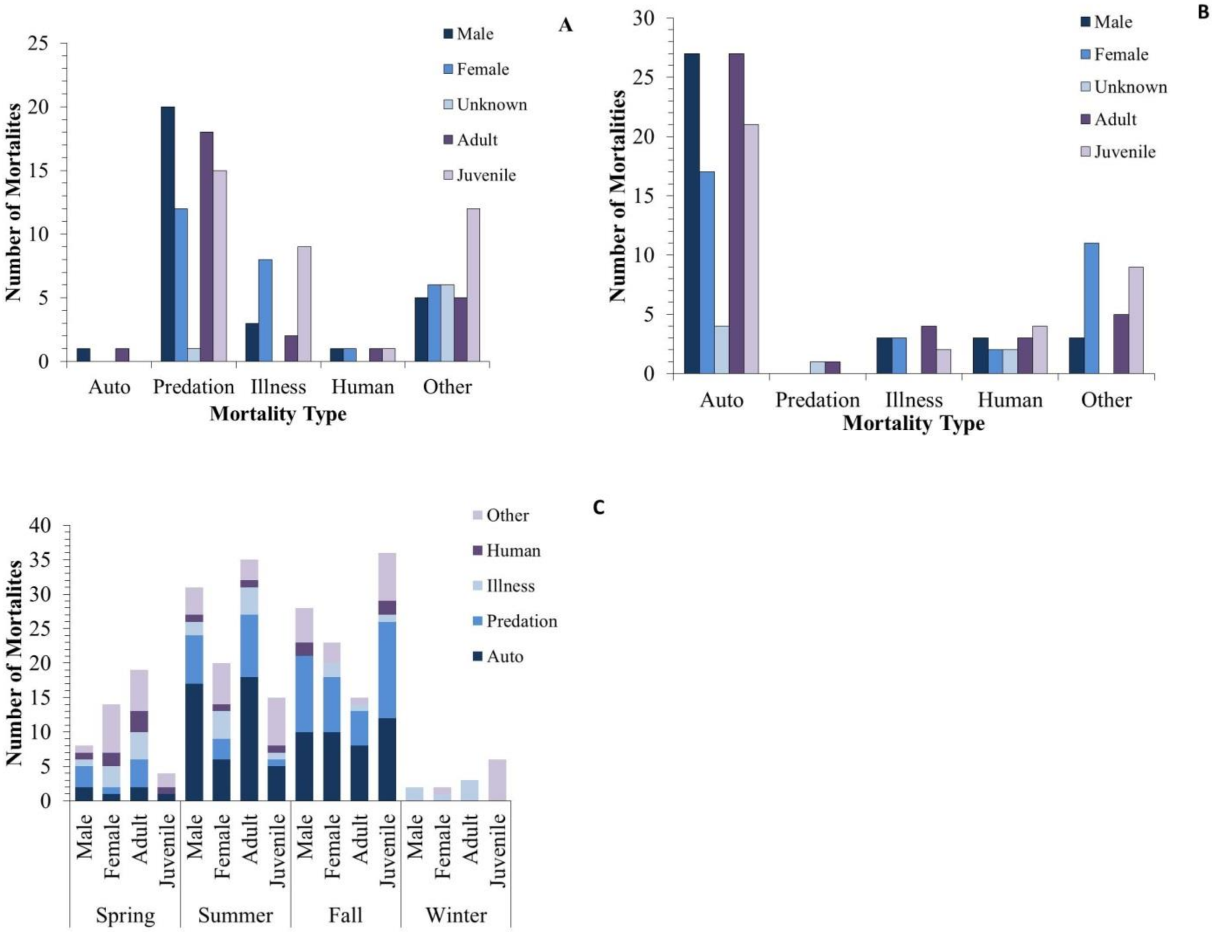

FIGURE 3.1. Eastern Massasauga (*Sistrurus catenatus*) mortality at Carlyle Lake, Clinton County, Illinois, USA broken down by sex (male, female, or unknown) and stage (adult or juvenile) for mortality documented during telemetry studies (panel A); mortality documented during observational studies (panel B); and mortality documented during each season (panel C).

CHAPTER FOUR

POPULATION VIABILITY ANALYSIS OF THE EASTERN MASSASAUGA AT CARLYLE LAKE, ILLINOIS

ABSTRACT

Conserving isolated populations of threatened and endangered species has become a necessity in the Anthropocene. To effectively achieve this, it is important to understand the population’s trajectory under current conditions and the demographic factors strongly influencing that trajectory. Population viability and sensitivity analyses are useful tools for providing conservation biologists with the necessary information to effectively manage populations. The Eastern Massasauga (*Sistrurus catenaus*) is a wide-ranging endangered snake species persisting in isolated populations throughout its historical range. Using long-term data from the sole extant population in Illinois, located at the species’ southern range limit, I conducted a viability and sensitivity analysis using the program Vortex. Results indicate the population has a 65% probability of extinction in 50 years, and its trajectory is heavily influenced by reproductive factors (proportion of breeding females, the mean number of offspring, and offspring sex ratio). The conservation implications indicate maintaining suitable conditions for reproductive females should be a priority as they are driving population persistence. Restoration or acquisition of additional habitat will also be critical to increase population size by increasing carrying capacity for the population. The results of this study provide information from the Eastern Massasauga’s southern range limit, and although demographic parameters differ across the species’ range, the PVA model can be applied to other populations within a similar geographic area without population-specific data. While the PVA model will have the most predictive power for the species or population from which the data

originated, it can still serve as a useful surrogate to provide a starting point for similar species lacking detailed demographic data.

INTRODUCTION

Preserving biodiversity is a central tenet of conservation biology (Meine et al. 2006). In the highly fragmented habitats of the Anthropocene, this often requires some level of conservation action, spanning a gradient from simple monitoring of self-sustaining populations, to captive propagation of entire species extirpated in the wild (Redford et al. 2011). Effective conservation actions require detailed knowledge about species ecology, distribution, evolutionary history, and threats (Schaffer et al. 2002). Population viability analysis (PVA) is a useful tool for coalescing these data under an integrated analytical framework. Applications of PVA include predicting population persistence, identifying factors affecting population dynamics, identifying critical habitats, weighing ecological and socioeconomic trade-offs, prioritizing management actions, communicating conservation problems to stakeholders, and identifying information gaps to direct future research (Coulson et al. 2001; Pe'er et al. 2013). As an essential counterpart to PVA, sensitivity analysis provides a mechanism for identifying the parameters with the most influence on population dynamics (Mills and Lindberg 2002). Unfortunately, the large amount of data required to construct accurate models is unavailable for many species (Coulson et al. 2001; Schaffer et al. 2002). Imperiled species may be at an increased disadvantage as populations or species may continue to decline or even become extirpated while sufficient data are collected or funding acquired to afford legal protection (Harris et al. 2012).

Reptiles are a globally imperiled taxa (Todd et al. 2010), with worldwide declines proposed to be of similar scale and geographic scope as have been documented in amphibians (Gibbons et al. 2000). Reptiles are under represented on the IUCN Red List with only 35% of described species evaluated (IUCN 2009), and in the United States the Endangered Species Act (ESA) under represents non-avian IUCN listed species by up to 80% (Harris et al. 2012). Recent efforts to conduct a global assessment of extinction risk to reptile species indicate that 15-36% of reptiles are threatened with extinction (Böhm et al. 2013). Additionally, 21% of species lack sufficient data to be evaluated, a much higher proportion than reported for birds and mammals (Böhm et al. 2013). Within the already under-represented reptiles, snake populations have received comparatively little attention (Todd et al. 2010) but face widespread localized declines (Reading et al. 2010), which could be a warning of species-level extinctions to come (Böhm et al. 2013). Vipers are an especially vulnerable group of snakes (Böhm et al. 2013) due in part to their life history characteristics, specialized habitat requirements (Todd et al. 2010), and undeserved bad reputation, particularly in Judeo-Christian cultures, that often yields outright persecution (Burghardt et al. 2009; Davis and Douglas *Accepted*). However, snakes have been identified as indicators of ecosystem functionality, and understanding their population demographic characteristics can provide insight into ecosystem-level processes such as global warming (Beaupre and Douglas 2009).

Given the lack of data for many reptile species and the vulnerability of venomous snakes to extinction or local extirpation, it is unlikely the time and resources exist to collect the necessary data to conduct specific PVAs for all populations, or even all species requiring them. Therefore, existing long-term data sets can be used as surrogates for other populations or closely related species (Schtickzelle et al. 2005). The Eastern Massasauga (*Sistrurus catenatus*) is declining throughout its range (Szymanski et al. 2015), and after 18 years as a candidate species for ESA protection it has been proposed for listing as threatened, pending public comment period and final ruling (USFWS 1998, 2015). Some research has been conducted on individual populations throughout the species range, mainly via short-term radio-telemetry studies, and considerable variation in survival and fecundity has been documented throughout the species range (Aldridge et al. 2008; Jones et al. 2012). However, detailed demographic data are lacking for most populations, and published PVAs representing the entire species range have been identified as a critical data need (Jones et al. 2012). In Illinois, only one Eastern Massasauga population remains extant, located at Carlyle Lake, Clinton County, the southern limit of the species range. Population monitoring has been ongoing since 1999, and long-term studies such as this provide invaluable information applicable to conservation throughout the species range (Todd et al. 2010). Therefore, the objectives of this study were to conduct a baseline population viability analysis for the Eastern Massasauga at Carlyle Lake to determine the predicted population trajectory over the next 50 years, conduct a sensitivity analysis to determine which parameters have the greatest influence on the population trajectory, and provide management recommendations based on the results of the model.

MATERIALS AND METHODS

Study Species and Site

The Eastern Massasauga is a prairie and bog species endemic to the eastern United States and Canada (Ernst and Ernst 2003). In Illinois, its distribution has been reduced to sparse, fragmented habitat around the southern perimeter of Carlyle Lake, Clinton County. The lake was constructed as an impoundment of the Kaskaskia River in 1967, and extensive habitat was lost as a consequence. Remaining individuals are now clustered in grasslands (native and non-native) with perched water tables containing crayfish burrow complexes that serve as hibernacula and represent the only remaining suitable habitat patches (Dreslik 2005; Dreslik et al. *in press*). The largest hibernacula is located at South Shore State Park (SSSP) and is isolated from nearby hibernacula by approximately 4.5 km of open water to the west, 5 km of highly developed recreation areas to the southwest, 5 km of agriculture fields and roads to the northeast, and to the east no significant amount of suitable habitat exists.

Capture-mark-recapture sampling has been ongoing at the SSSP hibernaculum since 1999 and previous studies have investigated population size (Dreslik 2005; Dreslik et al. *in press*), survival rates (Jones et al. 2012; Davis et al. *in review*), numerous aspects of reproduction (Jellen et al. 2007, Aldridge et al. 2008; Davis et al. *in review*; Chapter 2), and population genetics (Chiucchi and Gibbs 2010; Chapter 1). Because of both the quality and quantity of the data on the SSSP hibernaculum, only SSSP data were used to populate the PVA input parameters. Although mark-recapture also occurs at other hibernacula around the lake, sufficient numbers of recaptures are lacking to calculate robust population estimates. In the case of Eldon Hazlet State Park, the hibernacula possibly function as a metapopulation, based on observed migration among hibernacula, but rates of gene flow and migration have not been determined.

Data Analysis

I used Vortex 10.1.4 (Lacy and Pollak 2014) to conduct the population viability and sensitivity analysis. Vortex uses an individual-based modeling approach to follow the fate of individual animals through each year of their lifetime, and thus keeps track of the sex, age, and parentage of each individual. The input variables used were all derived from the long-term study of the SSSP population and are summarized in Table 1. Although previous survival estimates exist for the Carlyle Lake population (Jones et al. 2012), they are based on radio-telemetric data and thus carry the additional potential effects of radio-implantation. The discrepancy between the two data sets (for adult survival) potentially illustrates the impacts of radio-implantation on this species (Davis et al., in review). Given this, I opted to use survival rates that do not represent sustained radio-telemetric studies. Survival was estimated using a Bayesian capture-recapture state-space model (Clark, 2007) based on individual capture histories at SSSP from 1999-2010. Covariates, as either fixed or random effects, were incorporated into the model using a logistic regression function. Four candidate models were constructed and compared using Deviance Information Criteria (DIC). The null model included no covariates and the random effects model implemented the covariate year as a random source of variance. We then considered two mixed models where the first implemented the random year with the fixed effect of sex, and the third added stage (adult or juvenile) as a fixed effect (in addition to random year and fixed sex). Models were fit using OpenBugs (Lunn et al., 2009) through R (Team, 2008) based on standard Bayesian MCMC sampling methods. Thus, assuming survival rates are constant through adulthood state changes, transition probabilities were calculated as follows:

$$\text{if } S_{j\text{-}a} = S_j(S_a), \text{ then } S_j = S_{j\text{-}a}/S_a \qquad (1)$$

$$\text{and if } S_{n\text{-}j\text{-}a} = S_n(S_{j\text{-}a}/S_a)(S_a), \text{ then } S_n = S_{n\text{-}j\text{-}a}/S_{j\text{-}a} \qquad (2)$$

where S_a is the estimated survival of adults through adult transitions, $S_{j\text{-}a}$ is the estimated survival of juveniles through adulthood, $S_{n\text{-}j\text{-}a}$ is the estimate of survival of neonates through adulthood, S_j is the survival of juveniles for the juvenile to adult transition, and S_n is the survival of neonates for neonate to juvenile transition (Davis et al., in review) .

For all PVAs, the extinction threshold was set as only one sex remaining. The baseline PVA consisted of 50,000 iterations for a time horizon of 50 years. Demographic and environmental stochasticity were both incorporated. No evidence could be found in the literature that Eastern Massasaugas exhibit density dependence, so it was not included. Graphs were produced using qqplot2 (Wickham, 2009). The outputs of stochastic growth rate, probability of extinction, and size of extant populations were used as dependent variables in subsequent analyses conducted in R (R Core Team, 2015).

For sensitivity analyses, I varied initial population size, reproductive parameters, and mortality rates a relative +/- 50% of their input value (at 10% steps) and carrying capacity was varied from -50% to +500%. I conducted two types of sensitivity analyses, one varying each demographic parameter individually (single factor) and a second randomly sampling parameter values (multi-factor). These methods allowed me to single out the individual effects and then to identify interactions among demographic parameters. For both analyses, the time horizon was reduced to 20 years and because of the number of scenarios required, the number of iterations was reduced to 10,000. For the single factor sensitivity analysis, parameters were varied at 10 equal steps between the minimum and maximum relative values (totaling 240 scenarios including the base). For the multifactor sensitivity analysis, parameters were randomly selected between the minimum and maximum relative values, and I generated 25,000 unique scenarios based on this random selection.

For the single factor sensitivity analysis, I graphically portrayed the effects of each demographic parameter against each dependent variable: the probability of extinction (PE), stochastic growth rate (r_{stoch}), and size of extant populations (N_{extant}). Each parameter was varied across its range while all other parameters were held constant at their base values. Those showing wide variance can be considered sensitive parameters. For the multiple factor sensitivity analysis, I used an information theoretic approach to select among a candidate set of models (Burnham and Anderson, 2002) and linear regression in R (R Core Team, 2015) to evaluate the effects of varying multiple parameters simultaneously and investigate interactions (McCarthy 1995; Cross and Beissinger 2001). The candidate set of models for each dependent variable included: single, two, and three main effects with associated interactions, and the intercept only model (null). I did not examine any higher effects models as this candidate set already included 2,808 linear models for each dependent variable. I conducted model selection on the candidate set of models using the AICCmodavg package (Mazerolle 2015). I defined the top models as those within 2 ΔAIC units and conducted model averaging of parameters when necessary (Burham and Anderson, 2002). For the top models, I also calculated r^2s where necessary to assess fit. Finally, I used the effects package (Fox 2003) to assess each parameter and interactions in the top model set.

RESULTS

Population Viability

The baseline PVA model indicated a 65% probability of extinction for the SSSP population in 50 years with a mean time to extinction of 25.5±11.8 years, and median time to extinction of 36 years (Fig. 4.1). The stochastic population growth rate (r_{stoch}) was 0.12±0.0041, which was slightly lower than the deterministic growth rate of 0.14. For N_{extant}, the model predicted population size at SSSP will slightly increase over 50 years from the initial size of 54 individuals to a final estimate of 68 ±23 (Fig. 4.1).

Single-Factor Sensitivity Analysis

In the single factor sensitivity analysis, three reproductive variables had the greatest impact on PE (Fig. 4.2). As more females reproduced, PE decreased, approaching zero by ~28% of females reproducing (Fig. 4.2). The sex ratio of offspring has a modest impact, but still showed increasing PE with an increasing male-bias. Similarly, PE decreased as the mean number of offspring increased (Fig. 4.2). The probability of extinction showed little to no sensitivity to the remaining variables (Fig. 4.2).

The greatest sensitivity of r_{stoch} was to reproductive variables (Fig. 4.3). Percentage of breeding females had the largest impact on r_{stoch}, which is zero at ~12% of females breeding (Fig. 4.3). Offspring sex ratios also had a large impact, decreasing r_{stoch} to zero at about 75% males. Overall, r_{stoch} increased with an increased mean number of offspring from zero at ~3.5 offspring per litter (Fig. 4.3). Female survival rates had a moderate impact on r_{stoch}, especially age 0 and age 3 survival rates, the latter being the approximate age of first reproduction. The remaining variables had little impact on r_{stoch}.

For $N_{extant,}$ carrying capacity (K) had the greatest impact, showing a strong positive relationship, with simulated N_{extant} equal to ~50% of K (Fig. 4.4). Likewise, the percentage of breeding females and mean offspring number also showed a strong positive relationship with N_{extant} (Fig. 4.4). The sex ratio of offspring had a moderate impact on N_{extant}. The remaining variables had little or no impact on N_{extant} (Fig. 4.4).

Multi-Factor Sensitivity Analysis

For PE, the top model included the percentage of breeding females, mean number of offspring, and sex ratio of offspring (Akaike weight =1; r^2=0.94; Table 4.2). Main effects are identical to those described for single factor analysis. Lower PE resulted when the mean number of offspring was higher at all proportions of breeding females (Table 4.3; Fig. 4.5). Higher PE resulted when offspring sex ratios were male-biased, regardless of what proportion of females is breeding (Table 4.3; Fig. 4.5). Higher PE also resulted when the mean offspring number was smaller, and sex ratios were heavily male-biased (Table 4.3; Fig. 4.5). Finally, the three-way interaction indicated the highest PE occurred at lower proportions of breeding females, smaller mean number of offspring, and heavily male-biased offspring sex ratios (Table 4.3; Fig. 4.6).

For r_{stoch}, the top model included the percentage of breeding females, the mean number of offspring, and sex ratio of offspring (Akaike weight =1; r^2= 0.91; Table 4.2). Main effects were identical to those described for single factor analysis. Lower r_{stoch} resulted when the mean offspring number is smaller across all proportions of breeding females (Table 4.3; Fig. 4.7). Similarly, a lower r_{stoch} resulted from heavily male-biased sex ratios across all proportions of breeding females (Table 4.3; Fig. 4.7). Lower r_{stoch} also resulted from smaller numbers of mean offspring and a heavier male-biased offspring sex ratio (Table 4.3; Fig. 4.7). Finally, the three-way interaction resulted in the lowest r_{stoch} at high proportions of male offspring, smaller mean number of offspring and lower proportions of breeding females (Table 4.3; Fig. 4.8).

For $N_{extant,}$ the top model included the percentage of breeding females, mean offspring number, and K (Akaike weight = 1; r^2 =0.84; Table 4.2). Main effects were identical to those described for single factor analysis. A lower N_{extant} resulted from smaller mean number of offspring at lower proportions of breeding females (Table 4.3; Fig. 4.9). N_{extant} increased gradually across all levels of K at low proportions of breeding females (Table 4.3; Fig. 4.9). Similarly, N_{extant} increased gradually across all levels of K with smaller mean numbers of offspring (Table 4.3; Fig. 4.9). Finally, the three-way interaction showed the same pattern; N_{extant} increased with increasing K, but the rate of increase was determined by the proportion of breeding females and the mean number of offspring, with more breeding females and more offspring resulting in larger and faster rate of increase (Table 4.3; Fig. 4.10).

DISCUSSION

The importance of long-term data sets for construction of PVA models cannot be overstated (Zeigler et al. 2013), making these data extremely valuable for conservation. Unfortunately, despite evidence that snake populations are declining globally (Seigel and Mullin 2009; Reading et al. 2010) a comprehensive understanding of population dynamics is lacking for most species, limiting the development of effective conservation strategies (Dorcas and Willson 2009). The shortage of snake-focused studies can be attributed to a historical lack of interest in the taxa (Dodd 1993), and general difficulty in capturing and studying snakes in sufficient numbers (Fitch 1987; Parker and Plummer 1987). However, population viability has been examined for the federally listed New Mexico Ridge-nosed rattlesnake (*Crotalus willardi obscurus*) and Eastern Indigo snake (*Drymarchon couperi*).

The New Mexico Ridge-nosed rattlesnake exhibits similar life history and demography to the Eastern Massasauga and viability analysis indicated the modeled population (N= 151) had a 100% probability of extinction in 100 years with a mean time to extinction of 17 years (Davis et al. 2015). Unlike the Eastern Massasauga, sensitivity analysis indicated both adult and juvenile survival had a greater impact on population viability than fecundity, likely because of lower survival rates and negative population growth in the Ridge-nosed Rattlesnake (Davis et al. 2015). While numerically larger, the New Mexico Ridge-nosed rattlesnake population was found to have undergone a significant contemporary genetic bottleneck (Holycross and Douglas 2007; Davis et al. 2015), whereas the Eastern Massasauga at SSSP does not show signs of either a contemporary or historical genetic bottleneck (Chapter 1; Chiucchi and Gibbs 2010) nor significant levels of inbreeding (Chapter 1). These factors could be insulating the SSSP population from higher extinction probabilities. The Eastern Indigo snake is a larger and longer lived oviparous colubrid species. The probability of extinction was not estimated in this case, but adult survival was found to have the greatest impact on population growth rate (Hyslop et al. 2012). Ultimately, the viability of a population is determined by the balance between reproduction and mortality (Shine and Bonnet 2009), and the relative importance of these factors is likely to vary both among species and populations.

Population growth rate is a key determinant of viability by allowing the projection of future population sizes (Sibly and Hone 2002). Population size projections for SSSP indicate that N_{extant} is expected to remain stable over the next 50 years. Because this result mirrors the demographic data collected on the SSSP population (Dreslik et al. *in press*), it indicates population size is approaching K (Foley 1994). Projected stability in the population is likely a product of the species' historical adaptation to patchy habitat that insulates them from the negative genetic consequences of small population size (Chiucchi and Gibbs 2010). While overall the species is declining range-wide (Szymanski et al. 2015), individual populations may still exhibit positive growth rates, and be relatively stable despite their small size. The danger, however, is that stochastic events (both demographic and environmental) can lead to the rapid extinction of the population (i.e. the extinction vortex; Gilpin and Soulé 1986), and the high degree of isolation means habitat patches cannot be recolonized as likely occurred before anthropogenic habitat fragmentation.

Sensitivity analysis indicated viability is linked to reproductive biology (mean offspring number and proportion of breeding females), spatial resources (carrying capacity), and some demographic stochasticity (sex ratio of offspring). Multi-factor sensitivity analysis is not widely applied, likely due to the unavailability of this option in PVA software packages (Naujokaitis-Lewis et al. 2008). However, multi-factor analysis has been suggested as superior to traditional "one at a time" sensitivity analyses for its greater power to evaluate parameters simultaneously (Cariboni et al. 2007). The multi-factor models that included parameters related to reproduction had very high support and predictive power as indicated by high Akaike weights and high r^2 values. Fecundity has been found to factor prominently in population growth rates for many species (Heppell et al. 2000), and sex ratios play an important role in the viability of many species (Brook et al. 2000). Considerable variation exists in Eastern Massasauga litter size, and while offspring sex ratios within litters are sometimes skewed toward one sex, overall they do not differ from 1:1 (Chapter 2).

Reproduction in snakes, especially viviparous species, is often driven largely by the acquisition of resources (Nalleau and Bonnet 1996). The ability of a female to breed in a given year is often dependent upon body condition (Naulleau and Bonnet 1996), which is determined by an individual's ability to secure resources either before the onset of first reproduction, or following a reproductive event. Energy requirements to meet resting metabolic needs have been determined for the Eastern Massassauga and translated into approximate prey items needed per year (Baker et al. *in press*). Combining prey requirements with Eastern Massasauga population size would result in an approximate prey base estimate for population maintenance, which could be used in conjunction with small mammal monitoring/management programs to ensure prey populations remain well above maintenance levels. If the density of suitable prey is found to be inadequate, habitat manipulations that increase prey species abundance, or serve to attract prey to areas of high snake density have shown preliminary success for increasing population density of the Shedao Island Pit-viper (*Gloydius shedaoensis*; Shine and Bonnet 2009).

Carrying capacity appeared in the top model for population size projections, which was expected given that larger areas can support a larger number of individuals. Carrying capacity for a species depends on the area, quality, and connectivity of habitat available (Hodgson et al. 2009). Thus, as K figures significantly in population size projections, it should be feasible to increase population size at SSSP by increasing K via the restoration of low quality woodlots back to grassland habitat. Because dispersal ability of Eastern Massasaugas is limited (Dreslik 2005), priority should be given to land adjacent to currently occupied areas. As the Eastern Massasauga is a wide-ranging species, habitat preferences differ between populations (Weatherhead and Prior 1992; Johnson and Leopold 1998; Kingsbury 1999; Dreslik 2005). Therefore, habitat restoration actions should take into account local habitat preferences. In Illinois, the focus should be on maintaining grassland habitats with hydrology suitable for terrestrial burrowing crayfish. Future studies should utilize a population habitat viability assessment (PHVA) to quantify how much additional habitat is needed to reduce PE to an acceptable level.

Conservation Implications

Although reproductive characteristics are difficult to manipulate using management practices, because they are functionally linked with energetic resources, one possibility is to manage the prey base. Increased reproductive output has been previously documented in snakes in response to high prey density (Shine and Madsen 1997; Bonnet et al. 2001; Brown and Shine 2007). This could include creating or restoring quality habitat that can sustain a high density of small mammals while retaining microhabitat characteristics essential to the Eastern Massasauga. To maintain small mammal populations grassland habitats in a variety of successional stages should be available (Mulligan et al. 2013), and species specific requirements such as leaf litter and arthropod prey required for shrews, should also be considered (Genoways and Choate 1998). The prevalent roadside grassy ditches around Carlyle Lake should expedite colonization of restored areas by small mammals (Mulligan et al. 2013). By creating and restoring habitat, the net effect should be both an increase in K for the Eastern Massasauga population, and an abundant prey base. The efficiency of snakes to digest and assimilate energy from prey is temperature dependent (McNab 2002). Thus, providing favorable thermal sites within habitat areas could expand forage time, and concomitantly, increase energy assimilation via greater digestive efficiency (Shine and Bonnet 2009). Possible ways to achieve favorable thermoregulatory sites include retaining early successional grassland habitats with low canopy cover and the use of artificial items such as sheets of corrugated tin “cover-boards”, which heat up faster than the surrounding grass and provide cover in addition to enhanced thermal opportunities for both digestion and gestation. In addition, gravid females prefer open habitat away from roads and forest edges (Dreslik 2005), thus retaining an open canopy can achieve added benefits. Downed trees and brush piles also provide foraging and thermoregulatory opportunities and they should not all be removed following habitat management practices that produce them.

Supplemental feeding could increase the proportion of reproducing females each year. One mechanism for this is collecting gravid females and feeding them after parturition (Shine and Bonnet 2009). This technique increases reproductive events in a population of Aspic Vipers (*Vipera aspis*; Bonnet et al. 2002). In this population, individual females typically reproduce only once in their lifetime but were able to reproduce a second time 12-24 months after being supplementally fed, an outcome that is unlikely to have occurred without feeding in captivity (Bonnet et al. 2002). However, caution should be used when keeping individuals in captivity to avoid potential disease transmission (Allender et al. *in press*). Supplemental feeding in the field was found to increase reproductive occurrence in the Western Diamond-backed Rattlesnake (*C. atrox*; Taylor et al. 2005). However, this approach requires radio-transmitter implantation which has been found to adversely affect reproduction (Chapter 2) and potentially survival (Jones et al. 2012, Davis et al. in review) in the Eastern Massasauga.

Because the baseline PVA model predicts the population has positive growth potential, no direct manipulation of reproductive traits may be necessary. Instead, as K factors prominently in population size projections, the most beneficial use of limited conservation dollars is likely to restore and maintain grassland habitat adjacent to occupied hibernacula. Finally, the results of a PVA should not be seen as an endpoint, but rather an adaptive tool that should be updated with changing circumstances and data, with management altered accordingly (Ralls et al. 2002). For example, models of Eastern Massasauga vulnerability to climate change indicate that increases in extreme weather events such as drought/floods are likely to negatively affect population demographic parameters (Pomara et al. 2014). This underscores the need for continued monitoring as changes in demographic rates can be identified and incorporated into the PVA model to track progress and incorporate new threats.

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TABLES AND FIGURES

Table 4.1: Parameter settings for Eastern Massasauga baseline population viability analysis model in Vortex

Parameter setting	Value used	Citation/Justification
Number of iterations	50000/10000	Robust without exceeding computational power
Years	50/20	Realistic management timeline
Extinction definition	1 sex remains	Functional extinction
Species description		
Inbreeding depression	yes	No current inbreeding, but could be in the future
Lethal equivalents	6.29	O'Grady et al 2006
Percent due to recessive lethal alleles	50	Lacy et al. 1996
E.V. concordance of reproduction and survival	yes	Good years for survival should also good for reproduction
Reproductive system		
Type	Polygynous	Jellen et al. 2008
Age of first offspring females	3	Dreslik et al. in press (a)
Age of first offspring males	3	Dreslik et al. in press (a)
Maximum lifespan	11	Approximate age of oldest known-age individual
Maximum broods per year	1	Davis et al. in review
Maximum number of progeny per brood	20	Maximum recorded Anton 2000
Sex ratio at birth	50	Chapter 2
Maximum age of reproduction	11	No reproductive senescence
% adult females breeding	19.1	Davis et al in review
SD in % breeding due to EV	73.2	Davis et al in review
Mean offspring per brood	6.67	Chapter 2
Stdev offspring per brood	3.93	Chapter 2
Distribution of # offspring per brood	normal	Chapter 2
Mortality Rates		
Female Mortality from age 0 to 1	34.5	Davis et al. in review
SD in female 0 to 1 mortality due to EV	17.5	Difference in C.I. versus mean divided by two
Female Mortality from age 1 to 2	14.4	Davis et al. in review
SD in female 1 to 2 mortality due to EV	8.5	Difference in C.I. versus mean divided by two
Female mortality from age 2 to 3	6.2	Davis et al. in review
SD in female 2 to 3 mortality due to EV	2.8	Difference in C.I. versus mean divided by two
Female annual mortality after age 3	6.2	Davis et al. in review
SD in female mortality after age 3	2.8	Difference in C.I. versus mean divided by two
Male mortality from age 0 to 1	35.5	Davis et al. in review
SD in male 0 to 1 mortality due to EV	16	Difference in C.I. versus mean divided by two
Male mortality from age 1 to 2	15.8	Davis et al. in review
SD in male 1 to 2 mortality due to EV	8.8	Difference in C.I. versus mean divided by two
Annual male mortality after age 2	7	Davis et al. in review
SD in male mortality after age 2	3	Difference in C.I. versus mean divided by two
Male annual mortality after age 3	7	Davis et al. in review
SD in male mortality after age 3	3	Difference in C.I. versus mean divided by two
Population size		
Initial population size	54	Dreslik et al. in press (b) average of yearly *N*
Age distribution	stable	Initial age distribution close to A-J ratio
Carrying capacity (K)	62	Dreslik et al. in press (b)

Table 4.2: Top three candidate models for multi-factor sensitivity analysis examining impacts of demographic parameters on population probability of extinction, stochastic growth rate, and population size in the Carlyle Lake population of the Eastern Massasauga

Model	**K**	**AICc**	**ΔAICc**	***w*$_i$**
		Probability of Extinction		
SRBirth | %BF | Offspring#	9	-86148	0	1
%BF | MeanOff | N	9	-85482	666	0
SRBirth+PerBF+MeanOff	5	-85250	898	0
		Stochastic r		
SRBirth | %BF | Offspring#	9	-113926	0	1
SRBirth | N | K	9	-56606	57320	0
SRBirth | EVMalAge3Mort | K	9	-56607	57319	0
		Population Size		
%BF | Offspring# | K	9	252915	0	1
SRBirth | N | K	9	271292	18378	0
SRBirth | EVMalAge3Mort | K	9	271971	19057	0

Table 4.3: Parameter estimates and confidence limits from linear regressions conducted on the top model parameters in multi-factor sensitivity analysis of the Carlyle Lake Eastern Massasauga population

	Estimate	Std Error	t-value	p	Lower Confidence Limit	Upper Confidence Limit
Probability of Extinction						
(Intercept)	0.792	0.010	79.115	0	0.772	0.811
SRBirth	0.300	0.019	15.702	0	0.263	0.338
PerBF	-2.814	0.050	-55.845	0	-2.913	-2.716
MeanOff	0.005	0.001	3.542	0	0.002	0.008
SRBirth|PerBF	-0.421	0.096	-4.386	0	-0.609	-0.233
SRBirth|MeanOff	-0.032	0.003	-11.355	0	-0.038	-0.027
PerBF|MeanOff	0.001	0.007	0.197	0.844	-0.013	0.016
SRBirth|PerBF|MeanOff	0.042	0.014	2.938	0.003	0.014	0.069
Stochastic Growth Rate						
(Intercept)	-0.157	0.006	-27.331	0	-0.168	-0.146
SRBirth	-0.068	0.011	-6.206	0	-0.090	-0.047
PerBF	0.977	0.029	33.760	0	0.921	1.034
MeanOff	0.009	0.001	11.185	0	0.008	0.011
SRBirth|PerBF	-0.673	0.055	-12.212	0	-0.781	-0.565
SRBirth|MeanOff	0.000	0.002	-0.005	0.996	-0.003	0.003
PerBF|MeanOff	0.061	0.004	14.418	0	0.053	0.070
SRBirth|PerBF|MeanOff	-0.010	0.008	-1.281	0.200	-0.026	0.006
Population Size						
(Intercept)	34.987	5.635	6.209	0	23.942	46.032
PerBF	-10.053	28.516	-0.353	0.724	-65.947	45.840
MeanOff	-0.634	0.835	-0.759	0.448	-2.270	1.002
K	-0.293	0.017	-17.185	0	-0.327	-0.260
PerBF|MeanOff	-9.871	4.209	-2.345	0.019	-18.120	-1.621
PerBF|K	2.122	0.086	24.642	0	1.954	2.291
MeanOff|K	0.058	0.003	23.149	0	0.054	0.063
PerBF|MeanOff|K	-0.015	0.013	-1.157	0.247	-0.040	0.010

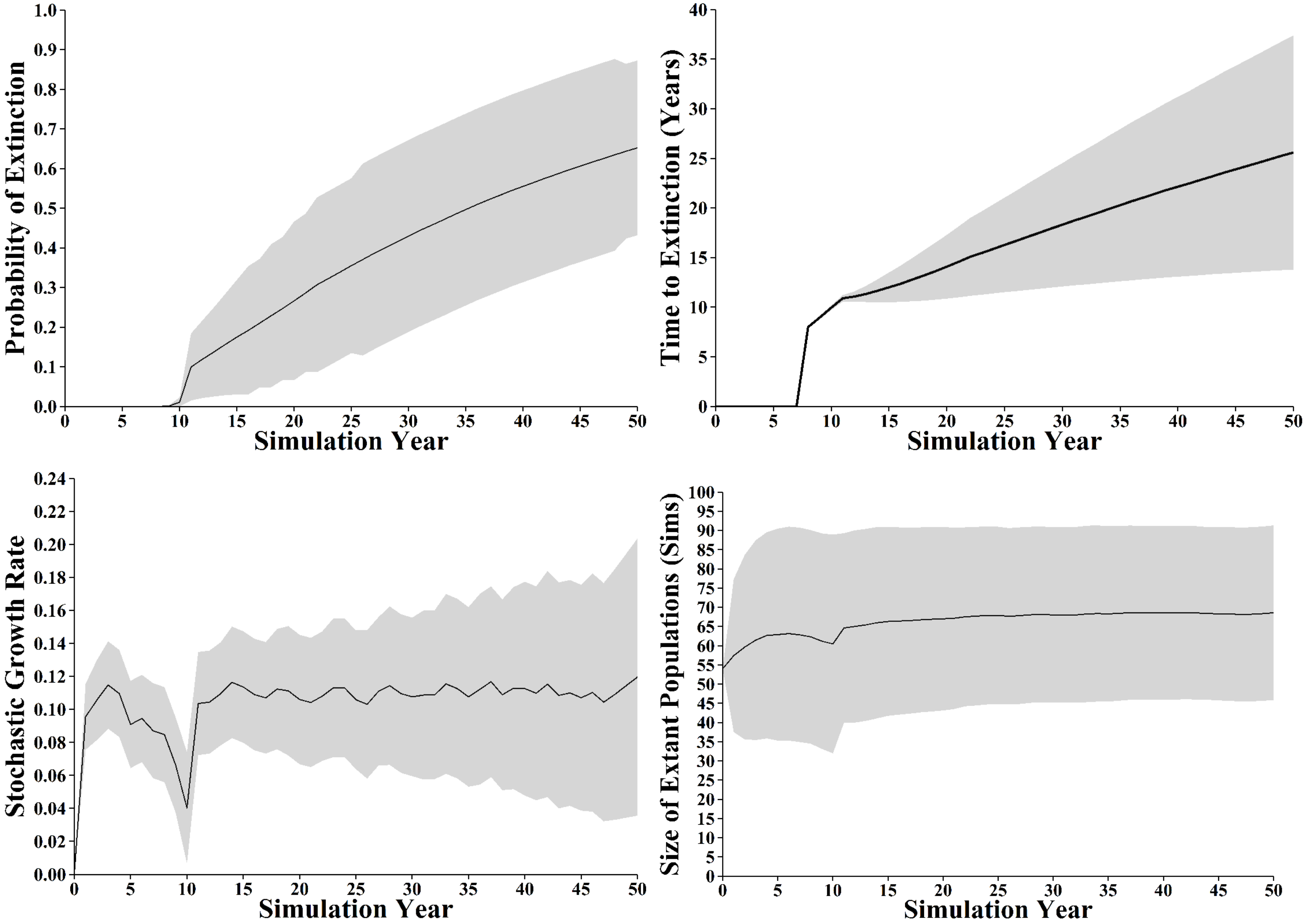

Figure 4.1: Results of baseline population viability analysis showing probability of extinction, mean time to extinction, stochastic growth rate, and size of extant populations of the Carlyle Lake population of Eastern Massasaugas projected for 50 years. Shaded area indicates variance

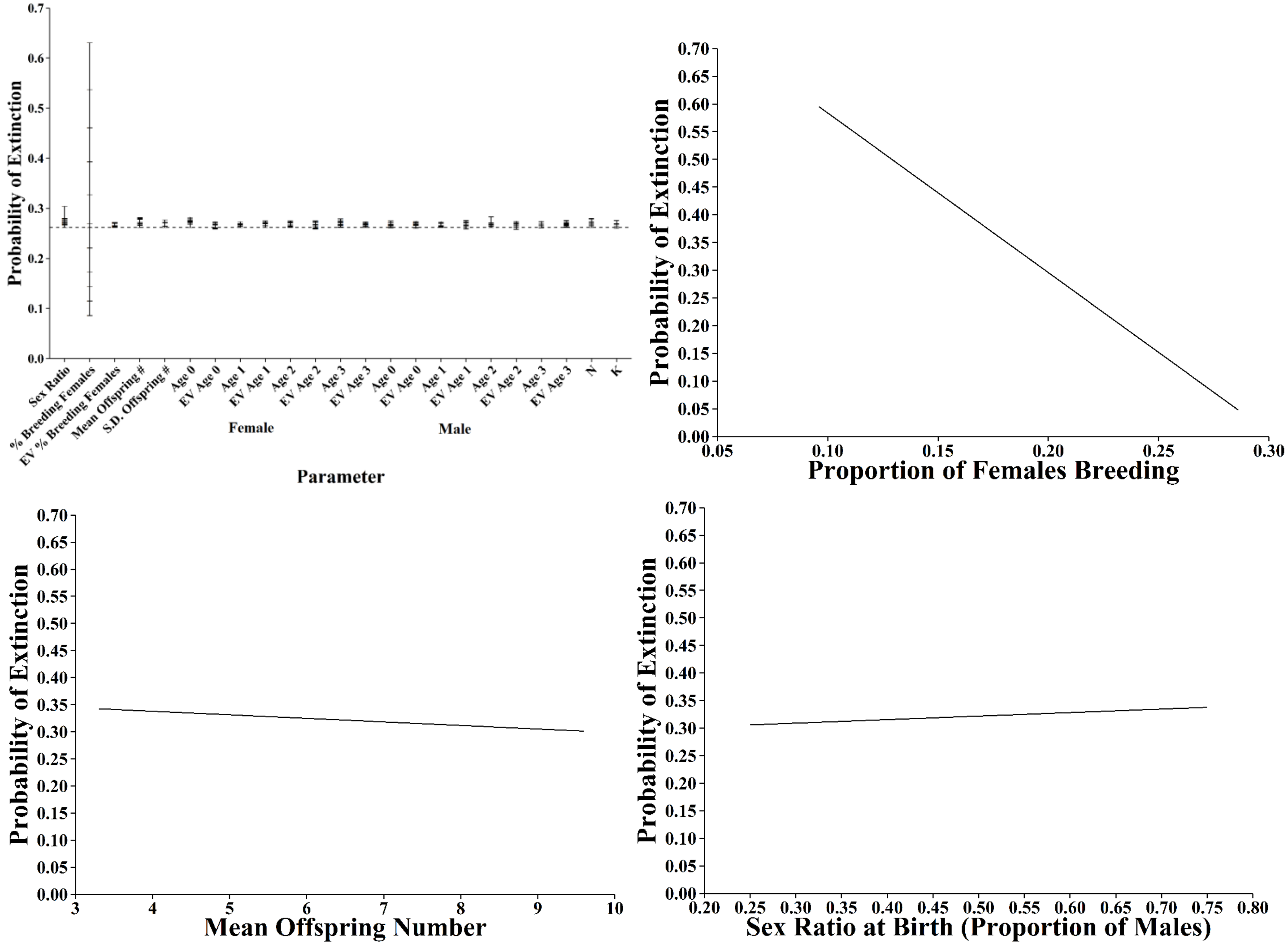

Figure 4.2: Results of single factor sensitivity analysis for probability of extinction showing impact of all demographic factors, and individual effects of the three parameters with the greatest impact for the Carlyle Lake population of Eastern Massasaugas

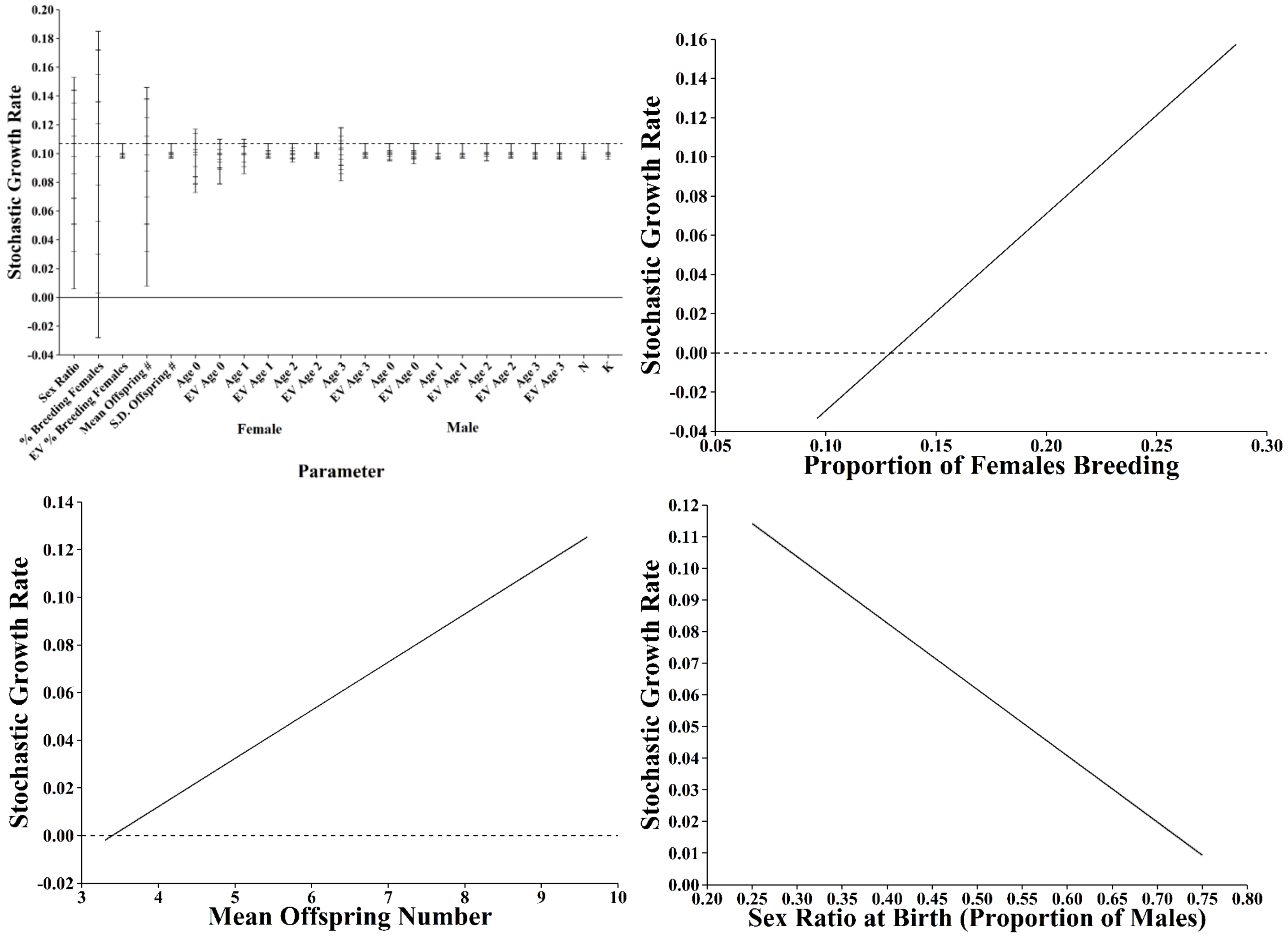

Figure 4.3: Results of single-factor sensitivity analysis for stochastic population growth rate showing impacts of all demographic parameters, and individual effects of the three parameters with the greatest impact on stochastic growth rate in the Carlyle Lake population of the Eastern Massasauga

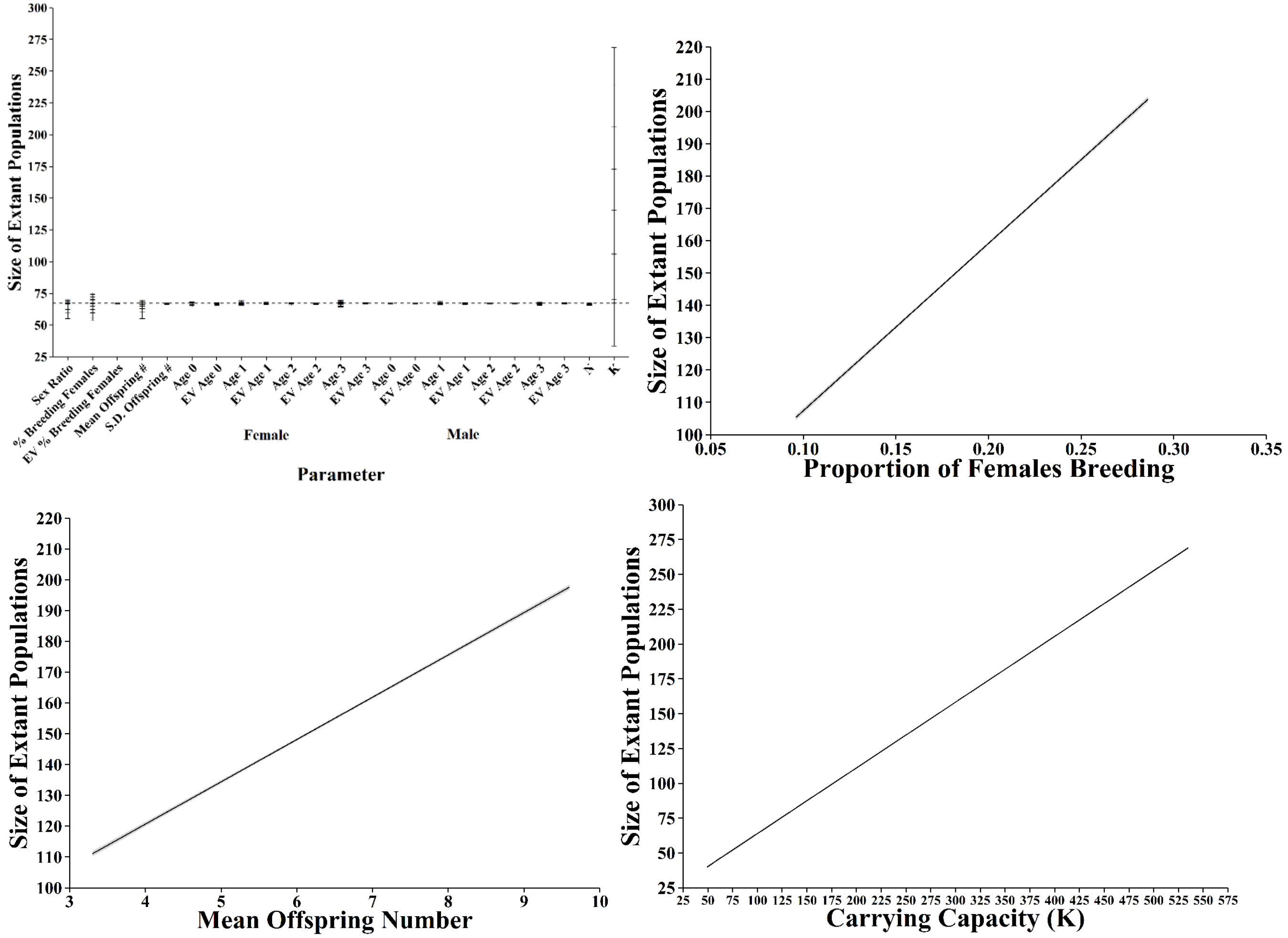

Figure 4.4: Results of single-factor sensitivity analysis for size of extant populations showing impacts of all demographic parameters, and individual effects of the three parameters with the greatest impact on population size in the Carlyle Lake Eastern Massasauga

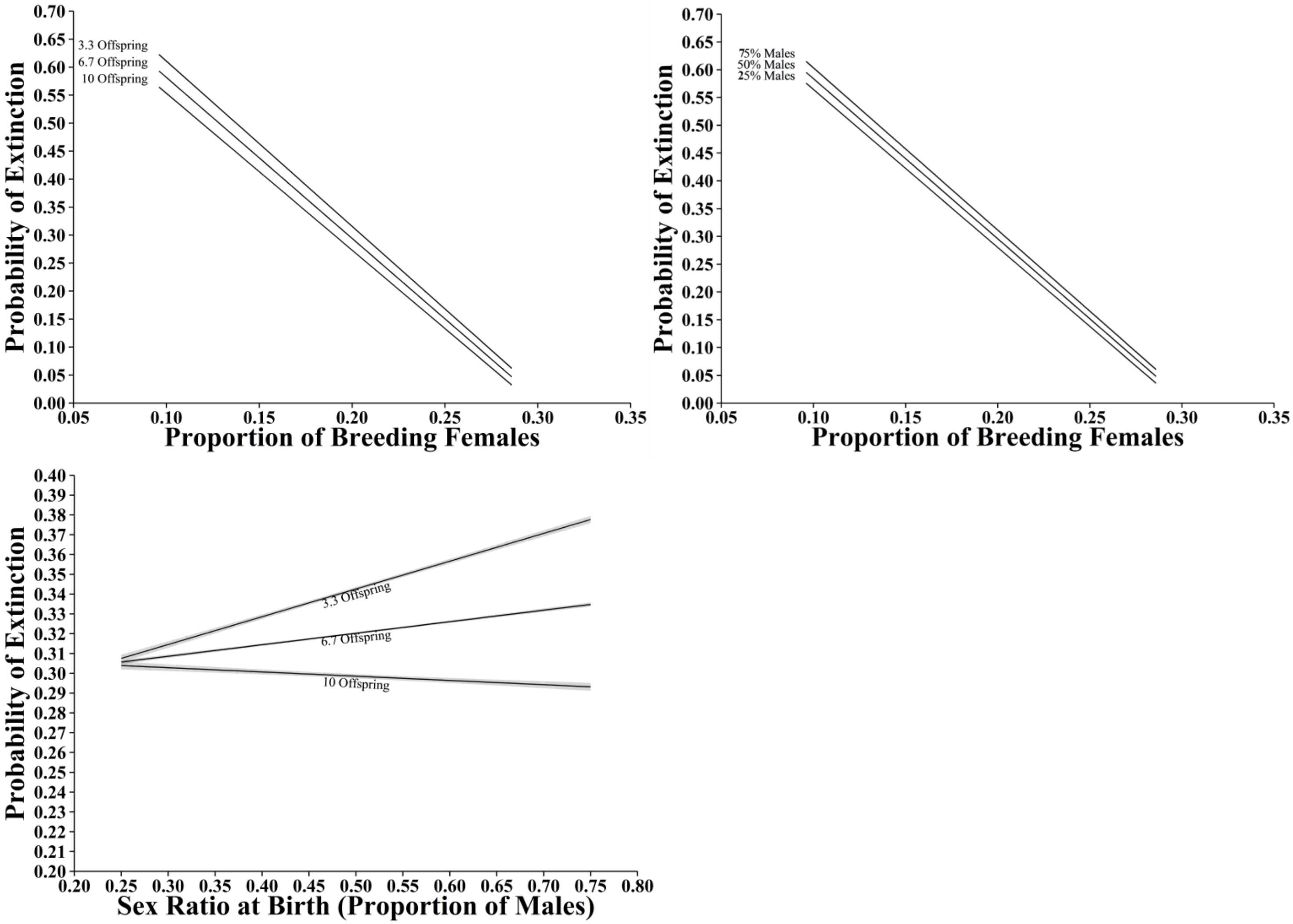

Figure 4.5: Results of multi-factor sensitivity analysis showing two-way interactions of top model parameters impacting probability of extinction in the Carlyle Lake population of the Eastern Massasauga

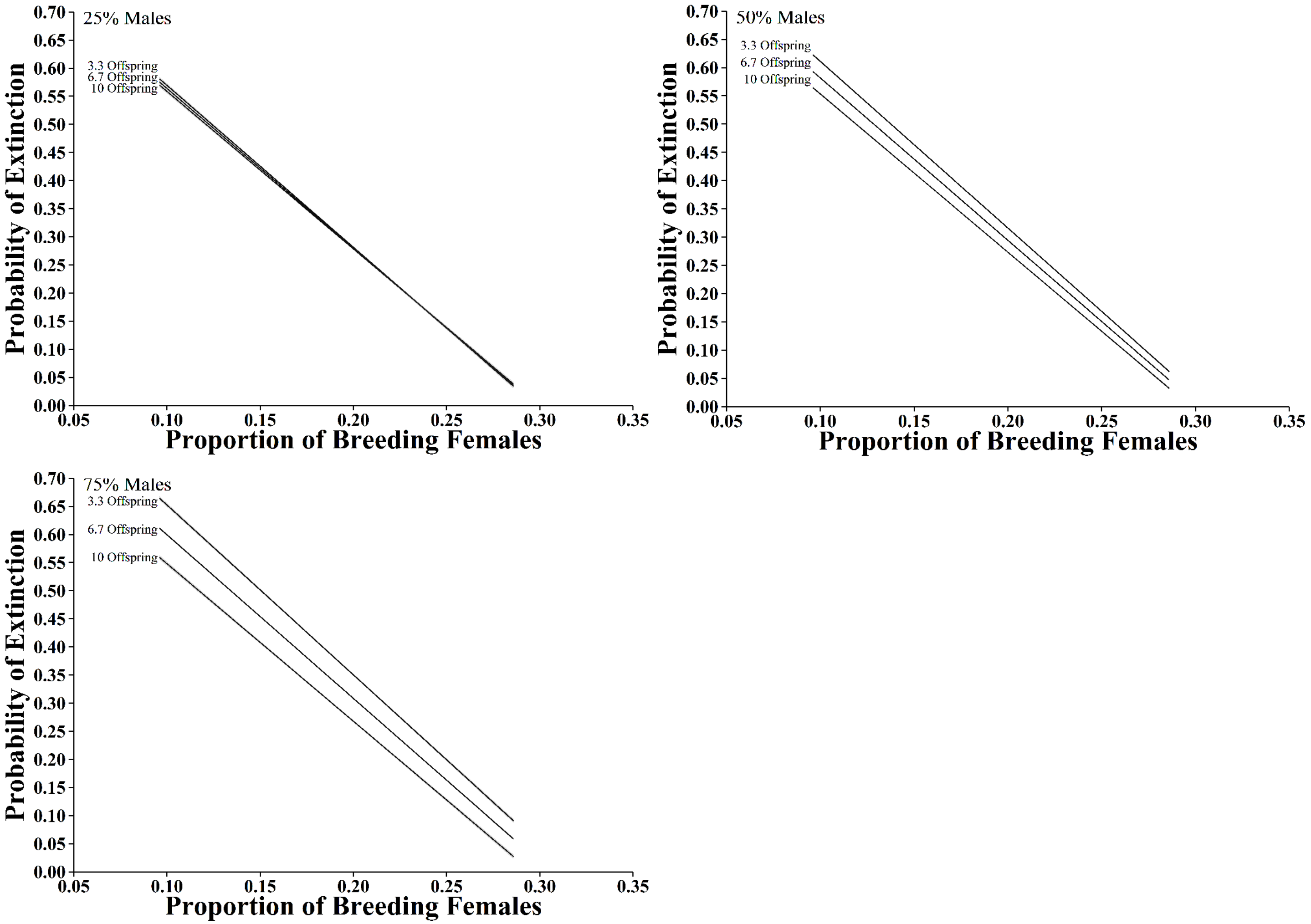

Figure 4.6: Results of multi-factor sensitivity analysis showing three-way interaction of top model parameters (proportion of breeding females, offspring sex ratio, and mean number of offspring) for probability of extinction in the Carlyle Lake population of Eastern Massasauga

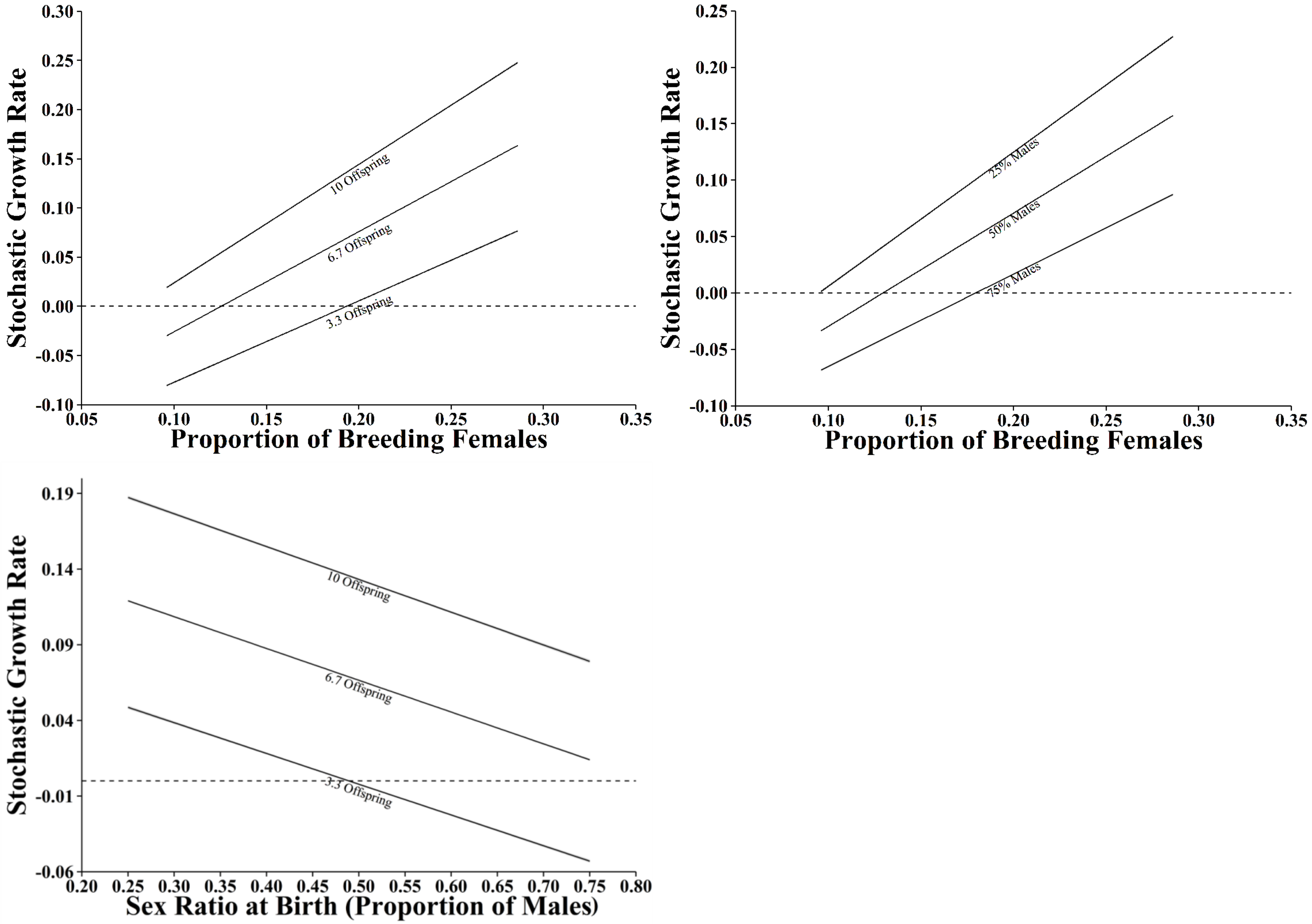

Figure 4.7: Results of multi-factor sensitivity analysis showing two-way interactions of top model parameters impacting population stochastic growth rate in the Carlyle Lake population of the Eastern Massasauga

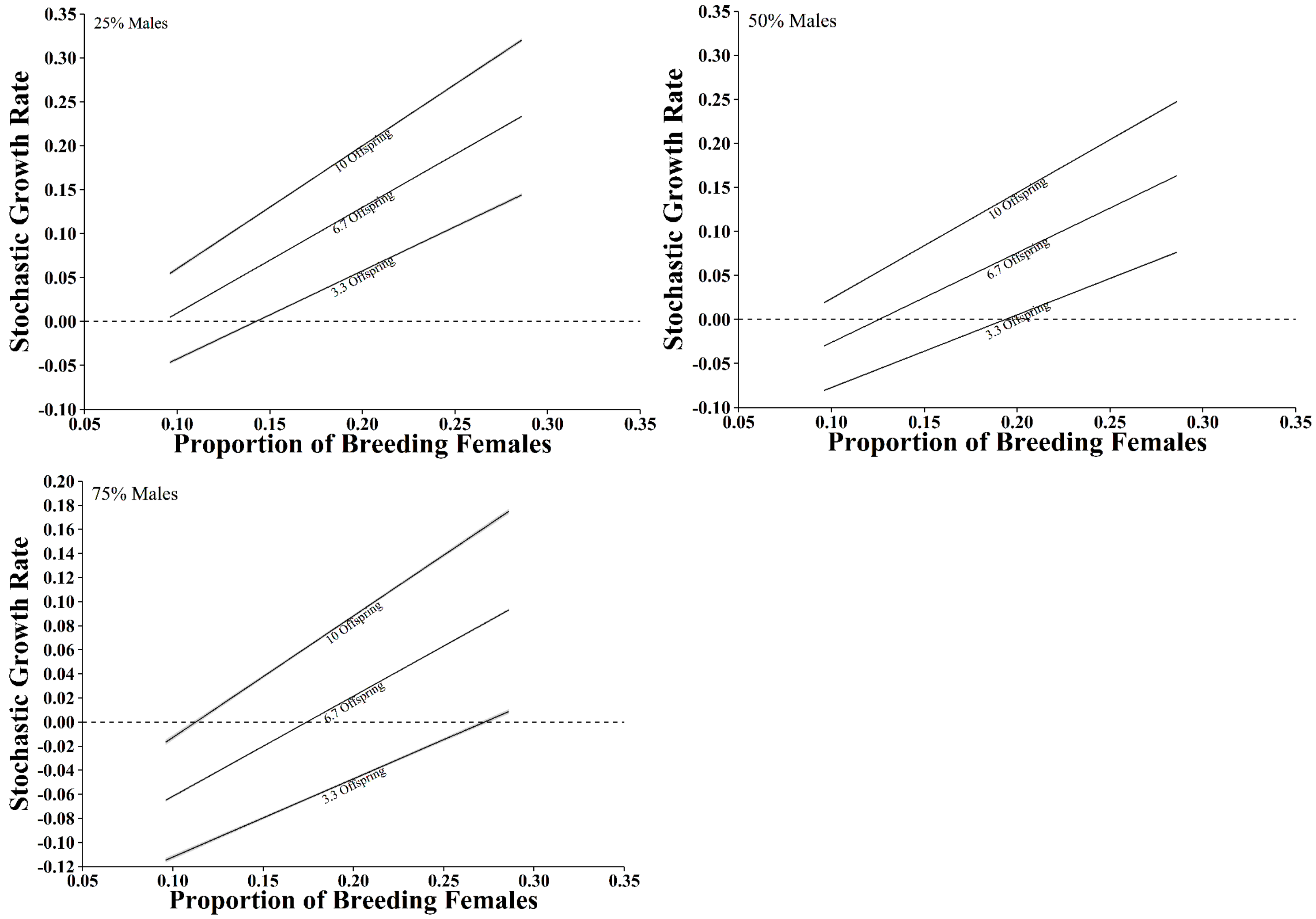

Figure 4.8: Results of multi-factor sensitivity analysis showing three-way interaction of top model parameters (proportion of breeding females, offspring sex ratio, and mean number of offspring) for population stochastic growth rate in the Carlyle Lake population of Eastern Massasaugas

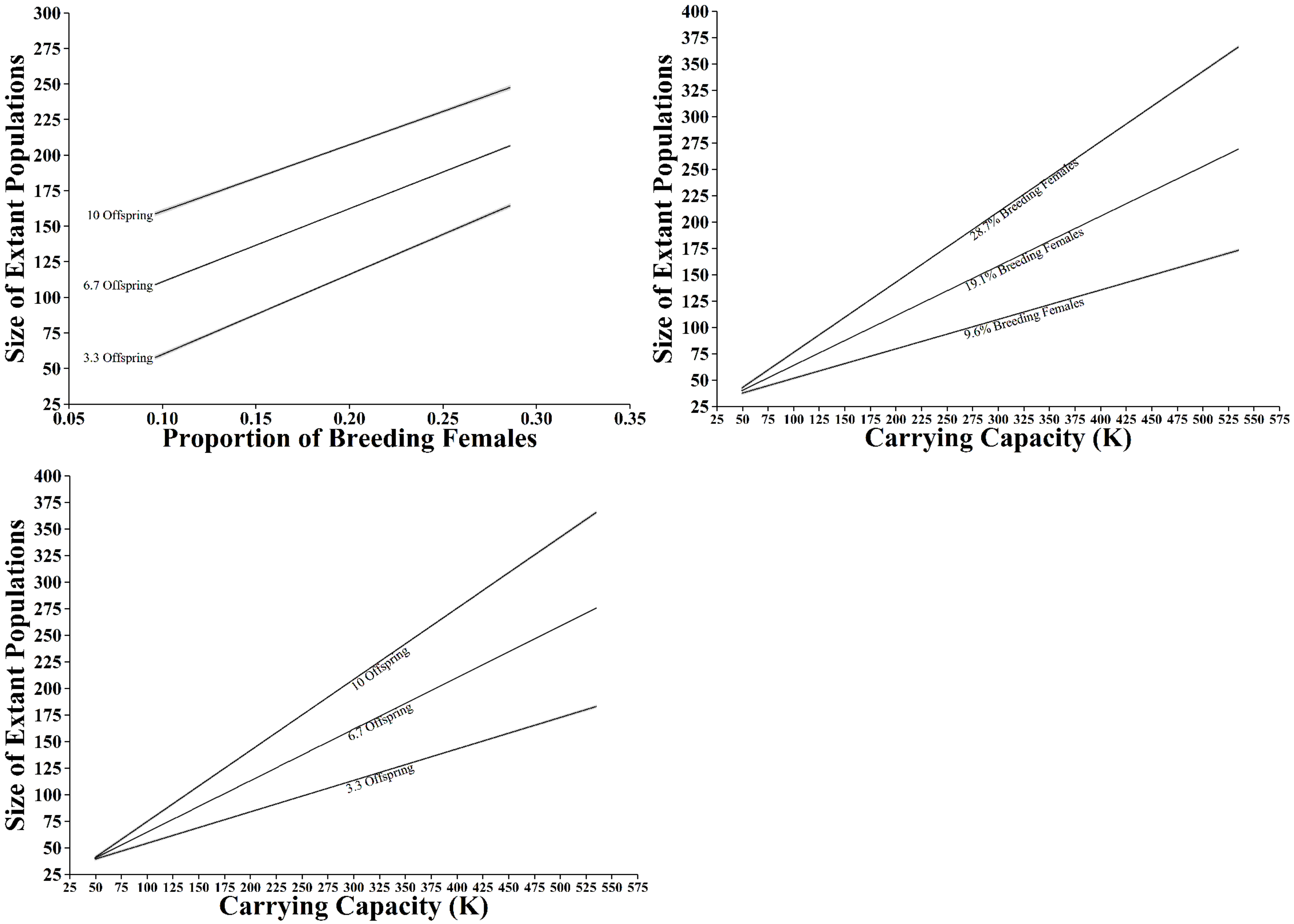

Figure 4.9: Results of multi-factor sensitivity analysis showing two-way interactions of top model parameters impacting size of extant populations in the Carlyle Lake population of the Eastern Massasauga

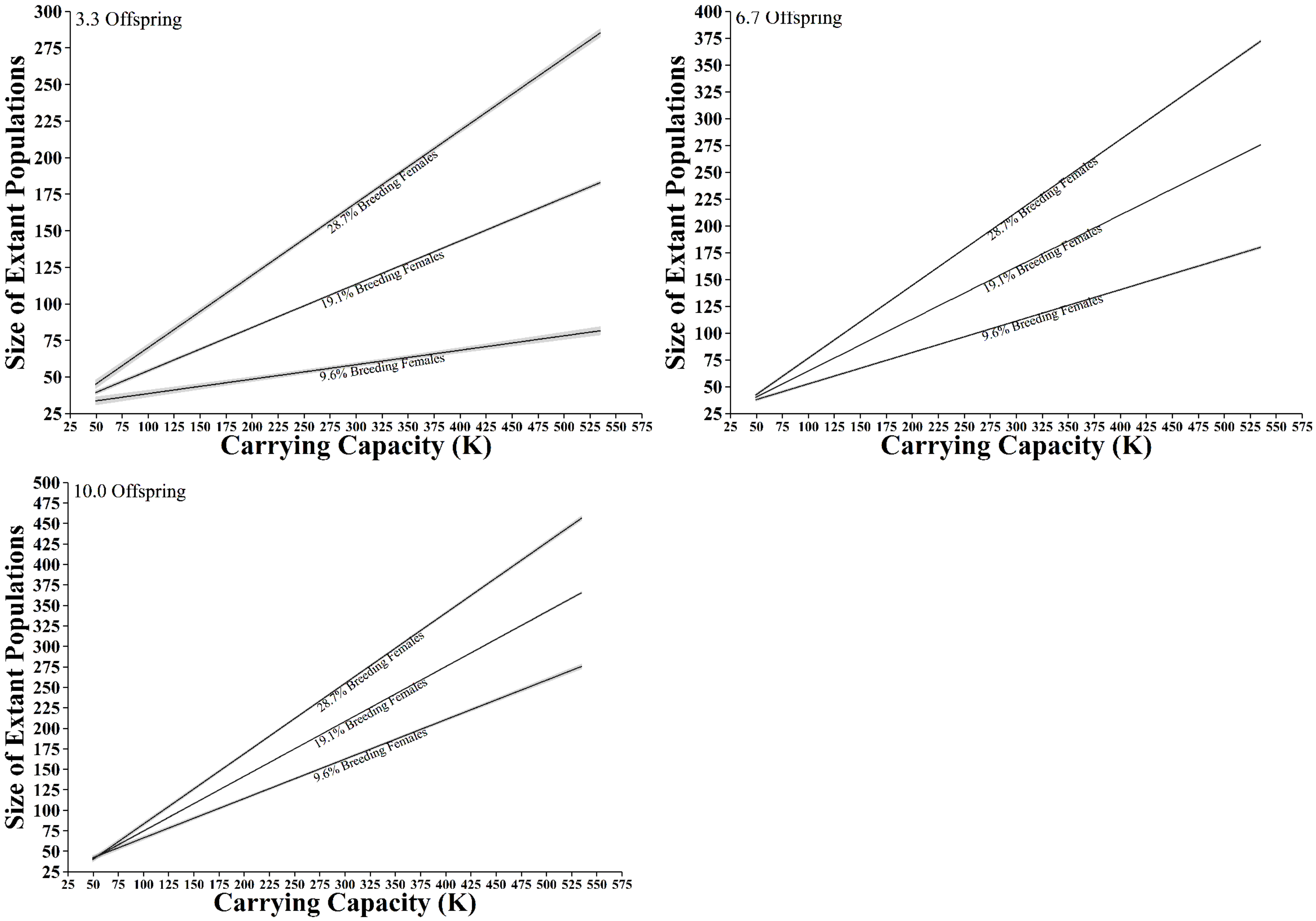

Figure 4.10: Results of multi-factor sensitivity analysis showing three-way interaction of top model parameters (proportion of breeding females, mean number of offspring, and carrying capacity) for size of extant populations in the Carlyle Lake population of Eastern Massasaugas

CONCLUDING SUMMARY

Anthropogenic habitat alterations have resulted in increasingly fragmented species distributions. When considering endangered species, the remaining populations are often numerically small in addition to being isolated. Small isolated populations result in conservation challenges consistent with the small population paradigm by which positive feedback loops hasten the population into the extinction vortex. In Illinois, habitat loss and fragmentation resulting from the conversion of prairie habitat to agriculture has negatively impacted prairie species. The Eastern Massasauga (*Sistrurus catenatus*) was formerly distributed throughout the northern two-thirds of the state, but its distribution has been reduced to a single extant population located at Carlyle Lake, Clinton County. Population monitoring has been ongoing at the Carlyle Lake population since 1999 and the long-term nature of the data is a rarity among snake studies. This study aimed to evaluate the viability of the Eastern Massasauga in Illinois through the integration of population genetics, reproductive biology, sources of mortality, and population viability and sensitivity analysis. Ultimately, this strategy provides the best data for use in developing conservation and management recommendations.

Specifically, this study documented standard population genetic metrics including heterozygosity, allelic diversity, inbreeding, and effective population size at three time points to assess temporal genetic variation (Chapter 1). Reproductive characteristics of litter size, offspring size, reproductive constraints and trade-offs, and developmental survival were evaluated (Chapter 2). The sources of mortality were identified and evaluated by sex stage-class, season, and study type (Chapter 3). Finally, an individual-based model of population viability to project population trajectory was constructed in conjunction with a sensitivity analysis to identify what demographic parameters have the biggest impact on viability (Chapter 4).

Small spatially isolated populations are expected to lose genetic diversity via inbreeding and genetic drift, ultimately resulting in the fixation of deleterious alleles that can decrease fitness, and result in the loss of adaptive potential, thus hampering population viability. As the remaining Eastern Massasauga population is numerically small, locally fragmented, and regionally isolated, it was expected genetic diversity would be low or declining. Additionally, the population is located at Carlyle Lake, which is a man-made impoundment created in the 1960s, and the resulting habitat contraction may have created a genetic bottleneck in the population. To capture temporal variation, the standard genetic diversity metrics of heterozygosity, allelic diversity, inbreeding (F_{IS}), and effective population size were measured at three time points over a 10-year span in conjunction with bottleneck tests. Despite expectations based on population size, Eastern Massasaugas at Carlyle Lake were found to display moderate genetic diversity and no significant inbreeding. A slight decline in allelic richness was noted and is likely the result of a loss of some rare alleles from the population, which can be a precursor to loss of heterozygosity. Effective population size was numerically low but high relative to census population size which may be the result of a polygynous mating system and multiple paternity within litters acting to buffer the loss of genetic diversity in the population.

As population viability is determined by the balance between reproduction and mortality, an understanding of reproductive biology, including reproductive output, selection, and trade-offs is informative for conservation. Data from 27 captive born litters was used to assess reproduction in the Eastern Massasauga and to identify if female body size is imposing constraints on reproductive output, or if there are trade-offs between litter characteristics. Offspring size and number are not associated with female body size and no trade-offs between offspring number, and size are apparent in the Eastern Massasauga suggesting the system is driven by female acquisition of energetic resources. Additionally, as stillborn or undeveloped follicles represent an energetic investment with no reproductive benefit, quantification of the survival rate of a follicle from deposition in the ovary to parturition can also be informative. Follicle survival is negatively impacted by radio-transmitter implantation. These data suggest reproductive output has the potential to increase.

Mortality counters reproductive output, and determining what causes mortality in the population provides the opportunity to decrease mortality rates via conservation management strategies. Using data collected from 155 Eastern Massasauga mortalities occurring from 2000-2011 during radio-telemetry and observational studies, automobiles and predation were determined to be the greatest sources of mortality. Season and mortality category significantly contribute to the number of observed mortalities. Reducing anthropogenic mortality through management strategies is feasible and should include closures of unnecessary roads and careful habitat management practices (e.g. burning).

Determining future viability of endangered species populations is of great interest for conservation. Because of the complex nature of projecting viability, PVA models require a large amount of data, and their accuracy is dependent upon the quality of data used to construct them. Given the lack of long-term data for most snake species, PVA models have seldom been conducted for them. The PVA for the Eastern Massasauga indicates a 65% probability of extinction in 50 years if the current population trajectory is maintained. Sensitivity analysis determines what population parameters have the greatest impact on viability. In the Eastern Massasauga, reproductive characteristics (proportion of breeding females, the number of offspring, and offspring sex ratio) and carrying capacity have the greatest impact on population trajectory.

Larger population sizes are more resistant to stochasticity. Thus, the conservation of the Eastern Massasauga in Illinois ultimately depends upon the restoration and maintenance of additional habitat. Increasing suitable habitat adjacent to currently occupied areas will provide connectivity between occupied patches to promote gene flow and maintain genetic diversity while simultaneously increasing carrying capacity allowing for larger population size. As reproductive output is not constrained by female body size, quality habitat with abundant resources may serve to increase fecundity, thus directly increasing the population growth rate. Reduction of anthropogenic mortality sources will further promote long-term persistence. The PVA itself is not an endpoint, but rather provides a useful tool that should be modified as conservation actions occur to assess progress and update strategies as necessary.