



Species Conservation Planning for the Gray Ratsnake – Carolinian Population (*Pantherophis spiloides*)



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Species Conservation Planning for the Gray Ratsnake – Carolinian Population (*Pantherophis spiloides*) (SCP|PVA)

Report from a workshop conducted on:
November 11-13, 2025
African Lion Safari
Cambridge, Ontario, CANADA



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Many people contributed to the 2026 Gray Ratsnake Species Conservation Planning | Population Viability Analysis workshops and to the completion of this Gray Ratsnake – Carolinian Population Species Conservation Plan. Their time and contributions are gratefully acknowledged here.

PVA Technical Team: Appendix 1 includes a list of Technical Session attendees and their affiliations.

Species Conservation Planning Workshop Participants: Appendix 2 includes a list of in person workshop attendees and their affiliations.

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Workshop host: African Lion Safari

Workshop facilitator and modeler: Amy Chabot (CPSG Canada)

A contribution of the IUCN/SSC Conservation Planning Specialist Group (CPSG) and CPSG's Regional Resource Center in Canada (CPSG Canada), in collaboration with African Lion Safari, Natural Resources Solutions Inc., the PVA Technical Team and workshop participants.

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Abbreviations and Acronyms

BMPs	Best Management Practices
CAZA	Canada's Accredited Zoos and Aquariums
CEMRRIG	Canadian Eastern Massasauga Rattlesnake Recovery Implementation Group
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
COSSARO	Committee on the Status of Species at Risk in Ontario
CPSG	IUCN SSC Conservation Planning Specialist Group
DU	Designatable Unit
ESA	Endangered Species Act (Ontario)
IRT	Infrared Thermography
IUCN	International Union for Conservation of Nature
NHIC	Natural Heritage Information Centre
NRSI	Natural Resource Solutions Inc.
PHVA	Population and Habitat Viability Assessment
PVA	Population Viability Analysis
RIG	Recovery Implementation Group
SAR	Species at Risk
SFD	Snake Fungal Disease
SSC	IUCN Species Survival Commission

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Summary

Gray ratsnakes (*Pantherophis spiloides*) are found at their northern range limit in Ontario, and, as a large-bodied snake species that are found at their northern range limit in Ontario. The species is slow to mature, taking up to 6 to 8 years to reach reproductive maturity. In Ontario, the species is divided into two Designatable Units (hereafter populations): the Carolinian, and the Great Lakes/St. Lawrence populations. In Canada, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) identifies a Designatable Unit as a "discrete" and "evolutionarily significant" population. The two gray ratsnake populations are listed as endangered and threatened, respectively, under Canada's *Species at Risk Act*, 2002. The Carolinian population is found within 100 km of the north shore of Lake Erie and is subject to extreme pressure from threats caused by human activities, including habitat destruction and road mortality. The population is characterized by six small, isolated subpopulations that are likely vulnerable to extirpation.

Natural Resources Solutions Inc. (NRSI) is an environmental consulting firm with strong ties to the Carolinian Zone in Ontario and expertise in native reptiles. Their work with the gray ratsnake in this region over the past 10 years suggests that preventing the eradication of this unique population requires immediate, collaborative, and data-driven conservation action. NRSI engaged CPSG Canada, a Regional Resource Center of the Conservation Planning Specialist Group (CPSG), part of the Species Survival Commission of the International Union for Conservation of Nature, to design and facilitate a conservation planning workshop for gray ratsnake in Carolinian Canada. A Population Viability Analysis (PVA) was developed to help guide the planning process.

The objective of the workshop was to develop an integrated, evidence-based Conservation Action Plan for the Carolinian population of the gray ratsnake. Planning was undertaken in two parts, a series of technical sessions were held between May and November 2025 (for a full list of participants and institutions, see Appendix A), followed by a three-day in person workshop, hosted by African Lion Safari, Cambridge, Ontario, November 11-13, 2025 (for a list of participants and institutions, see Appendix B). The workshop followed CPSG's Species Conservation Planning Principles and Steps, with the aim of bringing all relevant parties together and considering the full range of potential conservation actions, including intensive management options such as creating artificial habitat and integrating ex situ management (e.g. population augmentation) into conservation actions.

The PVA, specifically simulation modelling conducted in the software program Vortex, provided the scientific basis for the development of the evidence-based conservation planning effort for the Carolinian population of gray ratsnake. Despite uncertainty regarding the Carolinian population demographics, the analyses benefited from the large body of work on the Great Lakes St. Lawrence population.

Model scenarios were developed to help guide conservation planning by addressing the following questions:

1. What does the future of the six extant subpopulations¹ in the Carolinian population look like based on best available knowledge? How does model output compare to suggested rates of population decline based on the COSEWIC threat assessment?

2. What is the minimum population size required to maintain 90% of initial genetic diversity in a subpopulation for 30 years?
3. What are the most important demographic drivers of gray ratsnake population trends?
4. What demographic rates are required to achieve a stable or increasing population trend?
5. What would the impact be of augmenting populations? How would this most effectively be accomplished?
6. What would the impact be of combined management efforts to both decrease mortality and augment subpopulations?

Major takeaways and key results from the Population Viability Analysis include:

Q1: Future Outlook:

- The two largest subpopulations, Big Creek and Otter Creek, are likely to decline by more than 90% in the next 30 years.
- At current demographic rates, the probability of population persistence for 10 years is less than 50% for the four other, smaller subpopulations.
- At current demographic rates, the probability of population persistence for 20 years for large subpopulations is less than 50%.
- Small subpopulations are likely to fall below 80% of starting genetic diversity within 10 years.
- Large subpopulations are likely to fall below 80% of starting genetic diversity within 15 years.

Q2: Minimum Viable Population Size

- Breeding populations require at least 50 adults to maintain 80% of genetic diversity over 10 years, and 140 adults to maintain 90% of starting genetic diversity over 10 years. This may be an underestimate, as it is based on the assumption that all individuals are currently all equally genetically diverse and unrelated in each subpopulation.

Q3 and 4: Demographic Drivers

- Neonate mortality, sub-adult mortality and adult mortality are, respectively, the most important drivers of population trends.

Q5 and 6: Management Scenarios

- A decrease in mortality rates (i.e. increase in survival rates) is required for all age classes/stages in order to realize population growth.
- Large subpopulations may be able to achieve growth when mortality is reduced by 34% for all age classes. Smaller subpopulations will not realize the same results simply due to the dynamics that typically characterize small populations (e.g. risk associated with stochastic events).

¹ For the purposes of this report, subpopulations were delineated based on the spatial distribution of occurrence records and the presence of presumed geographic barriers to movement (e.g., high-density road networks and large river systems). Workshop discussions were organized around six distinct and apparently isolated subpopulations to facilitate recovery planning at a biologically meaningful scale. However, these subpopulations do not meet the Ontario Natural Heritage Information Centre (NHIC) criteria for formally recognized subpopulations.

- Augmentation will be most successful with older sub-adult age classes given the species' long subadult phase. It will not lead to long-term population growth for any subpopulation without threat abatement, but will help retain genetic diversity.
- Augmentation at low levels for 10 years coupled with a 20% decrease in mortality in all age classes will likely result in a stabilized population trend for large subpopulations and population growth for small subpopulations.
- Low levels of augmentation for 10 years will help ensure genetic diversity is maintained above 80% of starting levels for both small and large subpopulations over 30 years.
- Low levels of augmentation for 10 years will increase probability of survival to over 90% for large subpopulations and 60% for small subpopulations over 30 years.

Significant knowledge gaps exist regarding baseline demographic data, in particular, data on survival/mortality. Information on connectivity/dispersal; cost of dispersal (e.g. impact to survival); individual genetic diversity; and genetic diversity estimates within and among subpopulations is unknown. Given knowledge gaps, our Status Quo model used estimates population size and carrying capacity. However, the Status Quo model was based on consensus among species experts. Model parameterization was guided by results of research from the Great Lakes St. Lawrence population. We attempted to address uncertainty through use of sensitivity analysis, and by assessing results of simulations from both large and small subpopulations. As determined during the in-person workshop, knowledge gaps would best be addressed through implementation of a coordinated, long-term monitoring program using a standardized protocol across subpopulations.

As a result of the planning process, a subpopulation-specific Conservation Action Plan was drafted to complement the existing federal and provincial recovery strategies and provincial Government Response Statement for the Carolinian population of gray ratsnake. Activities were tailored to each subpopulation based on the scope and severity of identified subpopulation-specific threats identified through a threat assessment completed during the technical sessions. The group identified the goal for management action as: *achieve a self-sustaining population of Carolinian gray ratsnake with minimal conservation intervention*. The objectives required to achieve the goal included:

1. Prevent local extirpation
2. Maintain, improve and secure habitat
3. Secure adequate funding for management
4. Increase awareness and appreciation for the gray ratsnake, leading to human-ratsnake co-existence
5. Engage in advocacy to address obstacles to management.

Six management strategies were identified to achieve the group's goal and objectives for the Carolinian population of gray ratsnake, including:

1. Habitat protection and management
2. Monitoring
3. Awareness
4. Research
5. Augmentation of wild populations

6. Partnerships

For each identified strategy, targeted subpopulation specific management actions were subsequently developed and prioritized in order to mitigate identified threats, address key knowledge gaps, and support the long-term persistence and recovery of the Carolinian population. Workshop participants were then organized into five working groups to develop detailed, implementation-focused action plans for each of the six management strategies. Leads, partners and considerations/obstacles for development of a long-term monitoring plan were identified. A summary of the outcome of the working group's efforts is provided below.

1. *Habitat Protection and Management*: Identifying hibernacula was a central theme, given their importance as critical habitat elements for the species and their potential to inform targeted restoration and land securement activities, as well as targeted monitoring, research, outreach and partnership development. Additional key actions identified included a) protecting habitat, b) reducing threats, c) refining and producing Best Management Practices (BMPs), d) securing land and e) protecting hibernacula.
2. *Monitoring*: It was agreed that a long-term monitoring plan must be developed to address both research needs and knowledge gaps, and to gauge the impact of management actions. Ideally, a coordinated monitoring plan would: a) address the prevalence of snake fungal disease (SFD), b) identify habitat features and hibernacula, c) locate snakes and eggs to salvage for the establishment of a captive insurance population, which could, in future, be used as a source to augment wild populations, d) facilitate sampling to assess genetic diversity, and e) track subpopulation trends and snake abundance. Specific considerations, leads, partners and obstacles were identified, including the need for a long-term standardized protocol.
3. *Awareness*: The working group discussed opportunities/avenues to conduct outreach, and identified specific types of outreach and target groups. Leads were not identified but rather, outreach needs should be revisited once an outreach working group/outreach subcommittee of the Recovery Implementation Group has been established. The group identified the steps necessary to initiate outreach. Of particular importance was landowner-specific outreach, which needs to be focused on protecting hibernacula, edge habitat and implementation of BMPs. While additional planning is needed to identify who/where/how to deliver outreach, a variety of possible outreach approaches were identified, including signage, direct door-to-door outreach, surveys, community engagement and awareness for local farm workers.
4. *Research*: Eight knowledge gaps were identified as critical research needs: a) fine tune estimates of time to extinction for Sixteen Mile Creek, b) update the PVA as additional information becomes available, c) address knowledge gaps identified in PVA (see results of sensitivity analysis), d) determine level of genetic diversity and gene flow within among subpopulations, e) quantify and refine understanding of key habitat features, including development of a Habitat Suitability Model, f) quantify threats, in particular SFD and forestry activities, h) develop BMPs, and i) investigate the benefit of augmentation. Knowledge gaps would largely, with the exception of augmentation, be addressed through implementation of a monitoring program. Specific leads, partners and considerations/obstacles for research, other than augmentation, were identified.
5. *Ex Situ Management*: A cohort of experts from Canada's Accredited Zoos and Aquariums (CAZA) was present at the workshop. The group agreed that it had the capacity for and

could establish a bio-secure captive breeding population for use in augmentation. Collectively, it was decided that the best method would be to recruit founders from any subpopulation by collecting eggs and gravid females, which would be maintained until oviposition and subsequently returned to their respective habitat. Demographic augmentation, via the introduction of captive bred ratsnakes post-hatch, was the recommended approach. Contributing heavily to the decision to pursue ex situ management was the fact that well-established husbandry methods exist for gray ratsnake and the on-going effort in Ontario to include ex situ management in conservation actions for the Eastern Massasauga Rattlesnake (*Sistrurus catenatus*). Prior to concluding, the working session members of the group committed to performing actions that would lead to completion of the required materials.

6. *Partnerships*: A working group was convened that focused on identifying potential, and necessary partners to address threats and engage in management actions. Specific partners, and their potential role in conservation for the Carolinian population of gray ratsnake were identified, including a local college, First Nations located in the Carolinian Zone, provincial and federal governments, developers and other local industry with a focus on agriculture, and both not for profit and for-profit non-government organizations. Roles and responsibilities were assigned to specific leads or organizations. However, implementation is largely going to depend on accessing and securing funding. As such, it was not possible to develop detailed timelines. In reality, all actions are needed simultaneously, and immediately in order to recover this population.

The development of a governance structure to oversee implementation of the Conservation Action Plan was agreed upon, although it was recognized that implementation is contingent upon funding. A Recovery Implementation Group (RIG) will be established, similar to that for the Eastern Massasauga Rattlesnake. Participants noted their interest in volunteering on the RIG, which will meet annually, ideally in a hybrid format, to assess progress on conservation actions.

1. Introduction

This report was prepared to document the planning process and outcome of a two-part workshop process undertaken to identify priority management actions required to ensure that the Carolinian population of gray ratsnake (*Pantherophis spiloides*) does not become extirpated in Ontario. The decline of the taxon in this region is likely due to habitat loss and fragmentation and historical persecution, but the population is also at risk due to loss of genetic diversity and road mortality. Based on recent targeted population surveys, new information is available on extant subpopulations within the Carolinian Zone in Canada, and it is believed that there is a window of opportunity for action to prevent local extirpation of some of the six subpopulations that remain. Protection and recovery of the population is possible if urgent action can be initiated. A coordinated action plan was developed based on the International Union for Conservation of Nature (IUCN) Species Survival Commission (SSC)'s Conservation Planning Specialist Group's (CPSG) species conservation planning workshop process, including a Population Viability Analysis (PVA). CPSG's process provides a logical, transparent and collaborative decision-making process, based upon the best available data. By involving in situ and ex situ conservation experts, the process results in collaborative, practical and effective management recommendations (Figure 1).

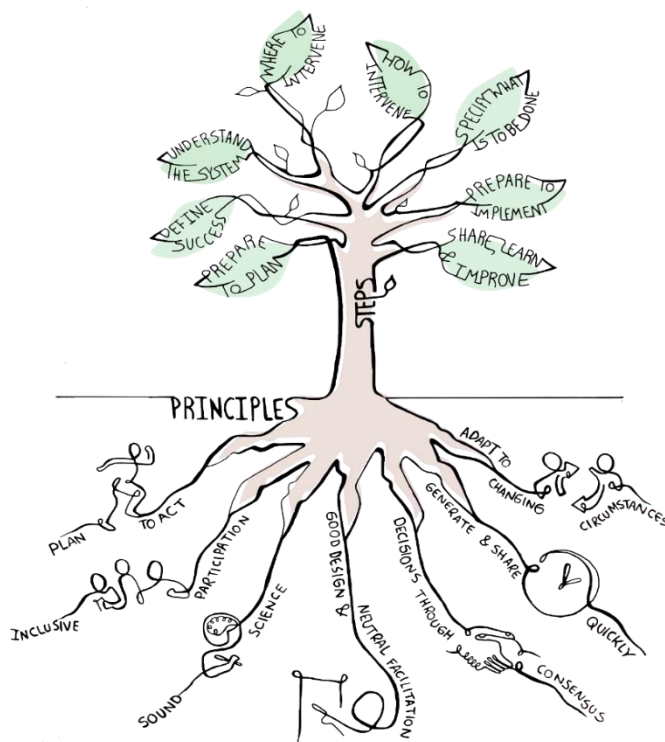


Figure 1. Graphical depiction of CPSG approach to species conservation planning (CPSG 2020). The process is rooted in a set of principles (the roots of the tree) and steps (the leaves of the tree) that emphasize sound science, neutral facilitation and the meaningful participation of all interested and impacted parties.

2. Preparing to Plan

2.1 Project Scope, Rationale and Outcome

Scope: The planning workshop and associated PVA were in aid of the conservation and recovery of the Carolinian population of gray ratsnake.

Rationale: An Integrated Collection and Assessment Planning (ICAP) workshop for all snake taxa in Canada conducted in 2022 found that, for this taxon, *“There is uncertainty around the abundance of the Carolinian Designatable Unit (DU), but the population appears to have declined severely to a very small size and is at risk of extirpation. A greater understanding of the demographics and population status is needed.”* As noted in the ICAP Final Report (Winton et al. 2021): *“It was agreed that the priority for the Carolinian DU is to augment the population (i.e., through demographic manipulation and/or reinforcement) over reintroduction.”*

While the Carolinian population of gray ratsnake is assumed to be declining, no formal population trend or size estimate has been determined, in large part due to the extremely small size of this population. Recent, intensive surveys in historic locations known to support the species have had limited success, with very few locations producing observations of gray ratsnake. The goal of the 2025 conservation planning effort was to identify those alternative approaches and management actions that would be most effective in conservation and recovery of the Carolinian population of gray ratsnake.

Outcome: The desired outcomes of the planning effort were as follows:

- Undertake a PVA to better understand the demographics, population status and trend of the Carolinian gray ratsnake.
- Following upon the ICAP recommendation, investigate potential approaches for augmenting the population.
- Based on knowledge gained through the PVA and from expert elicitation, update existing management actions and identify new actions to achieve objectives of the recovery strategy for the species.
- Develop a Conservation Action Plan with criteria for tracking implementation of management actions. Required funding and potential partners will be informed by the Conservation Action Plan. The action planning will also be used to identify governance requirements.

2.2 Overview of the Planning Process

The Conservation Action Plan was developed in two phases. The first phase focused on on-line technical sessions through which a subpopulation specific threat assessment and Population Viability Analysis were developed. The second phase was an in-person workshop in which the results of the threat assessment and PVA were used as a basis for science-based conservation planning.

Phase One: Technical Sessions

In 2025, a group of species experts came together under the leadership of Natural Resource Solutions Inc. (NRSI) and with the support of Amy Chabot (African Lion Safari), a member of CPSG Canada, to develop a PVA to help quantify the risks to the Carolinian population of gray ratsnake. During on-line sessions held from May to November 2025, a PVA was developed to

support the creation of the Conservation Action Plan (Phase Two). The full list of on-line participants is presented in Appendix A.

Phase Two: In Person Workshop

The results of the PVA were used to inform the development of a Conservation Action Plan for the Carolinian population of gray ratsnake. Over the course of a three-day, in-person workshop hosted by African Lion Safari and NRSI in Cambridge, Ontario, Canada (November 11-13, 2025), species experts, local knowledge holders and gray ratsnake supporters worked collaboratively to identify and develop management actions required to recover the Carolinian population of gray ratsnake. The full list of in-person workshop participants is presented in Appendix B. A summary of the species status, recent research results, subpopulation specific threat assessment and PVA results were presented. This information guided participants as they decided where and how to intervene to achieve subpopulation-specific goals. Working groups prepared detailed action plans for successful interventions that could be applied, as needed, within each subpopulation. Roles and responsibilities for specific management actions were identified, as possible. A Recovery Implementation Group (RIG) was established to provide support, monitor activities and serve as an informal governance body in the coming years. The in-person workshop was sponsored by the Leeds-Grenville Stewardship Council, NRSI, and African Lion Safari, with information technology equipment provided by the Canadian Herpetology Society. Dr. Phil Miller, Director of Single Species Conservation Planning for CSPG, supported the development of the PVA and facilitation of the in-person workshop.

3. Understanding the System

3.1 Species Overview

The gray ratsnake reaches the northern limit of its North American range in Ontario and is one of the province's largest snake species, typically measuring 1.5-2.0 m in length, with some individuals reaching lengths of up to 2.55 m. Within Ontario, the species is currently confined to two areas, which have been identified as separate Designatable Units by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2018): the Great Lakes St. Lawrence population, and the Carolinian population. The Carolinian population is particularly significant from a biogeographic perspective, as it occupies the transition between temperate and northern forest ecosystems. Once widespread across southern Ontario, the Carolinian population is now restricted to a few small, isolated areas north of Lake Erie.

Extensive habitat loss and fragmentation, high road mortality, persecution, and the species' naturally low reproductive rate have led to a severe population decline, with fewer than 300 individuals estimated to remain (COSEWIC 2018). Because the Carolinian population is both geographically isolated and genetically distinct from the other Ontario population, its loss would mean the disappearance of a unique edge-of-range population — one that may hold important adaptive traits for the species' long-term survival. Immediate, collaborative conservation action is therefore needed to prevent further declines and ensure the persistence of gray ratsnake in this ecologically and geographically critical region.

The Carolinian population of gray ratsnake is currently listed as Endangered both provincially under Ontario's *Species Conservation Act, 2025* (formerly, Ontario's *Endangered Species Act, 2007*) and federally under Canada's *Species at Risk Act, 2002*. In situ research and conservation projects, volunteer efforts, and landowner outreach over the past decade have revealed a sharp decline in both the number and size of known subpopulations. Many formerly occupied sites no longer support the species. Remaining subpopulations are mostly small and isolated, and thus vulnerable to extirpation. Ongoing threats continue to drive declines, and, without conservation intervention, the gray ratsnake faces a high risk of disappearing from Ontario's Carolinian Zone.

3.2 Subpopulation Descriptions

COSEWIC has designated two populations of gray ratsnake in Ontario. Previous work on the Carolinian population reflects on it as one panmictic population. In reality, the Carolinian population of gray ratsnake in Ontario is comprised of several small, geographically isolated subpopulations, varying in size and carrying capacity (Table 1), as well as other characteristics. The landscape context of each subpopulation range is briefly described below:

Sixteen Mile Creek (West Elgin County): The landscape within the range of this subpopulation is characterized by severely isolated, small forest blocks. Agricultural lands surround all natural lands, with one major, low-quality ravine and creek system in the area. The local area is intersected mainly by low-traffic backroads, with one highway traversing the major ravine. The area is almost entirely privately owned.

Big Creek and Otter Creek (Norfolk and Elgin Counties): This subpopulation falls within the two subwatersheds, Big Creek and Otter Creek. Tributaries within these subwatersheds are characterized by deeply incised valleys, particularly in areas where

sand plains are underlain by more resistant clay. Forest cover is relatively high, including along the steep-sided ravines. It is unknown whether these two subpopulations have any remaining physical and genetic connectivity. The area has a mixture of high and low-traffic backroads, as well as several very high-traffic highways. Agricultural lands surround all natural lands. A high proportion of land within the range of the Big Creek subpopulation consists of protected conservation lands, whereas the majority of the land within the Otter Creek subpopulation range consists of privately owned lands.

Ruthven and Oriskany Quarry (Haldimand County): These two subpopulations occur within a landscape characterized by isolated treed vegetation communities of varying sizes, including both upland forests and lowland swamps, within generally rolling topography. The local area has a mixture of high- and low-traffic backroads and several high-traffic highways bisect and border the natural forest blocks. Active quarries and agricultural lands surround all natural lands in the range of the Oriskany subpopulation, with mostly private landownership. Agricultural lands surround all natural lands in the range of the Ruthven subpopulation. The two subpopulation are separated by the Grand River, a highway, and agricultural lands.

Shorthills (Niagara Regional Municipality): The subpopulation occurs within an area characterized by a very large contiguous forested area featuring steep, rolling hills, deeply incised valleys, and ravines associated with the Fonthill Kame and the Niagara Escarpment. In the northern half of the forested area, habitat is largely restricted to protected conservation lands that have a high density of public trails. The southern half of the forested area occurs on private lands. The local area is characterized by mostly low-traffic backroads, with a couple high-traffic roads or highways bordering the forested area. Majority of adjacent lands consist of agricultural lands, with some residential areas.

For the purpose of the workshops, participants worked within the framework of six separate and isolated subpopulations. During the workshop, participants from the Ontario Natural Heritage Information Centre (NHIC) noted that neither Big Creek and Otter Creek, nor Ruthven and Oriskany meet their criteria as separate subpopulations as they were not over two kilometers apart. More correctly, in each case, they should be considered as two units within one subpopulation. In reality, all subpopulations are in fact comprised of several small units that likely vary in the degree of connectivity.

Carrying capacity (K) (Table 1) was estimated for each subpopulation by multiplying the total area of suitable habitat within the subpopulation range by an estimated density of 0.261 individuals per hectare. Suitable habitat was defined as deciduous and mixed forest and associated forest edge habitat. The subpopulation range was defined using both recent and historical occurrence records and the application of a 2 km buffer around each occurrence record, representing the typical maximum dispersal distance of individuals from hibernacula. Under this approach, all suitable habitat occurring within the resulting subpopulation range was assumed to be available to support the subpopulation. The estimated density of 0.261 individuals per hectare was based on research and long-term monitoring conducted by Queen's University Biological Station (QUBS), where this density was estimated for Gray Ratsnake in optimal habitat.

Population size (N) (Table 1) was estimated for each subpopulation by multiplying an assumed average of 10 individuals per hibernaculum by the estimated number of hibernacula within the occupied range of each subpopulation. Occupied range was defined using recent occurrence records (2000 - present). The estimated number of hibernacula was determined based on field observations and the application of a 2 km buffer around occurrence records, representing the typical maximum dispersal distance of individuals from hibernacula. Under this approach, recent observations were assumed to be associated with one or more hibernacula located within the surrounding landscape, and the number of hibernacula contributing to those observations was estimated accordingly.

These carrying capacity and population estimates should be considered preliminary and are subject to considerable uncertainty due to limited information on hibernacula distribution, subpopulation boundaries, occupancy, and demographic characteristics. While recent fieldwork has helped provide some estimates, there is an urgent need for this work to continue to improve characterization and gather demographic data required to update the PVA.

Table 1. Summary of the characteristics of the subpopulations that comprise the Carolinian population of gray ratsnake. These estimates are approximate and based on volunteer survey effort over several years.

Subpopulation	Estimated Population Size	Estimated Carrying Capacity
Sixteen Mile Creek	20	20
Big Creek	100	545
Otter Creek	120	300
Ruthven	20	350
Oriskany Quarry	20	60
Shorthills	20	350

3.3 Threat Assessment

During the on-line technical sessions, participants were asked to conduct a threat assessment, guided by the IUCN threat assessment calculator (Salafsky et al. 2025). The results were very similar to those of the 2018 COSEWIC threat assessment for the Carolinian population of gray ratsnake. COSEWIC's assessment was undertaken for the population as a whole, not at the subpopulation level, as was done during the technical sessions (Appendix C). COSEWIC has developed categories of decline to quantify likelihood and severity of the impact of varying threat levels as follows: Negligible impact = <1% decline; Low/Slight impact = 1-10% decline; Moderate/Restricted impact = 11-30% decline; High impact = 31-70% decline; Extreme/Pervasive impact = 71-100% decline. These categories were used in the technical sessions, with the category of risk and level of threat assigned on a subpopulation basis. The results were then transformed into a threat diagram for the population, and for each subpopulation (Appendix D), which guided planning during the in-person workshop.

Eight threats were noted as being of medium to high likelihood for all subpopulations: 1) increased farming activity, 2) increased/improved roads, 3) logging/inappropriate logging prescriptions, 4) inappropriate habitat stewardship/land management, 5) invasive non-native species, 6) problematic native species, specifically subsidized predation; 7) changes in temperature regimes; and 8) changes in precipitation regimes. All six subpopulations faced a high likelihood of threats due to climate change (changes in temperature and precipitation regimes), increased non-native species and increased subsidized predation, but the severity of the impact of these threats could not be quantified (i.e. severity was noted as 'unknown' in all

subpopulations). In all subpopulations, the severity of all but increased farming activity (low to moderate) and increased/improved roads (low to moderate) was considered low or was unknown. While increased rural housing was considered to have a low likelihood in all but the Shorthills subpopulation (where it was noted as moderate), the severity of the threat was believed to be high.

Assessing threats at the subpopulation level highlighted that each subpopulation faced somewhat unique threats, and thus conservation actions would need to be tailored for each subpopulation. The likelihood and severity of increased farming activity, increased/improved roads, logging/inappropriate logging prescriptions, and inappropriate habitat stewardship/land management varied among subpopulations. Of note, the Oriskany Quarry subpopulation is the only subpopulation that faces a threat due to a quarry operation. Increased residential housing is of greatest concern to the Shorthills subpopulation, reflecting its proximity to an urban setting, versus the rural surroundings of the other subpopulations. Big Creek faces fewer threats from farming than other subpopulations, but other land management practices are of concern, including recreational use. Recreational activities posed a threat, albeit low, in only Otter and Big Creek subpopulations. Inappropriate habitat management was considered less of a threat overall in Otter Creek than in other subpopulations. Of note, the greatest threat to Sixteen Mile Creek was not identified by the threat assessment but rather by the PVA – the constrained carrying capacity of this subpopulation is its greatest threat.

3.4 PVA Simulation Modeling

3.4.1 PVA Model Objectives

Each of the individual model scenarios was run using 1000 replicates. This iterative procedure is necessary as each replicate will yield a different outcome resulting from random annual variation in expected rates of breeding and survival in the simulated gray ratsnake population. Each scenario was projected forward for 30 years: 10 years is considered to be a ‘generation’ time for the species by COSEWIC and threat assessments are based on a 3 generation, or 30-year timeframe. Extinction was defined as when only one sex remained in a population. Model scenarios were developed to help guide conservation planning by addressing the following questions:

1. What does the future of the six extant subpopulations in the Carolinian population look like based on best available knowledge? How does model output compare to suggested rates of population decline based on the COSEWIC threat assessment?
2. What is the minimum population size required to maintain 90% of initial genetic diversity in a subpopulation for 30 years?
3. What are the most important demographic drivers of gray ratsnake population trends?
4. What demographic rates are required to achieve a stable or increasing population trend?
5. What would the impact be of augmenting populations? How would this most effectively be accomplished?
6. What would the impact be of combined management efforts to both decrease mortality and augment subpopulations?

3.4.2 Key Results

The full PVA model results are provided in Appendix E. Major takeaways and key results include the following:

Q1: Future Outlook:

- The two largest subpopulations, Big Creek and Otter Creek, are likely to decline by more than 90% in the next 30 years.
- At current demographic rates, the probability of population persistence for 10 years is less than 50% for the four other, smaller subpopulations.
- At current demographic rates, the probability of population persistence for 20 years for large subpopulations is less than 50%.
- Small subpopulations are likely to fall below 80% of starting genetic diversity within 10 years.
- Large subpopulations are likely to fall below 80% of starting genetic diversity within 15 years.

Q2: Minimum Viable Population Size

- Breeding populations require at least 50 adults to maintain 80% of genetic diversity over 10 years, and 140 adults to maintain 90% of starting genetic diversity over 10 years. This may be an underestimate, as it is based on the assumption that all individuals are currently all equally genetically diverse and unrelated in each subpopulation.

Q3 and 4: Demographic Drivers

- Neonate mortality, sub-adult mortality and adult mortality are, respectively, the most important drivers of population trends.

Q5 and 6: Management Scenarios

- A decrease in mortality rates (i.e. increase in survival rates) is required for all age classes/stages in order to realize population growth.
- Large subpopulations may be able to achieve growth when mortality is reduced by 34% for all age classes. Smaller subpopulations will not realize the same results simply due to the dynamics that typically characterize small populations (e.g. risk associated with stochastic events).
- Augmentation will be most successful with older sub-adult age classes given the species' long subadult phase. It will not lead to long-term population growth for any subpopulation without threat abatement, but will help retain genetic diversity.

3.4.3 Model Limitations and Next Steps

Significant knowledge gaps exist regarding baseline demographic data, in particular, data on survival/mortality. Information on connectivity/dispersal estimates and cost of dispersal (e.g. impact to survival) is lacking. Information on genetic diversity and the range of genetic diversity maintained within and among subpopulations is unknown. We were unable to create a Status Quo model that reflected the demographics of gray ratsnake in the Carolinian population with certainty, other than estimates of population size and carrying capacity, as demographic information is a knowledge gap for this population. However, species experts believe that the majority of life history parameters are likely similar to the well-studied Great Lakes St. Lawrence population – thus model parameterization was guided by results of research from this population. We attempted to address this large degree of uncertainty in our modelling approach through use of sensitivity analysis, and by assessing results of simulations from both large and small subpopulations.

As determined during the in-person workshop, knowledge gaps would best be addressed through implementation of a coordinated, long-term survey and monitoring program using a standardized protocol across subpopulations. During the in-person workshop, leads, partners and considerations/obstacles for development of a long-term monitoring plan were identified. Implementation is contingent upon funding.

3.5 Species Conservation Planning Workshop

3.5.1 Overview

The workshop was structured to first provide participants, which included individuals who had not participated in the technical sessions, with the necessary background information required for action plan development. Presentations were provided on the status and biological threats to the species, recent research activities, key knowledge gaps and previous recovery planning. In plenary, participants identified obstacles to recovery for the Carolinian population of gray ratsnake. Based on this foundation of knowledge, the participants then defined a goal (i.e. vision of success) and performance indicators for objectives by which the participants believed the goal for the population could be achieved. Subpopulation Working Groups were established in which participants identified the management action they believed would be most effective, specific to each subpopulation's unique characteristics, demographics and threats. The result was a suite of management strategies that would be effective across all subpopulations, but not necessarily a high priority for all subpopulations. Management actions were then prioritized within each subpopulation. Subsequently, management strategy based Working Groups convened to develop detailed action plans, and, as possible, roles and responsibilities required to achieve specific management interventions. Finally, in plenary, the participants discussed how best to collaboratively implement the action plan, including discussions of governance structure. As a result, a Recovery Implementation Group was established.

3.5.2 Background Presentations

A number of brief presentations at the start of the in-person workshop helped to summarize work to date and existing knowledge.

Oriskany Subpopulation (Anne Yagi)

Radio telemetry and habitat mapping was undertaken in 2001 and 2002. Telemetry was restricted to two individuals, with behaviour and movement patterns monitored over two seasons. Research determined home ranges, confirmed site fidelity and identified a hibernation area near an active quarry. The snakes were found to hibernate communally. An average number of days spent in hibernation was quantified. Since the study period, the quarry has expanded such that it is now located closer to the hibernation site.

Big Creek Subpopulation (Heather Fotherby)

NRSI conducted a study of habitat use, movement patterns and distribution using records of previous occurrence and radio telemetry from 2017 to 2020. A Habitat Suitability Model was employed and validated. Key habitat features (e.g. hibernaculum) were identified and characterized, and habitat preferences were quantified. The subpopulation distribution and potential road crossing hotspots were identified. Movement patterns were calculated using mean total and maximum distances moved, and home ranges determined based on mean estimated 100% Minimum Convex Polygons. Individuals of the subpopulation preferred edge habitats, and

selected habitats with higher vertical vegetation structure, a higher density of trees, a larger number of cavity trees, and areas near slopes that were greater than 20°. Based on quantified habitat preferences and available guidance on snake habitat creation, artificial habitat consisting of nest structures, and thermoregulation and foraging features (i.e. rock and brush piles, and logs, some of which were modified) were installed in early 2021 on several properties within the subpopulation range and regularly monitored between 2021 and 2023. Installed features were only documented to be used by other species of snake during monitoring efforts, but the modified logs were incidentally confirmed to be used by gray ratsnake in 2025.

Survey Efforts (Ryan Wolfe and Jon Wedow)

Available data, historic records, and review of historic and current aerial imagery were used to identify potential areas where gray ratsnakes might currently be found. Once a potential area was identified, landowners within the vicinity were contacted. Where permitted, field work included ground-truthing and site characteristic quantification (i.e. soil type, topography, vegetation age and quality, land usage and disturbance) was undertaken. Surveys focused on deciduous and mixed forest edges. Time of year was found to be important, with the best time for surveys being from mid-April to mid-June. Weather and time of day were also important. Multiple extant subpopulations were located, however, all were on privately owned, isolated lands and are unlikely to contain large numbers of individuals due to limited habitat availability.

Habitat Suitability Model (Center for Large Landscapes, Annika Keeley and Tyler Creech)

The Center for Large Landscapes is working with the Nature Conservancy of Canada, NRSI and other partners to conduct case studies across Canada for multiple species with different movement patterns and habitat needs, including gray ratsnake. The results will provide evidence regarding management actions needed to improve connectivity. Approximately 200 habitat patches larger than 8ha in size were identified based on a suitability model. Resistance models were developed based on landcover class and distance to nearest habitat patch to quantify the cost of movement through patches. Connectivity metrics (measures of how well the patches are connected in the study area) were developed. Connectivity metrics for the model suggest that habitat is fairly well connected for the species within the study area, but could be improved. Results of resistance models and connectivity metrics were used to identify potential management impacts. Additional models will be used to simulate landscape changes associated with management effects and to see how the identified connectivity metrics respond. Results are expected by mid-year of 2026 and can be shared at that time.

Climate-Land Use Interactions and Future Distributions (Andrea Gómez-Sánchez)

Research was conducted at Queen's University to assess how climate change may affect this species' distribution in Ontario. Gray ratsnake in Canada occur at the northern extent of the climatic conditions tolerated by the species across its range. Consequently, there may be potential for a northward expansion of the species' distribution under future climate scenarios. Modeling conducted to assess land-use change showed a likely reduction in range extent, whereas climate change models suggested the potential for range expansion. In summary, habitat loss and alteration associated with land-use change may pose a greater threat to gray ratsnake than increases in temperature resulting from climate change.

3.5.3 Obstacles to Conservation for the Carolinian Population of Gray Ratsnake

Conservation and recovery of the Carolinian population of gray ratsnake faces both biological threats and broader social, regulatory, and logistical challenges. Participants reviewed the threat

assessment developed during the technical sessions and identified additional factors to be considered during conservation planning. The factors incorporated into subsequent conservation action planning are summarized as follows:

A. Life History/Population Characteristics:

- Seasonal accessibility – there is only a short window of access to undertake management and conduct field surveys during the spring breeding season.
- Given the life history characteristics of the species (e.g. extended time as a subadult), research and management will need to be undertaken on a longer time frame than for other species, which is often not feasible under current funding models.
- The extended subadult stage and lack of knowledge on behaviour during this stage, compared to that of adults, makes it difficult to identify threats and plan.
- This population has already experienced a drastic decline over an extended period of time and is critically endangered. There is a risk that the species will become extirpated before management actions can be implemented. Conservation action is needed immediately.
- The population decline has led to the formation of small, isolated subpopulations that are particularly vulnerable to demographic and environmental stochasticity, as well as the loss of genetic diversity.

B. Knowledge gaps:

- Significant knowledge gaps exist regarding baseline demographic data, with data on survival of neonates and subadults being of particular importance. Information on connectivity/dispersal estimates and cost of dispersal (e.g. impact to survival) is lacking. Information on the degree of total genetic diversity and the specific distribution of that diversity within and among subpopulations is unknown.
- There is a lack of data and historical records, thus little is known about the demographics of the Carolinian population of gray ratsnake, in particular, survival and reproduction rates.

C. Funding

- Funding availability and existing dedicated funding is limited. The time it would take to secure funding for multi-year management activities is of concern given the species status, and long-term funding is unlikely given the currently available grants and funding sources. Additionally, funding for conservation is currently a low priority given the global economy and concerns for more urgent needs from a human perspective (e.g. housing, health care, etc.).

D. Emotion

- Successful conservation action requires public support and engagement; however, snakes are often viewed negatively by many people. Compared to other Species at Risk (SAR), snakes tend to be lower in priority due to lack of empathy or understanding. Current conservation education and staffing to undertake outreach is limited, in particular in the area in which the Carolinian population of gray ratsnake occurs. Conservation actions should include branding and other efforts to make the plight of the snakes of concern to the public, and foster interest and support for the species.

F. Habitat

- Most of the land in the Carolinian Zone is privately owned, requiring significant time and funding to legally access properties, and limited opportunity to change land management. Ultimately, conservation action will require landowner participation and cooperation. Coupled with this is a general lack of knowledge about programs that support land protections and could be used to support conservation of SAR in the area.

E. Bureaucracy, Government and Partnerships

- Despite the critically endangered status of the species, there is limited to no enforcement of legislation that protects the species, and limited political will to address this. Further, the government has competing interests, for example, with aggregate extraction and economic development, which is often prioritized over the needs of SAR.
- The whole of the Carolinian Zone in Ontario has been heavily impacted by human presence. Land trusts and other conservation organizations are working to secure and restore land but, currently, there is no area-wide action plan and limited public lands.
- Given the ‘at risk’ status of many species in the Carolinian Zone, a single-species focus is difficult to justify and find adequate resources (funding and personnel), especially where life history characteristics require a prolonged time frame for action.
- It would appear that there is limited political will at the federal and provincial levels to support management action required to ensure survival of the species in Canada. The legal framework is complex.
- Designation as a different Designatable Unit makes rescue from the Frontenac population impossible.
- There is currently a lack of collaboration from various sectors and among different organizations, whereas a collaborative approach will be needed to adequately address the subpopulation level management needs.
- The timeframe for academic studies is long compared to the immediacy of need to address knowledge gaps. Managers and researchers are often physically distant, making communication important.

F. Insights from the PVA

- None of the small subpopulations (Oriskany, Ruthven, Sixteen Mile Creek, Shorthills) are likely to persist for more than 30 years, despite having potential for growth.
- Carrying capacity in all but the Sixteen Mile Creek subpopulation is sufficient for growth to reach minimum viable population size requirements adequate to maintain genetic diversity at 80-90% starting levels.
- Probability of population survival in small subpopulations is likely to fall below 50% before the end of the next decade.
- Small subpopulations are likely to fall below 80% of starting genetic diversity in the next decade. Given the optimistic starting point used in Vortex, genetic diversity may already be a concern, as it may be inadequate to avoid inbreeding depression and allow for future adaptation.
- The two large subpopulations (Big Creek and Otter Creek) are likely to decline by more than 90% in the next 30 years.
- Large subpopulations are likely to fall below 80% of starting genetic diversity within the next two decades (~14 years based on simulations).

- Probability of population survival for large subpopulations is likely to fall below 50% within two decades.
- Breeding populations >50 individuals are likely needed to maintain 80% of genetic diversity over 10 years. If the subpopulations have already experienced reduced levels of genetic diversity as a result of population declines to date, this may be a conservative estimate.
- Neonate mortality, sub-adult mortality and adult mortality are, respectively, the most important drivers of population trends. However, reductions in mortality rates (i.e. increase in survival rates) across all life stages are required in order to realize positive population growth.

4. Defining Success

Participants were asked to identify concerns for the Carolinian population of gray ratsnake and then to envision what they would like to see as a future outcome, as a means to identify performance indicators, and metrics for judging the impact of management actions. The main concerns included:

1. The low/declining population due to high mortality rates
2. Negative public opinion regarding snakes
3. Lack of funding for management action
4. Reduced genetic diversity in the population
5. Landownership – almost all of the population is currently found on private lands

Based on these concerns, a list of desired outcomes for management action was developed, including:

1. Preventing extirpation and creating self-sustaining subpopulations
2. Ensuring adequate amount of habitat to support populations
3. Minimizing the need for conservation intervention for the population
4. Minimizing the cost/funding required to maintain a viable population
5. Increasing tolerance between humans and rat snakes

From these concerns and desired outcomes, the group identified their goal for success, or vision statement, was as follows: *Self-sustaining population of Carolinian population of gray ratsnake with minimal conservation intervention.*

Objectives identified by the group to achieve the goal included:

1. Preventing local extirpation
2. Maintaining/improving and securing habitat
3. Securing adequate funding for management needs
4. Increasing awareness and appreciation for the gray ratsnake, leading to human-ratsnake co-existence
5. Engaging in advocacy to address obstacles to management.

Based on this goal and objectives, the group identified the following metrics as measures of success of management actions:

1. Preventing local extirpation
 - a. Degree of connectivity among subpopulations (increasing)
 - b. Number of remaining subpopulations (stable or increasing)
 - c. Abundance (increasing)
 - d. Effective population size reflects census population size (increasing N_e)
 - e. Subpopulation growth trend (stable or increasing)
 - f. Genetic diversity (stable or increasing)
 - g. Development of an ex situ insurance population representative of the population
2. Maintaining/improving and securing habitat
 - a. Degree of connectivity (increasing)
 - b. Number of hectares/acres of suitable habitat (increasing)

- c. Number of key features e.g. hibernacula, nest sites, etc. (increasing)
 - d. Development of habitat suitability model and remote sensing to quantify habitat
 - e. Model and remote sensing results (increase of desired metrics)
- 3. Securing adequate funding for management needs
 - a. Number of grants/funding sources (increasing)
 - b. Cost of recovery (decreasing)
 - c. Cost of unit for each type of action (decreasing)
 - d. Need for resources (personnel, funding, etc.) (decreasing)
 - 4. Increasing awareness and appreciation for the gray ratsnake, leading to human-ratsnake co-existence
 - a. Public opinion – increasing positive attitude e.g. as measured in public surveys (increasing)
 - b. Increased percentage of population that know about and care about the species in the Carolinian Zone (increasing)
 - 5. Engaging in advocacy to address obstacles to management.
 - a. Level of financial support (increasing)
 - b. Engagement in conservation land tax incentive program (increasing)
 - c. Government support of action plan (increasing)

5. Decide Where to Intervene

What has already been identified, and what can we add or alter knowing what we know now for the Carolinian population?

The COSEWIC conducted a status assessment in 2007, with a subsequent review in 2018, which resulted in the Carolinian population of gray ratsnake being listed as endangered in Canada. In 2020, the federal government adopted and slightly expanded the 2010 Ontario Provincial Recovery Strategy.

The Committee on the Status of Species at Risk in Ontario (COSSARO) conducted a status assessment in 2009, with a subsequent reassessment in 2020, which resulted in the Carolinian population of gray ratsnake being listed as endangered in Ontario. A provincial Recovery Strategy for the species was developed in 2010, which provides generalized advice to the Province of Ontario on the recommended approach to recovery for both populations. Subsequently, the province released a Government Response Statement (GRS) that listed a fairly comprehensive set of government-led and government-supported actions for the species in Ontario.

The provincial government's goal for the recovery of the gray ratsnake is to halt the decline of the Carolinian population. The government supports investigating the feasibility of increasing the distribution and size of the Carolinian population. A summary of government led and government supported actions is provided in Appendix F.¹

Under Canada's *Species at Risk Act*, 2002, population and distribution objectives for the species must be established. Consistent with the goal set out in the Government of Ontario's Government Response Statement, Environment and Climate Change Canada's population and distribution objectives for the gray ratsnake in Canada for the Carolinian population is *to maintain and, where biologically and technically feasible, increase the current abundance, area of occupancy and habitat connectivity within the subpopulations of the gray ratsnake in the Carolinian population*.

During the workshop, participants were asked to review the list of government-led and government-supported actions and identify which actions would best address the threats for individual subpopulations based on their unique characteristics and demographics. Participants were also asked to identify additional activities required for the recovery of the Carolinian population of gray ratsnake. No additional conservation actions were suggested beyond those identified in existing recovery planning documents. All of the actions identified in previous

¹ It should be noted that since the workshop, the *Endangered Species Act*, 2007 (ESA) has been repealed and replaced with the *Species Conservation Act*, 2025 (SCA). Under the old ESA, the Government of Ontario was legally obligated to review scientific advice, draft a recovery strategy, and publish a Government Response Statements (GRS). The new SCA explicitly removes the requirement for the province to prepare recovery strategies, management plans, and GRS. Under the new SCA, recovery strategies and GRS are no longer legally mandated or required to be followed, but the province retains the flexibility to use previously published GRS documents as voluntary guidance or reference materials. It is also worth noting that many species previously protected under the old ESA are no longer protected under the SCA; however, gray ratsnake (*Pantherophis spiloides*) remains to be listed on the new Protected Species in Ontario List and currently continues to be afforded protection under the new Act.

recovery planning were noted as being required/suitable management interventions. Innovative methods for implementing some of the actions were noted as follows:

Monitoring:

- Develop monitoring methods focused on scent or pheromones. Shed skins could be used as a training tool in scent tracking. This method would likely best be implemented at specific times in the annual cycle, such as when snakes are entering or leaving hibernacula.
- Develop monitoring methods focused on use of infrared thermography (IRT). Research is needed to determine if an exothermic animal would be detectable. Methods could include use of a drone with IRT capabilities, or use of hand-held IRT cameras.

Genetic Connectivity:

- Artificial insemination could be used as means of increasing connectivity among subpopulations, or between ex situ and wild subpopulations. Research is needed to refine sperm storage protocols and develop suitable methods.

Reduce Threats:

- The IUCN Human Wildlife Conflict Specialist Group has resources regarding snake/human conflict. Resources are available at <https://www.hwctf.org/> and <https://www.hwctf.org/snakes>.

6. Decide How to Intervene

Which actions will most effectively address each subpopulation needs?

Participants in the workshop were asked to identify actions that would be most effective in each subpopulation, given the area and species-demographic characteristics. Subpopulation-specific experts were identified in advance and were assigned to a specific working group. Workshop participants with broadly applicable knowledge were split among working groups to ensure equal representation of specific expertise within each group. Action planning for Ruthven and Oriskany Quarry was completed together, in a single working group, due to the close proximity and similarity between the two subpopulations in habitat characteristics and threats. The outcome of working group discussions is summarized in the following sections. A list of prioritized actions for each subpopulation as identified by the working groups is summarized in Table 2.

Table 2. Prioritization of management actions among Carolinian subpopulations of gray ratsnake. Priority is indicated using stars, with more stars denoting higher priority. The number of priority actions varied by subpopulation based on the area's characteristics and gray ratsnake demographics, thus, for example, three stars in one column may denote a highest priority activity in one subpopulation while seven stars will denote the highest priority in another subpopulation.

Management Action	Sixteen Mile Creek	Shorthills	Big Creek	Otter Creek	Ruthven/Oriskany
Protection and Management					
Protect Habitat					**
Reduce Threats	**	**	*****	****	**
Refine BMPs		*		*	
Secure Land			***	***	
Protect Hibernacula					*****
Monitoring					
General	***			**	*
Distribution			*****		
Extend Monitoring			**		
Awareness					
General	*	****	****	****	***
Research					
PHVA ¹					
Genetic Connectivity			*****		
Habitat Use		***	*		
Quantify Threats	***		***		
Ex Situ Management	***	*			**
Partnerships					
General					****

¹PHVA: Population and Habitat Viability Analysis

6.1 Sixteen Mile Creek

Subpopulation Characteristics

Sixteen Mile Creek is a subpopulation believed to consist of no more than 20 snakes. It is believed to be at carrying capacity, which makes the area of lesser value for in situ focused recovery efforts. In this area, the most recent observations of gray ratsnake have been documented close to a creek system and within a small, isolated forested block that is surrounded

by agricultural land. The entirety of the habitat is privately owned, which could present options for land purchase and subsequent management. The surrounding agricultural landscape may act as a barrier to movement, which may have the benefit of reducing the likelihood of road mortality. However, while the habitat is of high quality, it is insufficient to sustain the population and there is no room for growth. The working group participants felt that this subpopulation was functionally extirpated and management action in this area would have limited benefit to the Carolinian population. However, the individual animals could provide benefit for the larger population. For example, if they could be used to help maintain, or improve genetic diversity in other subpopulations through an augmentation program.

Major Threats to the Subpopulation

Based on results of the PVA, the greatest threat to the Sixteen Mile Creek subpopulation is the constrained carrying capacity of this subpopulation. Based on the results of the threat assessment completed during the online technical sessions (Appendix C), increased farming activity (likelihood = high, severity = medium) was identified as the major threat to the survival and recovery of this subpopulation. Additional threats identified to have a high likelihood but low or unknown severity included increased roads/road improvements, logging and inappropriate logging prescriptions, inappropriate habitat stewardship and land management, invasive species, subsidized predators, changes in temperature regimes, and changes in precipitation and hydrological regimes. Threats with low likelihood but which would have high severity included increased rural housing.

Subpopulation Specific Objectives

The subpopulation specific goal is to ensure persistence in the immediate future while efforts are undertaken to establish an augmentation program, with the long-term objective of using this subpopulation as a source to enhance the genetic diversity in other subpopulations.

Subpopulation Specific Actions

Four actions were identified as being of importance to the Sixteen Mile Creek subpopulation. For each, several options were identified that could help sustain the current population.

1. Protection and Management

a. Protect habitat: Actions to ensure implementation of BMPs and other beneficial stewardship actions would help protect key habitat features. The action was believed to have moderate risk, as it involves alerting farmers to the presence of an ‘at risk’ species on their property.

b. Reduce threats:

i. Protect hibernacula and key habitat features: Working with farmers to reduce edge disturbance around agricultural fields and to limit or stop the removal of hedgerows would help to reduce risk faced when snakes move within the area. The action was believed to have moderate risk, as it involves alerting farmers to the presence of an ‘at risk’ species on their property.

ii. Create artificial habitat: The installation of nest boxes and, if possible, hibernacula could prove beneficial. The action was believed to be low risk but had a moderate potential for failure based on research to date (i.e. it can take many years for snakes to find and utilize the nest sites and hibernacula).

2. Monitoring: Monitoring of artificial habitat such as nest boxes or hibernacula could assist in monitoring species abundance/persistence of this subpopulation. Risk was

identified as low to medium as the monitoring is fairly non-invasive, but monitoring hibernacula could influence behaviour.

3. **Awareness:** Actions that would improve or build landowner relationships would help ensure that landowners understand the species needs, and could lead to reduced persecution. The action was noted as being of medium risk due to the fact that landowners would be alerted to the presence of an ‘at risk’ species on their property.
4. **Research**
 - c. **Quantifying threats:** Research could be conducted to assist in identification of key habitat features and habitat use. This action was noted as having high risk due to potential risk of mortality or change in behaviour associated with radio telemetry work that would likely be required.
 - d. **Ex Situ Management:** Individuals/eggs could be used to establish a source population to enhance and maintain genetic diversity in other subpopulations. This action would likely pose considerable risk to both the individuals involved and the donor subpopulation. However, it was noted that this population is likely functioning as a ‘sink’ rather than a ‘source’ within the broader Carolinian population. Given the results of the PVA and estimated carrying capacity of the subpopulation, removal of animals could potentially be considered a form of population rescue.

6.2 Shorthills

Subpopulation Characteristics

Shorthills is the subpopulation for which the least current information is available regarding the presence of gray ratsnake. It is believed to consist of approximately 20 snakes, or less, and have a carrying capacity of $K = 350$. The majority of the habitat in which the species may be found is public land. A privately owned area of potentially suitable historic habitat that used to support gray ratsnake is located nearby. The core area of this subpopulation is not bisected by any roads, however, road mortalities of individuals of the species have been documented on the surrounding, intense road network.. Given the area’s proximity to an urban center, the human pressure (e.g. recreational use, traffic volume on surrounding roads) is high. Within the core area, recreational trail density is high, with trail users consisting of hikers, mountain bikers, and equestrians. High levels of trail use by a variety of user groups may contribute to the spread of non-native, invasive species, habitat fragmentation and degradation, and increased mortality risk, similar to the effects associated with roads. Non-native, invasive plant species, including Japanese Stiltgrass (*Microstegium vimineum*), are present within the area and represent a localized management concern. Recent shifts in vegetation species composition resulting from invasive forest pests (e.g. Emerald Ash Borer) have also been observed, including increased dominance of Black Walnut (*Juglans nigra*) and Multiflora Rose (*Rosa multiflora*), and associated reductions in understory structure complexity and species diversity. Nearby Niagara College is a potential partner for habitat stewardship and monitoring. Ontario Parks and the Niagara Peninsula Conservation Authority have initiated restoration activities and management of non-native, invasive vegetation on their properties in the area. While habitat restoration and enhancement is underway, it will take some time for any benefits to ratsnake to be realized (e.g. newly planted trees will take time to mature).

Major Threats to the Subpopulation

Based on results of the threat assessment (Appendix C), the major threats (likelihood = medium/high, severity = medium/high) to the subpopulation were identified to be increased rural

housing, increased farming activity, and increased or improved roads. These are reflective of the subpopulation's proximity to a major urban center in southern Ontario. Threats that have a high likelihood but low or unknown severity included logging and inappropriate logging prescriptions, invasive non-native species, subsidized predators, changes in temperature regimes, and changes in precipitation regimes.

Subpopulation Specific Objectives

The subpopulation-specific goal is to focus on restoration and enhancement of existing habitat to support future reintroduction or augmentation efforts.

Subpopulation Specific Actions

Three management actions were identified as being of particular importance to the Shorthills subpopulation, with several methods identified for each.

1. Protection and Management

- a. **Reduce threats:** Local partners (e.g. Niagara Peninsula Conservation Authority, Ontario Parks, Niagara College) could assist with habitat restoration efforts, as the subpopulation faces many threats due to human impacts, including:
 - i. **Invasive species** – the area could be restored by managing invasive species, which is a low-risk activity to the species. However, complete removal would likely not be possible.
 - ii. **Loss of suitable habitat** – existing habitat is degraded and requires restoration.
 - iii. **Road mortality** – efforts to mitigate road mortality are needed, however, at some risk due to potential negative response from the public. Similarly, an awareness campaign for public recreational areas might be beneficial but comes with some risk due to public pushback.
 - iv. **Policy/Advocacy** – efforts to develop local policy that would benefit gray ratsnake, for example, where it does not already exist, a bylaw restricting the cutting of large, mature trees, may help to address habitat loss.
- b. **Refine BMPs:** adapting existing regulations and outreach to private landowners regarding land management practices may help to increase/improve ratsnake habitat. However, workshop participants felt that while this was a low-risk activity, it was likely not the best use of time and limited resources.

2. **Awareness:** Signage and events could be developed that include information on how to report sightings to help improve awareness of the species and distribution/abundance in the subpopulation. The area offers potential for partnerships with the Niagara Peninsula Conservation Authority, Ontario Parks, Niagara College and other organizations responsible for management of public lands. Partnerships could be leveraged to assist with outreach.

3. Research

- c. **Habitat use:** Partners such as Niagara College and other local organizations could be engaged to assist in survey and research efforts. Research is needed to determine if the area can support a viable population of gray ratsnake, identify specific restoration activities to improve habitat suitability, and refine quantitative assessment of the area's ability to support ratsnake.
- d. **Ex Situ Management:** Augmentation of the species is a lower priority, after more pressing needs to address threats and assess habitat suitability. This information is needed to assess the feasibility for augmentation. Augmentation was considered a

high-risk activity in the immediate future due to potential for disease transmission, possible negative public opinion and knowledge gaps related to habitat suitability.

6.3 Big Creek

Subpopulation Characteristics

Big Creek is a subpopulation believed to support approximately 100 snakes, with a carrying capacity of $K = 350$. Connectivity with the Otter Creek subpopulation may be possible. However, monitoring within the Big Creek subpopulation documented limited road crossings by individuals, and several roadways occur between the two nearest known occurrences within these subpopulations, potentially limiting movement and connectivity between them. It is believed that it is critical for the area of habitat to be large enough to sustain local seasonal movements within its boundaries, without being fragmented by roads. The majority of the habitat in this subpopulation occurs on public land, which is subject to human pressure due to recreational activities. The land is managed by multiple organizations, which may offer opportunities to coordinate and adapt land management practices to improve habitat conditions for ratsnake. One land trust has already expressed interest in partnering on conservation efforts. However, it may be challenging to implement broad-scale management across multiple partners. Further, land managers may have other priorities that are not beneficial for ratsnake.

Major Threats to the Subpopulation

The results of the threat assessment (Appendix C) identified the major threat (likelihood = high, severity = medium) to the survival and recovery of this subpopulation to be increased/improved roads. Threats identified to have a medium or high likelihood but low or unknown severity included increased farming activity, logging/inappropriate logging prescriptions, inappropriate habitat stewardship and management, invasive species, subsidized predators, pathogens, and changes in both temperature and precipitation regimes.

Subpopulation Specific Objectives

Big Creek, alongside Otter Creek, is considered to be the last stronghold for the species in this population. The goal for the subpopulation is to build upon previous habitat conservation and enhancement efforts for the species and implement actions to address road mortality. Future land securement or restoration should focus on improving connectivity to Otter Creek and surrounding historic habitat.

Subpopulation Specific Actions

Four actions were identified as being of importance to the Big Creek subpopulation. For each, several options were identified that could help sustain or improve the viability of the subpopulation. In all cases, lack of funding was identified as a risk.

1. Protection and Management

- a. **Reduce threats:** Roads are assumed to present the main threat to this subpopulation. There are some limited road closures by local government to reduce road mortality. This comes with a socio-economic impact and risk of losing public support for conservation.
- b. **Secure lands:** Habitat modelling being undertaken by the Center for Large Landscapes will identify priority areas for land securement. Securement should focus on increasing connectivity between habitat patches, and eventually with the Otter Creek subpopulation. The priority for habitat restoration on secured lands is

- thinning/removal of pine plantations and creating edge habitat. Land securement faces risk if information on priority sites was made public, which might result in raised cost for securement. This activity runs the risk of not being beneficial if management to benefit the species is not possible.
2. **Monitoring:** Increased monitoring comes with associated risk of increased human impact on potentially critical habitats (e.g. disease spread, invasives, presence changing snake behaviour, poaching, disturbance).
 - a. **Distribution:** Monitoring should focus on identifying the current distribution of ratsnake (e.g. through mark-recapture) and on identifying suitable habitat (e.g. through habitat assessment), with results being focused on informing land procurement to increase connectivity.
 - b. **Extend monitoring:** Monitoring should initially focus on identifying hibernacula, with subsequent monitoring directed toward improving estimates of subpopulation size and trends.
 3. **Awareness:** Before awareness programs can be implemented, audience(s) and delivery method(s) need to be identified. Use of ambassador animals could assist in awareness campaigns. The risk exists that feasibility of being able to implement awareness programs is low, in particular as costs to adequately implement an awareness campaign is relatively high. There is also a risk from competing, incorrect messaging from detractors. Health and safety considerations exist for volunteers/staff involved in awareness campaigns. The following target audiences were identified as priorities for outreach:
 - a. **Foreign Workers/Mennonite Population:** outreach should consider targeting these audiences with a trusted partner and potentially a translator. Persecution is likely related to a lack of knowledge.
 - b. **Landowners:** Pamphlets could be developed and distributed strategically by target audience. The *Black Rat Snake: A Landowner's Guide to Helping it Recovery* developed for the Frontenac population could be used as a template, and revised for the Carolinian population. Farmtario is a local newspaper delivered to farmers – articles could be submitted for publication to target this audience. Ideally effective conservation would result in landowner led conservation efforts, for example, similar to efforts for Bobolink elsewhere in Ontario.
 - c. **Local Government:** Efforts are underway to develop a Memorandum of Understanding with Norfolk County to promote the use of appropriate wildlife crossing designs as part of any future road and bridge improvements and replacements to address road mortalities. A brief was delivered to the local government council to promote this effort.
 - d. **Community:** Events or volunteer opportunities exist with local high school/elementary students geared to encourage participation in conservation.
 4. **Research**
 - a. **Genetic connectivity:** Connectivity among populations should be quantified to help inform augmentation efforts. This action requires substantial effort and cost and results may be compromised due to small sample sizes.
 - b. **Habitat use:** A Habitat Suitability Model, or similar spatial prioritization tool, should be developed to identify and prioritize areas for land securement, habitat restoration, and enhancement.

- c. **Quantify threats:** Threats to this subpopulation are still not fully known. A better understanding of which threats are acting upon the subpopulation, interactions and severity is needed. For example, the impact of logging practices and wild turkeys are unknown. Risks include the time and effort required to quantify threats (e.g. snake fungal disease) that may result in delays to conservation action, and the identification of current actions (e.g. logging practices) as inappropriate could lead to decreased public support.

6.4 Otter Creek

Subpopulation Characteristics

Otter Creek is a subpopulation believed to support 120 snakes with a carrying capacity of $K = 350$. There is potential for connectivity to the Big Creek subpopulation. The majority of the habitat is private land. Road mortality rates are the highest in this subpopulation, comparative to the other subpopulations. Land management is largely suitable, which is evident in the subpopulation size. However, private landownership may provide limited opportunities to change existing land management practices that are not beneficial to ratsnake.

Major Threats to the Subpopulation

Based on the threat assessment (Appendix C), the primary threats to the subpopulation were identified as increased/improved roads, and increased farming activity, both of which were assigned a high likelihood of occurrence and medium severity rating. Additional threats assessed as having a medium to high likelihood of occurrence, but low or unknown severity, included logging/inappropriate logging prescriptions, inappropriate habitat stewardship and management, invasive species, subsidized predators, and changes in temperature and precipitation regimes.

Subpopulation Specific Objectives

Otter Creek, alongside Big Creek, is considered to be the last stronghold for the species in this population. The subpopulation specific goal is to promote population viability to reach carrying capacity.

Subpopulation Specific Actions

Three actions were identified as being of importance to the Otter Creek subpopulation. For each, several options were identified that could help sustain the current population.

1. Protection and Management

a. Reduce threats:

- i. **Road mortality** is considered the major threat to this subpopulation. While road mitigation for this species can be more challenging than for many other reptile species, as exclusion fencing must account for their climbing ability, extensive mitigation measures may not be necessary. If snakes can be encouraged to use existing ravine systems as movement corridors, only limited fencing may be required. However, road mitigation should be carefully planned and implemented, as inappropriate mitigation strategies could result in unintended negative impacts on the subpopulation.
- ii. **Predation** by species such as wild turkey, American mink, cats, raccoon, and coyote may pose a threat to the subpopulation, although its significance remains poorly understood and requires further investigation. Management actions

targeting predation are considered moderately risky due to low feasibility and uncertainty regarding the magnitude of the threat.

- b. **Refine BMPs:** Priority should be placed on identifying hibernacula, basking sites and other important habitat features, and focusing BMPs on these. Further information on subadult snake habitat requirements is needed.
- c. **Secure lands:** For ratsnake, land purchase should be focused near ravines. This activity is low risk, but requires work to identify suitable partners and funding sources.

2. Monitoring:

- a. **Snake fungal disease (SFD)** is prevalent in this region. Monitoring should be focused in this area. Efforts should be made to integrate with existing monitoring for the fungal pathogen. This action potentially comes with high risk due to inadvertent spread of the disease.
 - b. **A long-term monitoring program** should be established for this and the other subpopulations where the species has recently been found. Currently, monitoring is focused largely on only the Big Creek subpopulation. Research priorities (e.g. genetic connectivity) could be incorporated into a monitoring program. Monitoring and research efforts should be strategically designed to address key knowledge gaps and support effective conservation decision-making. This action has a low risk associated with surgery to implement radio telemetry tracking devices.
3. **Awareness:** The small, tight-knit community provides a network to promote BMPs through word-of-mouth communication. A concise, community-specific BMPs fact sheet could be developed to support outreach efforts. While likely a low-risk activity, this action has the risk of creating negative public perception if messaging is not well-crafted. Identifying a local champion and novel marketing strategies could mitigate the risk.

6.5 Ruthven/Oriskany Quarry

Subpopulation Characteristics

These two subpopulations are each estimated to consist of up to 20 snakes. The Oriskany Quarry area has an estimated carrying capacity of approximately 60 snakes, while the Ruthven area is believed to have a carrying capacity of up to 350 snakes. There is limited opportunity for expansion of either subpopulation.

Habitat associated with the Oriskany Quarry occurs on private land owned by two landowners, one of whom has a negative perception of snakes. The properties are also associated with an active quarry operation. The Grand River separates the Oriskany Quarry and Ruthven areas, while surrounding agricultural lands and road network further limit connectivity between the two subpopulations. Limestone extraction at the quarry is nearing the end of its operational cycle, creating a potential opportunity for habitat restoration that could benefit gray ratsnake. Although uncertain, there is also potential for the property to be acquired by a land trust in the future, which could provide additional opportunities for habitat conservation and management.

The Ruthven area consists primarily of a large tract of low-lying, forested wetland surrounded by agricultural lands. Based on recent observations of gray ratsnake, suitable habitat may extend further east along small, fragmented forest patches associated with riparian corridors.

Major Threats to the Subpopulations

Based on the combined likelihood and severity ratings presented in Appendix C, the major threats to these two subpopulations, characterized by medium to high likelihood and medium to high severity, include increased farming activity, increase quarrying activity, and increased or improved roads. Additional threats assessed as having a high likelihood of occurrence but low or unknown severity include logging or inappropriate logging prescriptions, invasive species, subsidized predators, and changes in temperature and precipitation regimes. For the Oriskany subpopulation specifically, inappropriate habitat stewardship or land management was assessed as having a high likelihood of occurrence, although the severity of this threat is expected to be low if realized.

Subpopulation Specific Objectives

Recent observations are limited to road kills. The main goal for this subpopulation is to identify, and subsequently protect hibernacula.

Subpopulation Specific Actions

Five actions were identified as being of importance to the two subpopulations. Several options were identified for each action.

1. Protection and Management:

- c. **Protect habitat:** Given private ownership and negative perception of the species held by some landowners, this action is considered a high priority with moderate risk due to the complexities associated with establishing partnerships with the quarry. Feasibility is considered high in the Oriskany Quarry area but remains unknown at Ruthven. Before this action can be undertaken, critical habitat features and priority areas for protection should be identified. Efforts should also consider the protection and restoration of hedgerows, as well as the creation of artificial habitat features, such as hibernacula and nesting sites.
- d. **Protect hibernacula:** Identifying and protecting hibernacula is the most important action for this subpopulation
- e. **Reduce threats:** Threats reduction can be opportunistically undertaken in association with habitat protection. Focus should be on known threats, including persecution, road mortality mitigation, and farming practices. Research is needed to better understand the severity of disease and predators – threat abatement needs and methods should be informed by the outcome of the research. The activity has low risk, but also low feasibility.

- 2. **Monitoring:** The small subpopulation size will make development of a monitoring program difficult. Monitoring should include efforts to gather samples for genetic analysis. This action has low risk and high feasibility, but will require substantial effort.
- 3. **Awareness:** Initial development of an awareness campaign should focus on identifying appropriate partners and audiences, as well as determining how the program can be most effectively implemented. Messaging should address key issues including logging and forest management practices, persecution of snakes, and the management and modification of natural systems. This action is considered highly feasible and low risk; however, outreach efforts should account for the potential risk of poaching or illegal collection of wild snakes and the sensitivity of species occurrence data. Careful consideration should therefore be given to the information shared publicly and how it is communicated.

4. Research:

a. **Ex Situ Management:** Given the very small size of this subpopulation, consideration could be given to using snakes from the area to develop an insurance population.

5. Partnerships: Partnerships will be key to the success of all management actions. In addition to the quarry, efforts should be made to engage the municipality to address road mitigation. This action comes with moderate risk and is only moderately feasible due to potential for miscommunication and potentially differing values.

7. Specify What is to be Done

Participants convened in five management focused working groups to develop detailed action plans: 1) Habitat protection and management, 2) Monitoring, 3) Awareness, 4) Research and 5) Partnerships. Action plans developed are summarized below.

7.1 Habitat Protection and Management

Identifying hibernacula was a central theme as this is both a critical habitat element for this species and would also help focus targeted land restoration or securement efforts, as well as facilitating targeted monitoring, research, outreach and partnership development (Table 5). Key actions for habitat protection and management efforts were identified as follows:

1. *Protect Habitat*
 - a. Identify and protect critical habitat features
 - b. At identified hibernacula, establish and maintain appropriate buffer zones to protect the feature, including through local awareness campaigns, policy development and enforcement, etc.
 - c. Identify which lands would be beneficial to secure, based on presence of habitat features
 - d. Protect and restore hedgerows and other habitat features, informed by knowledge of key habitat requirements and features
 - e. Where feasible, review and provide input into quarry management and rehabilitation plans to identify and incorporate opportunities for habitat protection and future site restoration that would benefit gray ratsnake
2. *Reduce Threats*
 - a. Install artificial habitat in subpopulations (e.g. nest boxes, hibernacula)
 - b. Identify both beneficial and harmful farm practices (e.g. edge disturbance, removal of hedgerows)
 - c. Quantify invasive species and their habitat
 - d. Identify areas with high road mortality and identify mitigation strategies
 - i. Work with municipalities to implement mitigation as part of on-going road repairs
 - ii. Implement awareness campaign for the Ministry of Transportation and municipalities
 - iii. Communicate with Conservation Authorities and other organizations that can assist in road mitigation efforts
 - e. Identify areas where recreational use of public land is a problem and develop a plan to reduce impact
 - f. Identify required policy/advocacy to address specific threats
 - g. Identify methods to reduce persecution of snakes
 - h. Identify native subsidized predators and develop management plans to reduce predation
 - i. Determine severity of SFD
3. *Refine/Produce BMPs*
 - a. Public – identify and develop appropriate (e.g. layperson) recommendations to increase/improve habitat, using BMPs developed for other populations or species, as possible

- b. Private
 - i. identify and develop suitable land management practices for specific industries, (e.g. time of hay harvest on farms), using BMPs developed for other populations or species, as possible
 - ii. identify key partners for implementation and dissemination (e.g. Ontario Woodlot Owners Association, municipal government)
 - c. Key elements of BMPs were identified as including:
 - i. Maintaining hedgerows and edges
 - ii. Leaving old trees/stumps
 - iii. Timing for edge management e.g. recommendations for burn, vs mowing
 - iv. Educating landowners about legality of certain actions
 - v. Incorporate Indigenous knowledge/stories
 - d. Develop subpopulation-specific BMPs and hold workshops to raise awareness and build support
 - e. Ensure BMPs are easily accessible/widely disseminated
4. *Secure Land*
- a. Identify priority lands to secure to protect subpopulations and enhance connectivity
 - b. Identify key partners e.g. First Nations, land trusts, to secure priority lands in each subpopulation
 - c. Develop policy (e.g. conservation easements) or move toward government ownership to ensure land management is regulated
 - d. Identify existing incentive programs for farmers, specifically focused on habitat features such as maintaining hedgerows
5. *Protect Hibernacula*
- a. Find/confirm locations of hibernacula in each subpopulation
 - b. Identify method to ensure compliance with bylaws that would negatively impact hibernacula.

Table 3. Summary of target groups, methods and partnerships for habitat protection, restoration and threat reduction for gray ratsnake.

Category	Target Group	Method	Partnerships
Habitat protection/ restoration/ threat reduction	Land owners and managers	Policy/Compliance Voluntary restoration Tax incentive programs Land securement	Quarry Operators Landowners Conservation Authorities Land Trusts
<i>Specific Threats</i>			
Road Mortality	Municipalities, consultants, local residents	Design, and install wildlife road crossing structures and associated fencing Education Signage Close roads at key crossings	Municipalities, including road works and communications staff
Persecution	Temporary Foreign Workers Farmers Landowners	Targeted outreach to communities with higher impacts Targeted outreach for Foreign Workers	Agency for Temporary Foreign Workers Agricultural Association
	General Public Naturalist Groups	Multi species awareness campaign.in the Carolinian Zone	Governments– funding Ontario Nature Naturalist groups iNaturalist Scales Reptile Hotline
Recreational use of public land	Trail Users	Educational Signage Dog leash requirement Encourage users to report sightings	Ontario Parks (Shorthills) Ruthven Park National Historic Site Nature Conservancy of Canada Norfolk County (Backus Woods) Conservation Authorities Land Trusts
Subsidized predation	Landowners	Outreach regarding pets, garbage, etc.	General Public

7.2 Monitoring

A long-term, standardized monitoring plan should be developed to address priority research needs and knowledge gaps, while also evaluating the effectiveness and impacts of management actions. Ideally, a coordinated monitoring plan would be designed to: 1) assess the prevalence of SFD, 2) identify important habitat features and locate hibernacula, 3) locate snakes and eggs that may be suitable for collection to establish a captive insurance population, which could potentially be used to augment wild populations in the future, 4) facilitate sampling to assess genetic diversity, and 5) monitor subpopulation trends and estimate snake abundance. Specific considerations, potential leads and partners, and anticipated obstacles were identified by workshop participants:

1. Long-term Monitoring Protocol: A phased approach may be the most practical and effective strategy in the development of a long-term monitoring protocol. For example, the initial phase could focus on developing a habitat suitability model to guide targeted field surveys. Subsequent surveys could prioritize locating snakes for use in mark-recapture or telemetry studies aimed at identifying hibernacula. Once hibernacula have been identified through more intensive field efforts, monitoring could transition to long-term focus on hibernacula occupancy, use, and population trends.
 - a. *Lead*: NRSI
 - b. *Partners*: Jon Wedow, local conservation partners (to assist in surveys or funding), landowners (access to land, report sightings), general public (to report sightings), Ontario Parks (access and permits), Queen's University/African Lion Safari (genetics research), African Lion Safari/Reptilia/Little Rays/Riverview Park and Zoo (CAZA facilities that would accept and care for salvaged snakes/eggs to develop an insurance population), veterinarians/researchers (for comment on methods), iNaturalist (to report sightings, and potentially include focus on ratsnake in existing road-focused projects).
 - c. *Considerations*:
 - i. Partners should be allowed time to review and comment on the plan
 - ii. Review plans for other snake species, e.g. Queensnake, for insights/guidance
 - iii. Surveys should not rely on volunteers for monitoring
 - iv. The protocol should take into consideration all management and research needs and coordinate accordingly (e.g. ensure swabbing for SFD is undertaken, samples are collected in a suitable fashion for genetic analysis).
 - v. Results should be used to update the PVA as results become available.
 - vi. The protocol may need to be refined based on the implementation of specific management actions. For example, if augmentation is undertaken, the survey should include a method to quantify success of this action.
 - vii. Surveys focused on hibernacula have the potential to create disturbance in sensitive areas. In particular, assessing use of hibernacula will likely require the installation of fencing and other monitoring equipment.
 - d. *Obstacles*
 - i. Funding may be difficult to leverage
 - ii. Given the structure of the population into several isolated subpopulations, communication will be important to ensure protocol is followed. Ideally, there would be centralized administration/oversight of the survey effort among subpopulations.
 - iii. Landowner access will need to be granted, which is time intensive.
2. Snake Fungal Disease (SFD): Initial efforts should focus on determining the prevalence and distribution of SFD within and among subpopulations. Depending on the findings, more detailed research may then be undertaken to better understand the impacts of the disease and inform potential management responses.
 - a. *Lead*: Coordinate with Dr. Christina Davy/Rachel Dillion and on-going study of SFD
 - b. *Partners*: Coordinate with field survey effort for all subpopulations
 - c. *Considerations*: A training video could assist in detailing protocol and ensuring biosafety procedures are followed so that the disease is not accidentally spread

- d. *Obstacles:* Costs are associated with this activity, including for administration, analysis, reporting, mailing, equipment, lab time, etc.

7.3 Awareness

The working group discussed opportunities and avenues to conduct outreach, and identified specific types of outreach and target groups. Leads were not identified but rather, outreach needs should be revisited once an outreach working group or outreach subcommittee of the Recovery Implementation Group has been established. The group identified the following steps as necessary to initiate outreach:

1. Establish a Working Group/Subcommittee focused on Outreach and Awareness
2. Organize a stakeholder meeting
 - a. Identify the stakeholders for overall Carolinian Zone and for each subpopulation
 - b. Identify existing connections for each stakeholder
3. Leverage existing initiatives and partnerships
 - a. Identify existing funding, SAR initiatives, and restoration efforts that could be expanded on to include ratsnake needs
4. Establish Partnerships and Sponsors
 - a. Funding support
 - b. In-kind support

Landowner-specific outreach should be focused on protecting hibernacula, edge habitat and implementation of BMPs. While additional planning is needed to identify who/where/how to deliver outreach, a variety of possible outreach approaches were identified, including:

1. Signage
2. Partnerships with conservation authorities, Niagara college, Ontario Parks
3. Direct door-to-door outreach
4. Surveys
5. Community based engagement at public events, either existing or new (e.g. ratsnake festival)
6. Develop local champions (landowners) that can act as the centre of a circle of influence
7. Engaging specialist to develop targeted, and novel marketing
8. Awareness for seasonal farm workers

Potential leads for targeted outreach, and specific outreach efforts were suggested as follows:

1. Municipal Government
 - a. Road mortality mitigation infrastructure
 - b. Policy development
 - c. Landowner outreach
 - d. Land restoration/stewardship on public lands
2. Provincial Government
 - a. Land management
 - b. Policy development
 - c. Compliance enforcement
 - d. Funding
3. Federal Government

- a. Temporary Foreign Worker education requirement
 - b. Land management
 - c. Funding
4. Private Landowners/Landowner Associations, Organizations (e.g. Ontario Federation of Agriculture, Ontario Woodlot Association, Naturalist groups, etc.)
 - a. Land restoration/stewardship
 - b. Awareness to reduce persecution/increase reporting of observations
 - c. Surveys to help quantify threats (e.g. assess extent of risk due to outdoor cats)
5. Non-Government Organizations/Public Landowners (Land trusts, Conservation Authorities, Institutional organizations such as schools)
 - a. Land restoration/stewardship
 - b. Land securement
 - c. Conduct outreach
 - d. Fundraising
6. First Nations
 - a. Land restoration/stewardship/securement
 - b. Community engagement
 - c. Conduct research and monitoring
7. Consultants
 - a. BMPs development and implementation
 - b. Expertise
 - c. Conduct research and monitoring
 - d. Ensure compliance
8. Industry (Quarry/Agricultural operations)
 - a. Provide education for workers, including Temporary Foreign Workers
 - b. Participate in/facilitate research and monitoring
 - c. Land restoration / stewardship
9. Targeted media (News/TV, Print/Web, Newsletters/Magazines, Influencers/YouTube)
 - a. General public awareness (reduce persecution, reporting sightings, volunteer)

7.4 Research

Eight knowledge gaps were identified as critical research needs:

1. Fine tune estimates of time to extinction for Sixteen Mile Creek
2. Update the PVA as additional information becomes available
3. Address knowledge gaps identified in PVA (see results of sensitivity analysis in Appendix E)
4. Determine level of genetic diversity and gene flow within and among subpopulations
5. Quantify and refine understanding of key habitat features, including development of a Habitat Suitability Model
6. Quantify threats, in particular SFD and forestry activities
7. Develop BMPs
8. Investigate the benefit of augmentation

Addressing knowledge gaps would largely (i.e. with the exception of augmentation) be addressed through implementation of a monitoring program. Potential leads and partners, considerations,

and anticipated obstacles for research, other than for augmentation, were identified by participants as follows:

1. *Leads:*
 - a. Update PVA: Amy Chabot
 - b. Genetic analysis: Amy Chabot and Queen's University
2. *Partners:*
 - a. Individuals and organizations involved in development of the monitoring program
 - b. BMPs – conservation authorities, organizations involved in logging, local municipal government, provincial parks
3. *Considerations:*
 - a. Addressing key knowledge gaps through targeted research and information collection is expected to require a minimum of five years to complete.
 - b. The Habitat Suitability Model should be developed to inform conservation planning by identifying areas where habitat restoration, enhancement, or protection could improve connectivity within and among subpopulations.
4. *Obstacles:*
 - a. Funding will be required to cover costs associated with research activities.

7.5 Ex Situ Management

A cohort of experts from Canada's Accredited Zoos and Aquariums (CAZA) was present at the workshop. They convened in a working group focused on development of ex situ management. The group agreed that it had the capacity for and could establish a bio-secure captive breeding population for use in augmentation. Collectively, it was decided that the best method would be to recruit founders from any subpopulation by collecting eggs and gravid females, which would be maintained until oviposition and subsequently returned to their respective habitat. Rescue of all age classes was deemed a method of collection should their habitat become uninhabitable. Population rescue may be warranted for the Sixteen Mile Creek subpopulation, as its estimated carrying capacity is believed to be insufficient to support long-term population viability without intervention.

The working group discussed methods of population augmentation and the feasibility of each option. Demographic augmentation, via the introduction of captive bred ratsnake post-hatch, was the recommended method. Using information from previous aspects of the workshop, the group suggested that the Big Creek subpopulation would benefit most from releases, as it appeared to possess the most intact habitat and room for population growth. Further, the PVA suggested that this subpopulation is the most likely to persist over 100 years, if genetic diversity could be maintained and survival rates improved. Contributing heavily to the decision to pursue ex situ management was the fact that well-established husbandry methods exist for gray ratsnake and the on-going effort in Ontario to include ex situ management in conservation actions for the Eastern Massasauga Rattlesnake.

Prior to concluding the working session, members of the group committed to performing actions that would lead to completion of the required materials.

1. *Leads:*
 - a. Ginger Elliott – Feasibility Study, Husbandry Manual coordination
 - b. Jeff Hathaway – Husbandry Manual: Transportation

- c. Rick Vos – Husbandry Manual: Housing and Nutrition
- d. Cheryl Sheridan – Husbandry Manual: Health and Veterinary Care
- e. Tom Eles – Husbandry Manual: Reproduction
- f. Wyatt Thorton & Emma Legge – Husbandry Manual: Biosecurity
- g. Ariana Ermani – Husbandry Manual: Staff Training
2. *Partners:*
 - a. CAZA and breeding facilities in southern Ontario, including African Lion Safari, Little Rays, Riverview Park and Zoo, Reptilia and Scales Nature Park.
3. *Considerations/Obstacles:*
 - a. Challenges with demographic augmentation include establishing a biosecure captive breeding population.
 - b. Salvage and temporary holding of gravid females, eggs and neonates will require government permits. Based on work to establish an ex situ population of Eastern Massasauga Rattlesnake, it is likely that a species at risk wildlife collection permit would be needed. Requirements for this permit include: 1. a detailed husbandry manual; and 2. a project feasibility study.

7.6 Partnerships

A working group was convened that focused on identifying potential, and necessary partners to address threats and engage in management action. Specific partners, and their potential role in the conservation of the Carolinian population of gray ratsnake include:

1. Niagara College
 - a. In kind support
 - b. Assistance with on the ground restoration and research, in particular for the Shorthills subpopulation
2. First Nations
 - a. Engagement in management action on the ground where suitable habitat exists or can be secured
3. Provincial Government
 - a. Engagement with the Ontario Ministry of Environment, Conservation and Parks, Ministry of Natural Resources and Forestry for permits, and enforcement
 - b. Financial support through Species Conservation Program funding
4. Federal Government
 - a. Financial support through programs such as the Habitat Stewardship Fund, AFSAR, SARPAL
5. Developers
 - b. Implementing habitat stewardship programs
 - c. Subsidizing land securement by land trusts
6. Birds Canada
 - a. Potential for collaboration in combination with conservation of bird species in the Carolinian Zone
7. Nature Conservancy of Canada
 - a. On the ground habitat stewardship/restoration and land securement
8. Influencers
 - a. Assist in engaging the public, raising awareness, and potentially fundraising
9. Trade Shows/Conventions

- a. Assist in raising awareness, engagement with on the ground restoration/stewardship
- 10. Ontario Nature
 - a. Raising awareness and building public support through publication of article(s) in magazine
 - b. Potential assistance with fundraising through donations for management or land securement
 - c. Communication expertise and support via Membership
- 11. NRSI
 - a. Continue to lead research, funding applications, etc.
 - b. Chair/Lead Recovery Implementation Group

8. Prepare to Implement, Share, Learn and Improve

The participants reviewed the structure and activities of the Canadian Eastern Massasauga Rattlesnake Recovery Implementation Group (CEMRRIG). There was unanimous agreement that a similarly structured Recovery Implementation Group (RIG) would facilitate and help ensure management actions for the Carolinian population of gray ratsnake were implemented and could be reviewed periodically.

An initial and critical goal for the RIG will be to further develop this species conservation plan into a detailed and actionable implementation plan. While good progress was made in identifying the priority actions, potential partners, and, in most cases, appropriate leads for implementation, additional work is required to provide the level of detail needed to facilitate implementation and broad participation. This should include identifying anticipated budgets, timelines, potential funding sources, and all necessary partners and resources required to implement the management actions identified during the workshops. A process for annual or periodic review of progress and adaptation of management actions should also be established to ensure the plan remains responsive to new information, emerging threats, and changing conditions.

A good first step may be to identify a lead or co-leads for specific management actions who can then convene partners and delegate further refinement of the management needs identified during the workshop. Heather Fotherby and Ryan Wolfe, NRSI, were inspired to action and will lead efforts to hold a follow up meeting, essentially establishing the RIG, or taking the first step to do so in 2026, after the workshop report has been finalized. Efforts will be made to facilitate participation, for example, by ensuring meetings are hybrid. The majority of workshop participants were interested in participating in the RIG, suggesting there will be adequate support and leadership for a model similar to CEMRIG.

Acknowledgements

The author of this report is grateful for the time and energy brought to this effort by all the participants that contributed to the online meeting technical sessions and the in-person workshop. Particular thanks go to the Heather Fotherby, Ryan Wolfe and Natasha Sawatzky, NRSI, for their expertise and insights from recent field work on the Carolinian population of gray ratsnake that was so important in the workshop and PVA development. Thanks to Ontario's Natural Heritage Inventory Committee for making data available to NRSI for use in the PVA and for the workshop. Thanks to Ginger Elliott and Stephanie Winton, for assistance with technical sessions. Finally, a special note of thanks to the Honorable Dr. Phil Miller, CPSG's Director of Science and Data, and my mentor in species conservation planning utilizing Population Viability Analysis with Vortex, for critical assistance in model construction and validation, and for assistance in the development of the workshop agenda.

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Appendix A. Participation in on-line technical sessions for development of a Population Viability Analysis for the Carolinian population of gray ratsnake

Name	Affiliation	Session 1 (25-5-30)	Session 2 (25-6-24)	Session 3 (25-7-9)	Session 4 (25-7-30)	Session 5 (25-10-30)
Amy Chabot	African Lion Safari, CPSG Canada	Yes	Yes	Yes	Yes	Yes
Andy Gomez	Queen's University	Yes	Yes	Yes	Yes	Yes
Anne Yagi	8Trees Inc.	Yes				
Calder Schweitzer	Thousand Islands National Park	Yes	Yes	Yes		
Campbell McKay	Chippewas of the Thames First Nation					Yes
Cathy Blott	Haldimand Stewardship Council		Yes	Yes		Yes
Cheryl Sheridan	Little Ray's Nature Center					Yes
Gabriel Blouin-Demers	University of Ottawa	Yes				
Ginger Elliott	African Lion Safari, CPSG Canada	Yes	Yes		Yes	Yes
Grace Robillard	Haldimand Stewardship Council	Yes	Yes		Yes	
Hannah McCurdy-Adams	Wildlife Preservation Canada	Yes	Yes	Yes	Yes	Yes
Heather Fotherby	Natural Resource Solutions Inc.	Yes	Yes	Yes	Yes	Yes
Jeff Hathaway	Scales Nature Park		Yes			Yes
Jeff Row	Minnow Environmental Inc.	Yes				
Jenna Quinn	Ontario Nature					Yes
Jenni Kaija-Bershatsky	Ontario Parks	Yes	Yes			
Jennifer McCarter	Parks Canada					Yes
Jennifer Thompson	Canada Wildlife Service-ON, ECCC					Yes
Jenny Pearce	Amphibian and Reptile Conservation Canada (ARCC)					Yes
Jessica Ferguson	Clean Annapolis River Project (CARP)	Yes	Yes	Yes	Yes	Yes
Joe Crowley	Ontario Ministry of the Environment and Parks Species at Risk branch	Yes		Yes		Yes
Jon Wedow	Local Expert	Yes	Yes	Yes	Yes	Yes
Karolyne Pickett	Environment and Climate Change Canada (ECCC)					Yes

Katherine Yagi	8Trees	Yes	Yes			
Kelsey Moxley	Scales Nature Park, Amphibian and Reptile Conservation Canada (ARCC)					Yes
Kent Prior	Parks Canada	Yes				
Lauren Jones	Six Nations of the Grand River Elected Council	Yes				
Maddy Perkins	Reptilia Vaughan					Yes
Mary Gartshore	Long Point Basin Land Trust	Yes	Yes			Yes
Natasha Sawatzky	Natural Resource Solutions Inc.	Yes	Yes	Yes		Yes
Nathan Grant	Natural Resources Solutions INC					Yes
Peter Gartshore	Nature Conservancy of Canada	Yes	Yes	Yes	Yes	Yes
Phil Miller	CPSG		Yes	Yes	Yes	Yes
Rick Vos	Toronto Zoo					Yes
Ron Gould	Ontario Parks	Yes				Yes
Ryan Archer	Natural Resources Solutions Inc.	Yes				Yes
Ryan Wolfe	Natural Resource Solutions Inc.	Yes	Yes	Yes	Yes	Yes
Scott Gillingwater	Upper Thames Conservation Authority	Yes				
Shaun Thompson	Retired		Yes	Yes		Yes
Stephanie Winton	Wildlife Preservation Canada, CPSG Canada	Yes	Yes	Yes	Yes	
Stephen Lougheed	Queen's University	Yes	Yes			
Tom Eles	Little Ray's Nature Center					Yes
Victoria Tawa	Queen's University	Yes	Yes	Yes		Yes
Winona Drouin	Haldimand Stewardship Council	Yes	Yes			

Appendix B. Participation in the in-person species conservation planning workshop for the Carolinian population of gray ratsnake

First Name	Affiliation	Day 1	Day 2	Day 3
Amy Chabot	CPSG	Yes	Yes	Yes
Andrea Gómez-Sánchez	Queen's University	Yes	Yes	Yes
Anne Yagi	8Trees Inc	Yes		
Ariana Emami	Reptilia Whitby	Yes	Yes	Yes
Ashley Cantwell	NOTETAKER		Yes	
Campbell McKay	Chippewas of the Thames First Nation	Yes	Yes	Yes
Cheryl Sheridan	Little Rays Exhibitions	Yes		Yes
Emma Legge	African Lion Safari	Yes	Yes	Yes
Ginger Elliott	African Lion Safari	Yes	Yes	Yes
Hana Thompson	NOTETAKER	Yes		
Hannah McCurdy-Adams	Wildlife Preservation Canada	Yes	Yes	Yes
Heather Fotherby	NRSI	Yes	Yes	Yes
Jeff Hathaway	Scales Nature Park	Yes	Yes	Yes
Jennifer McCarter	Parks Canada	Yes	Yes	Yes
Jennifer Thompson	Canadian Wildlife Service - Ontario Region	Yes	Yes	Yes
Jenny Pearce	Amphibian and Reptile Conservation Canada	Yes	Yes	Yes
Jessica Ferguson	Clean Annapolis River Project	Yes	Yes	Yes
Jillyan Birtch	NOTETAKER	Yes	Yes	Yes
Jocelyn Mennen	NOTETAKER	Yes	Yes	Yes
Joe Crowley	Ministry of Natural Resources		Yes	Yes
Jon Wedow	Local Expert	Yes		Yes
Kendra Gardner	African Lion Safari	Yes		Yes
Liza Kucheryava	NRSI		Yes	
Maddy Perkins	Reptilia Vaughan	Yes	Yes	Yes
Mary Gartshore	Long Point Land Basin Trust	Yes	Yes	Yes
Nathasha Sawatsky	NRSI	Yes	Yes	Yes
Phil Miller	CPSG	Yes	Yes	Yes
Rick Vox	Toronto Zoo	Yes		Yes
Ryan Archer	NRSI	Yes		Yes
Ryan Wolfe	NRSI	Yes	Yes	Yes
Shaun Thompson	NA	Yes	Yes	Yes

Tom Eles	Little Rays	Yes		Yes
Tyler Creech	Center for Large Landscape Conservation	Yes		
Cathy Blott	Haldimand Stewardship Council	Yes		Yes
Victoria Tawa	Queen's University	Yes	Yes	Yes
Wyatt Thorton	African Lion Safari	Yes	Yes	Yes

Appendix C. Threat assessment results for the six subpopulations of the Carolinian population of gray ratsnake

Note, where details are not provided by subpopulation, workshop participants believed that the threat was not applicable to a specific subpopulation.

Threat Impact	COSEWIC 2016 Scope	COSEWIC 2016 Severity	Workshop 2025 Likelihood	Workshop 2025 Severity
A. Use of Lands & Water				
1. Residential, Commercial & Recreation Areas	Small (1-10%)	Extreme (71-100%)		
1.1. Residential areas: increased rural housing	Small (1-10%)	Extreme (71-100%)		
Area 1: Sixteen Mile Creek			Low (1-30%)	High (71-100%)
Area 2: Otter Creek			Low (1-30%)	High (71-100%)
Area 3: Big Creek			Low (1-30%)	High (71-100%)
Area 4: Oriskany-Quarry			Low (1-30%)	High (71-100%)
Area 5: Oriskany-Ruthven			Low (1-30%)	High (71-100%)
Area 6: Shorthills			Medium (31-70%)	High (71-100%)
1.2 Commercial & Industrial Areas	Negligible (<1%)	Extreme (71-100%)		
1.3 Tourism & Recreation Areas	Negligible (<1%)	Extreme (71-100%)		
2. Agriculture & Aquaculture				
2.1. Annual & Perennial Non-Timber Crops: increased farms/farming activity	Pervasive (71-100%)	Moderate-Slight (1-30%)		
Area 1: Sixteen Mile Creek			High (71-100%)	Medium (31-70%)
Area 2: Otter Creek			High (71-100%)	Medium (31-70%)
Area 3: Big Creek			Medium (31-70%)	Low (1-30%)
Area 4: Oriskany-Quarry			High (71-100%)	Medium (31-70%)
Area 5: Oriskany-Ruthven			High (71-100%)	Medium (31-70%)
Area 6: Shorthills			High (71-100%)	Medium (31-70%)
2.2. Wood & Pulp Plantations			N/A	N/A

Threat Impact	COSEWIC 2016 Scope	COSEWIC 2016 Severity	Workshop 2025 Likelihood	Workshop 2025 Severity
2.3 Terrestrial Animal Farming, Ranching & Herding	Negligible (<1%)	Negligible (<1%)		
2.4 Marine & Freshwater Aquaculture				
3. Energy Production & Mining	Restricted (11-30%)	Extreme-Serious (31-100%)		
3.1 Oil & Gas Exploration & Extraction				
3.2 Mining & Quarrying	Restricted (11-30%)	Extreme-Serious (31-100%)		
Area 1: Sixteen Mile Creek			Low (1-30%)	Low (1-30%)
Area 2: Otter Creek			Low (1-30%)	Low (1-30%)
Area 3: Big Creek			Low (1-30%)	Low (1-30%)
Area 4: Oriskany-Quarry			Medium (31-70%)	High (71-100%)
Area 5: Oriskany-Ruthven			Medium (31-70%)	High (71-100%)
Area 6: Shorthills			Low (1-30%)	Low (1-30%)
3.3 Renewable Energy	Negligible (<1%)	Slight (1-10%)		
4. Transportation, Service & Security Corridors	Pervasive (71-100%)	Serious-Moderate (11-70%)		
4.1. Roads, Trails & Railroads: increased/improved roads	Pervasive (71-100%)	Serious-Moderate (11-70%)		
Area 1: Sixteen Mile Creek			High (71-100%)	Low (1-30%)
Area 2: Otter Creek			High (71-100%)	Medium (31-70%)
Area 3: Big Creek			High (71-100%)	Medium (31-70%)
Area 4: Oriskany-Quarry			High (71-100%)	Medium (31-70%)
Area 5: Oriskany-Ruthven			Medium (31-70%)	Medium (31-70%)
Area 6: Shorthills			Medium (31-70%)	Medium (31-70%)
4.2 Utility & Service Lines	Negligible (<1%)	Slight (1-10%)		

Threat Impact	COSEWIC 2016 Scope	COSEWIC 2016 Severity	Workshop 2025 Likelihood	Workshop 2025 Severity
4.3 Shipping Lanes				
4.4 Atmospheric & Space Activities				
4.5 Fencing & Walls				
B. Use/Management of Species & Ecosystems				
5. Biological Resource Use & Control	Small (1-10%)	Extreme (71-100%)		
5.1. Hunting, Collecting & Controlling Terrestrial Animals: persecution of snakes	Small (1-10%)	Extreme (71-100%)		
Area 1: Sixteen Mile Creek			Low (1-30%)	Low (1-30%)
Area 2: Otter Creek			Low (1-30%)	Low (1-30%)
Area 3: Big Creek			Low (1-30%)	Low (1-30%)
Area 4: Oriskany-Quarry			Low (1-30%)	Low (1-30%)
Area 5: Oriskany-Ruthven			Low (1-30%)	Low (1-30%)
Area 6: Shorthills			Low (1-30%)	Low (1-30%)
5.2 Gathering, Harvesting & Controlling Terrestrial Plants and Fungi				
5.3. Logging, Harvesting & Controlling Trees: Logging and inappropriate logging prescriptions	Small (1-10%)	Slight (1-10%)		
Area 1: Sixteen Mile Creek			High (71-100%)	Low (1-30%)
Area 2: Otter Creek			High (71-100%)	Low (1-30%)
Area 3: Big Creek			High (71-100%)	Low (1-30%)
Area 4: Oriskany-Quarry			High (71-100%)	Low (1-30%)
Area 5: Oriskany-Ruthven			High (71-100%)	Low (1-30%)
Area 6: Shorthills			High (71-100%)	Low (1-30%)
5.4 Fishing, Harvesting & Controlling Aquatic Species				
6. Human Intrusions & Disturbances	Negligible (<1%)	Negligible (<1%)		

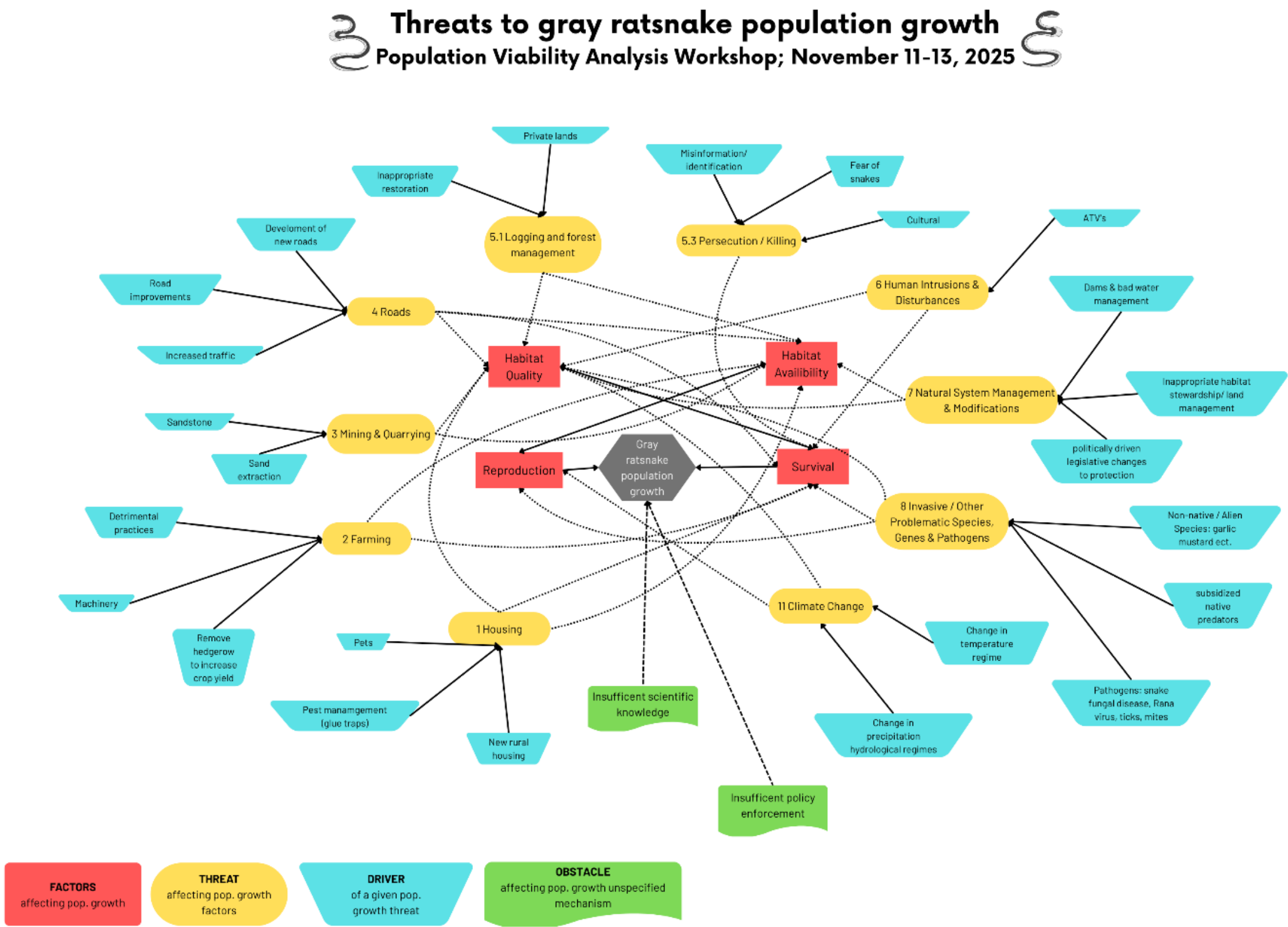
Threat Impact	COSEWIC 2016 Scope	COSEWIC 2016 Severity	Workshop 2025 Likelihood	Workshop 2025 Severity
6.1. Recreational activities: ATVs, biking trails	Negligible (<1%)	Negligible (<1%)		
Area 1: Sixteen Mile Creek			N/A	N/A
Area 2: Otter Creek			Low (1-30%)	Low (1-30%)
Area 3: Big Creek			Low (1-30%)	Low (1-30%)
Area 4: Oriskany-Quarry			N/A	N/A
Area 5: Oriskany-Ruthven			N/A	N/A
Area 6: Shorthills			N/A	N/A
6.2 Conflict, Civil Unrest & Security				
6.3 Other human disturbances: species research				
Area 1: Sixteen Mile Creek			N/A	N/A
Area 2: Otter Creek			N/A	N/A
Area 3: Big Creek			N/A	N/A
Area 4: Oriskany-Quarry			N/A	N/A
Area 5: Oriskany-Ruthven			N/A	N/A
Area 6: Shorthills			N/A	N/A
7. Natural System Management & Modifications	Small (1-10%)	Slight (1-10%)		
7.1 Fire & Fire Management/Suppression	Negligible (<1%)	Slight (1-10%)		
7.2 Dams & Water Management/Use: Field drainage	Small (1-10%)	Slight (1-10%)		
Area 1: Sixteen Mile Creek			Low (1-30%)	Low (1-30%)
Area 2: Otter Creek			Low (1-30%)	Low (1-30%)
Area 3: Big Creek			Low (1-30%)	Low (1-30%)
Area 4: Oriskany-Quarry			Unknown	Unknown
Area 5: Oriskany-Ruthven			Unknown	Unknown
Area 6: Shorthills			Unknown	Unknown
7.3 Earth & Sediment Management				
7.4 Weather & Climate Management				
7.5 Biological System Management: Inappropriate habitat stewardship/land management				

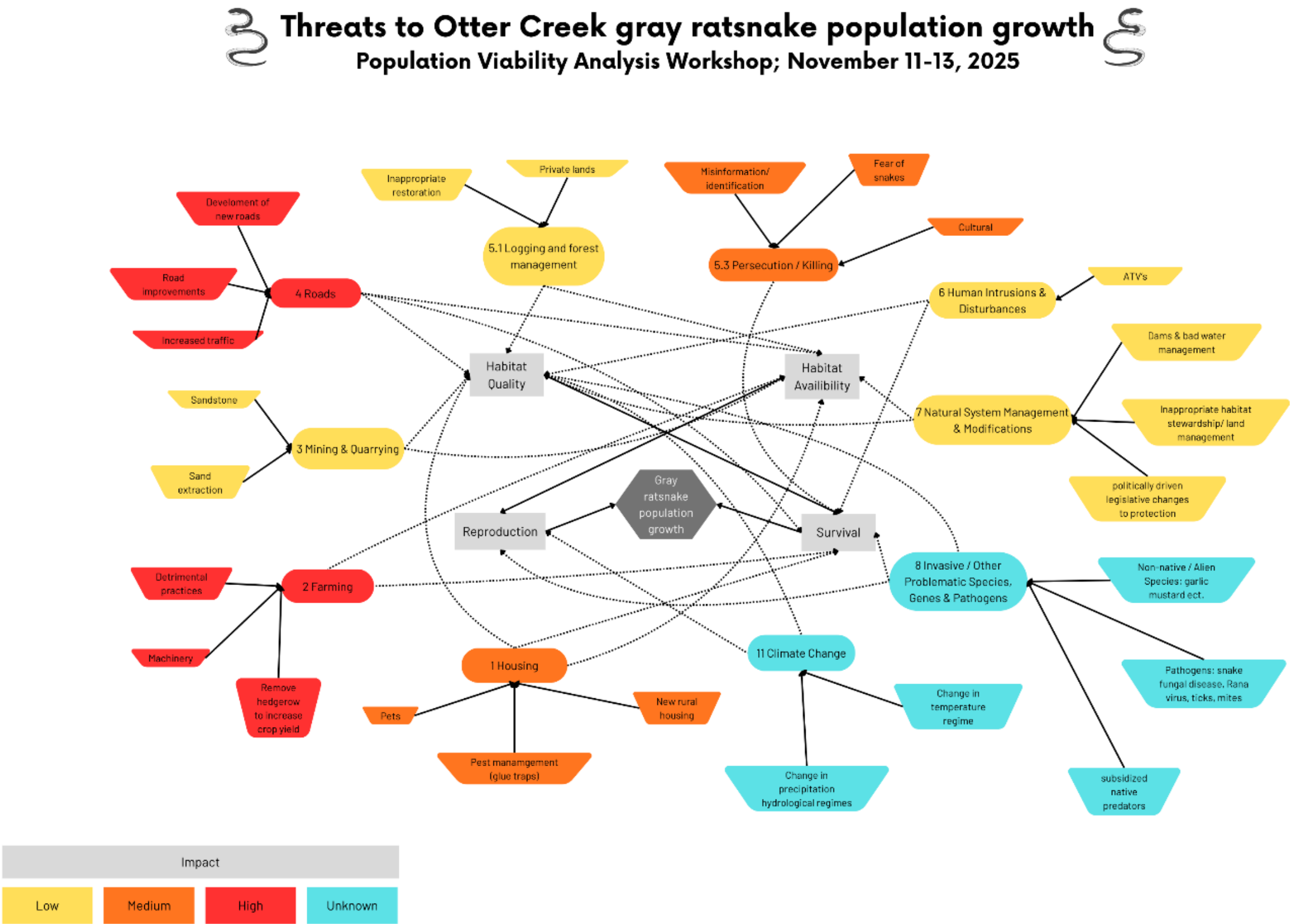
Threat Impact	COSEWIC 2016 Scope	COSEWIC 2016 Severity	Workshop 2025 Likelihood	Workshop 2025 Severity
Area 1: Sixteen Mile Creek			High (71-100%)	Low (1-30%)
Area 2: Otter Creek			Medium (31-70%)	Low (1-30%)
Area 3: Big Creek			High (71-100%)	Low (1-30%)
Area 4: Oriskany-Quarry			High (71-100%)	Low (1-30%)
Area 5: Oriskany-Ruthven			Low (1-30%)	Low (1-30%)
Area 6: Shorthills			Unknown	Unknown
7.6 Removing/Reducing Human Management				
C. Additional Sources of Stress				
8. Invasive/Other Problematic Species, Genes & Pathogens	Pervasive (71-100%)	Not Known		
8.1 Invasive Non-native/Alien Species: Garlic mustard, etc.	Unknown	Unknown		
Area 1: Sixteen Mile Creek			High (71-100%)	Unknown
Area 2: Otter Creek			High (71-100%)	Unknown
Area 3: Big Creek			High (71-100%)	Unknown
Area 4: Oriskany-Quarry			High (71-100%)	Unknown
Area 5: Oriskany-Ruthven			High (71-100%)	Unknown
Area 6: Shorthills			High (71-100%)	Unknown
8.2. Problematic Native Species: subsidized predators (mink, fisher, wild turkey, coyote, etc.)	Pervasive (71-100%)	Unknown		
Area 1: Sixteen Mile Creek			High (71-100%)	Unknown
Area 2: Otter Creek			High (71-100%)	Unknown
Area 3: Big Creek			High (71-100%)	Unknown
Area 4: Oriskany-Quarry			High (71-100%)	Unknown
Area 5: Oriskany-Ruthven			High (71-100%)	Unknown
Area 6: Shorthills			High (71-100%)	Unknown

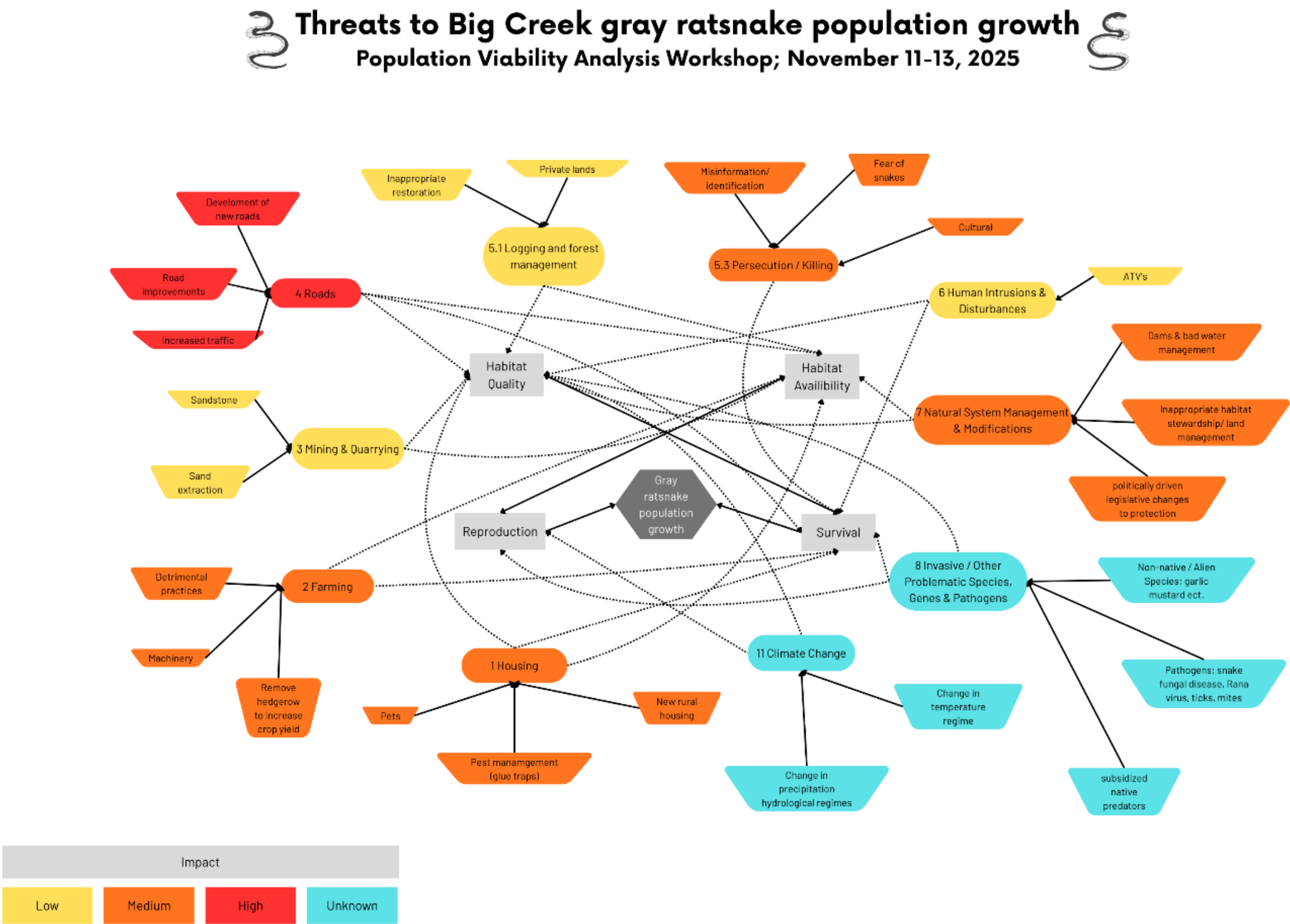
Threat Impact	COSEWIC 2016 Scope	COSEWIC 2016 Severity	Workshop 2025 Likelihood	Workshop 2025 Severity
8.3 Introduced Genetic Material				
8.4 Pathogens: snake fungal disease, Rana virus, ticks, mites				
Area 1: Sixteen Mile Creek			Unknown	Unknown
Area 2: Otter Creek			Unknown	Unknown
Area 3: Big Creek			Medium (31-70%)	Unknown
Area 4: Oriskany-Quarry			Unknown	Unknown
Area 5: Oriskany-Ruthven			Unknown	Unknown
Area 6: Shorthills			Unknown	Unknown
9.0 Pollution				
9.1 Water-Borne & Other Effluent Pollution				
9.2 Garbage & Solid Waste				
9.3 Air-Borne Pollutants				
9.4 Energy Emissions				
10. Natural Disasters				
10.1 Geological Events				
10.2 Severe Weather Events: increased floods, drought, etc.				
Area 1: Sixteen Mile Creek				
Area 2: Otter Creek				
Area 3: Big Creek				
Area 4: Oriskany-Quarry				
Area 5: Oriskany-Ruthven				
Area 6: Shorthills				
11. Climate Change	Pervasive (71-100%)	Not Known		
11.1 Changes in Physical & Chemical Regimes				
11.2 Changes in Temperature Regimes: changing winter seasonality, heat waves, etc.	Pervasive (71-100%)	Unknown		
Area 1: Sixteen Mile Creek			High (71-100%)	Unknown
Area 2: Otter Creek			High (71-100%)	Unknown

Threat Impact	COSEWIC 2016 Scope	COSEWIC 2016 Severity	Workshop 2025 Likelihood	Workshop 2025 Severity
Area 3: Big Creek			High (71-100%)	Unknown
Area 4: Oriskany-Quarry			High (71-100%)	Unknown
Area 5: Oriskany-Ruthven			High (71-100%)	Unknown
Area 6: Shorthills			High (71-100%)	Unknown
11. 3 Change in Precipitation & Hydrological Regimes: increased flooding, drought, precipitation				
Area 1: Sixteen Mile Creek			High (71-100%)	Unknown
Area 2: Otter Creek			High (71-100%)	Unknown
Area 3: Big Creek			High (71-100%)	Unknown
Area 4: Oriskany-Quarry			High (71-100%)	Unknown
Area 5: Oriskany-Ruthven			High (71-100%)	Unknown
Area 6: Shorthills			High (71-100%)	Unknown

Appendix D. Threat diagrams for the Carolinian population of gray ratsnake and individual subpopulations





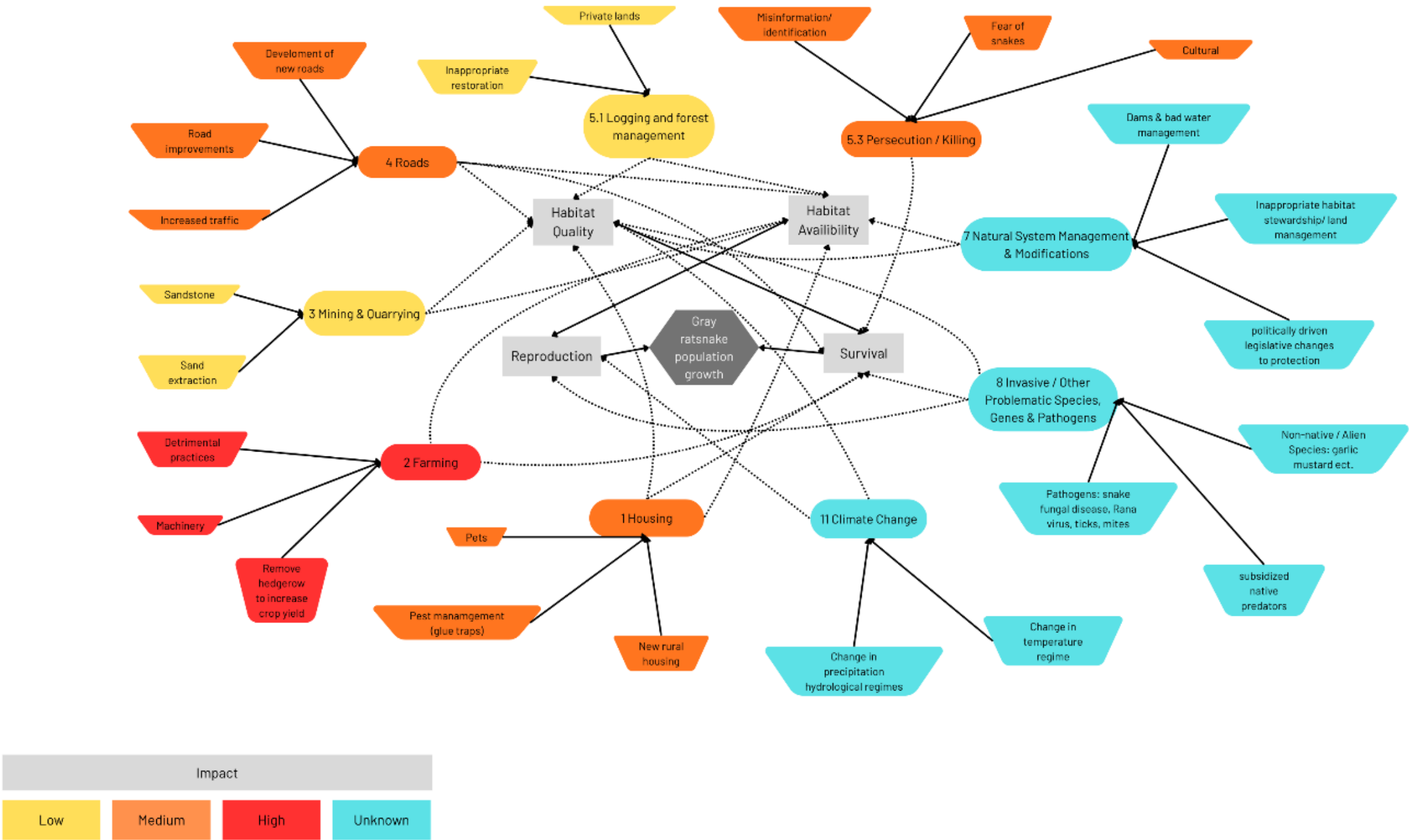


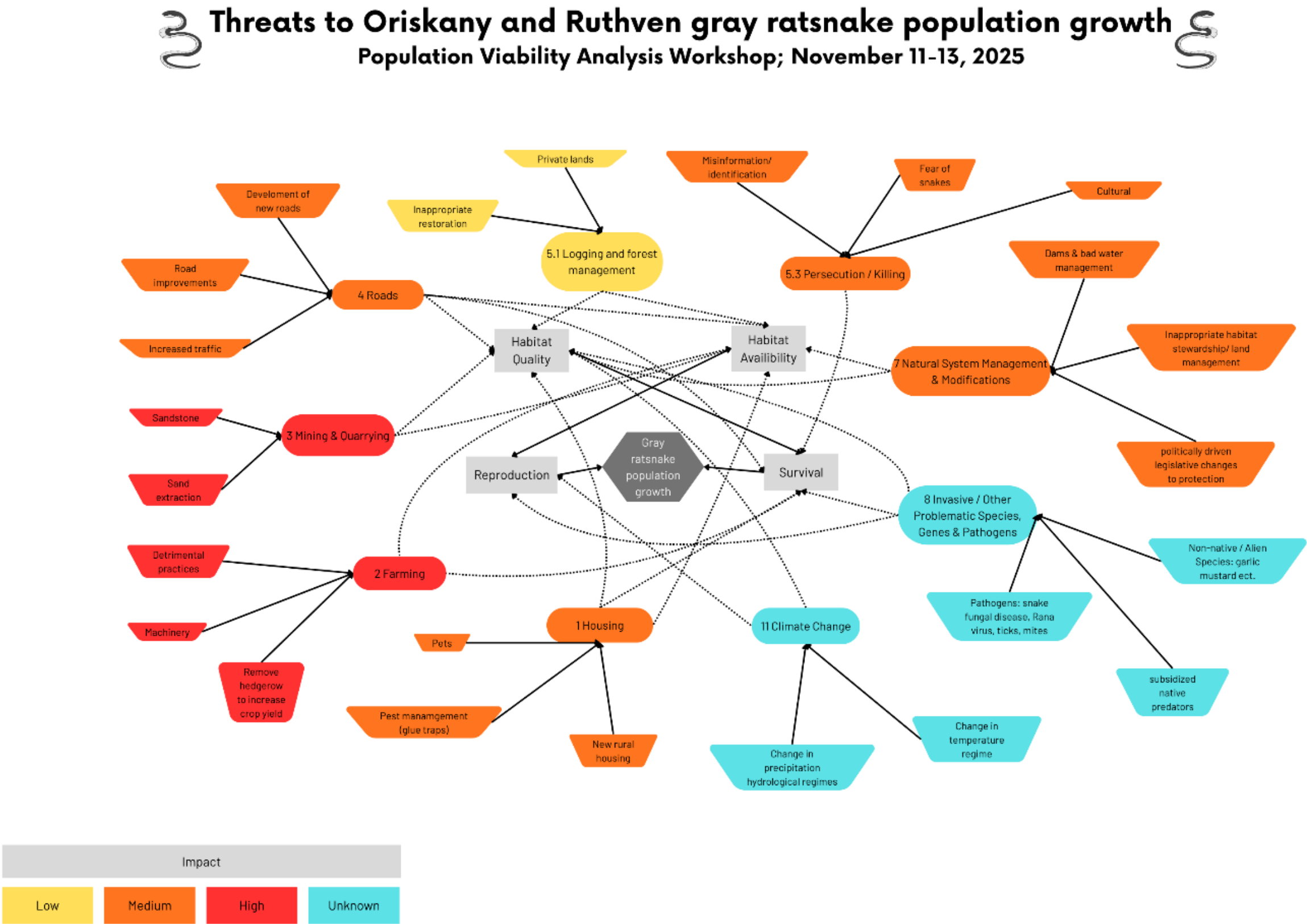


Threats to Shorthills and 16 Mile Creek gray ratsnake population growth

Population Viability Analysis Workshop; November 11-13, 2025







Appendix E. Population Viability Analysis for the Carolinian population of gray ratsnake

Population Viability Analysis Model Development Methods

Approach to Simulation Model Development

Population Viability Analysis (PVA) is a powerful suite of tools used by wildlife conservation researchers, non-government organizations, and national and regional government agencies to assess threats to endangered wildlife populations and to evaluate management options designed to improve population or species status in the wild (Beissinger and McCullough 2002; Morris and Doak 2002). These analyses typically rely on computer simulation modeling to project wildlife population abundance into the future and create a valuable framework for assessing the extent of species data available and how these data interact to drive population growth or decline. In particular, the process of conducting a PVA helps to stimulate information sharing and discussion among species experts and provides a neutral environment where assumptions about our collective understanding of the system of interest can be expressed and clarified.

That said, it is important to recognize that PVA methodologies are not intended to give absolute and accurate “answers” for what the future will bring for a given wildlife species or population. Many practitioners caution against the exclusive use of absolute results from a PVA when using the tool to recommend specific management actions for threatened populations (e.g., Reed et al. 2002; Ellner et al. 2002; Lotts et al. 2004; Lacy 2019). Instead, we often rely on PVA results to make comparisons of the relative performance of a simulated population under alternative management activities or different assumptions of environmental conditions. Even in this comparative framework, however, results from PVA efforts can provide a critical base of evidence when deriving meaningful and justifiable quantitative targets for endangered species recovery.

Choice of software to conduct the PVA

As noted previously, PVA is typically conducted using computer simulation modeling tools. The tool chosen for the analysis of gray ratsnake presented herein is Vortex (version 10.10.0; Lacy et al. 2021, Lacy and Pollak 2025). Vortex simulates the fate of each individual in a population, usually based on an annual cycle that features breeding, mortality, incrementing individual age, dispersal among subpopulations, removals (or emigration from the population), supplementation (managed or natural immigration), and truncation of population abundance if the abundance of individuals exceeds the specified carrying capacity (ceiling density dependence) (Figure 1). Stochasticity in demographic processes is modeled as annual variation in each demographic rate at the population level (environmental variation) and random, binomial sampling variation in the fates of individuals (demographic stochasticity) (Lacy 2000a, 2000b). The software includes a facility to incorporate metapopulation structure, with multiple populations linked through natural or managed dispersal. Individual fates are aggregated to output describing projected population sizes (mean, standard deviation, and distribution across independent iterations), population growth rates, population age and sex structure, and probabilities and times to local extinction or quasi-extinction (abundance falling below a user-defined threshold size). Vortex projects the effects of population structure on loss of genetic diversity through drift, and in turn the impacts

of loss of diversity on fecundity and survival and, hence, population growth, via processes such as inbreeding depression. This emphasis on population genetic components of long-term population viability is a key defining feature of Vortex in comparison to other PVA simulation software. For more detailed information on the features of Vortex and its application to wildlife population management, see Lacy (2000a; 2000b; 2019) and Lacy et al. (2021).

General overview of gray ratsnake PVA model structure

This section gives additional detail on the numerical input and settings used in the PVA models used for simulations.

General simulation characteristics

Each of the individual model scenarios was run using 1000 replicates. This iterative procedure is necessary as each replicate will yield a different outcome resulting from random annual variation in expected rates of breeding and survival in the simulated gray ratsnake population. Each scenario was projected forward for 30 years: 10 years is considered to be a ‘generation’ time for the species by COSEWIC and threat assessments are based on a 3 generation, or 30-year timeframe. Extinction was defined as when only one sex remained in a population. The order of events was as follows: Breeding, Mortality, Age, Disperse, rCalculations (the annual rate of change in population trend) and Ktruncation (the probabilistic truncation across all age classes, unless otherwise specified, when K, carrying capacity, is exceeded – in this case, at the end of the year). Where augmentation was used in a scenario, it was implemented before Breeding.

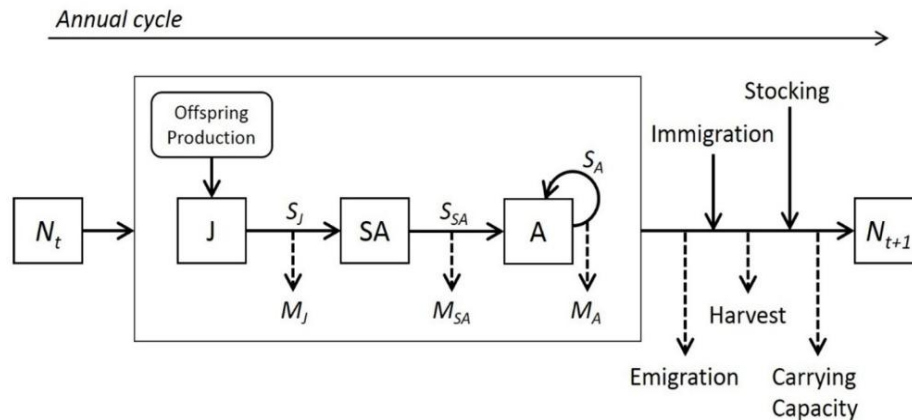


Figure 1. Generalized diagram showing the series of events making up a typical annual cycle (timestep) in the PVA software package Vortex, representing simulated change in population abundance N from year t to year $t+1$. Enclosed portion shows the production of juveniles (J) and the transition of individuals among the juvenile, subadult (SA) and adult (A) stages, determined by annual age-specific survival (S_x) and their complementary mortality rates M_x . Processes displayed above the timeline to the right of the diagram increase population abundance, while those below decrease abundance. For more information on Vortex, see Lacy and Pollak (2025) and Lacy et al. (2021).

Demographic Inputs

A PVA model was developed by Row et al. (2007) using information from extensive research that has been conducted on the Great Lakes St. Lawrence population. Row et al. (2007) used

their model to assess the impact of road mortality on population persistence. However, given the absence of accurate information on mortality rates, the authors chose to create a baseline model in which mortality rates, which were largely unknown, were adjusted to achieve a stable to slightly increasing population trend characterized by a $\lambda = 1.03$ (i.e. representing a mean 3% rate of population growth per year).

As little information exists on the Carolinian population of gray ratsnake, we had to rely on research undertaken on the Great Lakes St. Lawrence population. During the first on-line session of the gray ratsnake PVA development, participants reviewed Table 1 and discussion focused on the need to adjust these parameters to better reflect the Carolinian population. Generally, participants believed that the two populations likely had similar life history parameters. However, in later discussions, technical experts suggested that mortality rates should be revised. Broadly, it was believed that the more southerly location of the Carolinian population would likely reach sexual maturity earlier due to the longer active season. Where data was altogether lacking (i.e. mortality rates), data from other North American species of snake were used as guidance from which the technical experts made informed estimates. The final demographic data used to develop the PVA for the Carolinian population is presented in Table 1.

Initial population size, age structure and carrying capacity

The 2018 COSEWIC status assessment considered the Carolinian population of gray ratsnake as one panmictic population, isolated from the other population of ratsnake in Canada, and from populations in the USA. However, in part due to extensive field research by NRSI, the Carolinian population of gray ratsnake is more correctly characterized as six isolated subpopulations that vary in size and carrying capacity. Initial population size (N) and carrying capacity (K) were derived independently for each subpopulation in the model – see Section 3.2 ‘Subpopulation Description’ in the main report for details. The age structure of the subpopulations is unknown, and, for the purposes of the model simulations, was characterized as being stable. A stable age structure means a population’s age distribution (the proportion of individuals in each age group) remains constant over time because age-specific fertility and mortality rates have remained constant. This results in a roughly equal proportion of young and middle-aged individuals, often appearing as a column-shaped age pyramid. Neonates were described as being Age-0 (birth) to Age-1 year. Subadults were described as being Age-1 to 7 years. Adults were age-8 years and older.

Inbreeding depression

All populations are assumed to be subject to the detrimental effects of inbreeding. The strength of inbreeding depression is expressed as the number of lethal equivalents distributed across the species’ genome. In the absence of species-specific research, analyses like this one rely on meta-analyses across many species for which data on inbreeding levels and accompanying detrimental impacts can be measured, typically in controlled ex situ environments. An analysis by O’Grady et al. (2006) concluded that a typical wildlife population would carry approximately six lethal equivalents, with about half of those composed of recessive lethal genes. Relatively little is known about inbreeding depression in snakes. Therefore, it is prudent to adopt a conservative approach in an analysis of this type and assume it could be present. Consequently, a value of 6.29 lethal equivalents is used in all populations (see Lacy and Pollak 2025 and references therein for more information). However, detrimental impact occurs only when inbreeding becomes

relatively more pronounced in a population; if rates of inbreeding are negligible, inbreeding depression is not a factor influencing future population growth.

Correlation of environmental variation in demographic rates within and among populations

Vortex allows for correlation of the direction and strength of environmental variation between reproduction (probability of breeding) and age-specific survival. In this way, years that are good or bad for reproduction can also be similarly good or bad for survival as a general property of the species' life history. The level of correlation in the gray ratsnake models was set at 0.5, indicating a moderate level of correlation. Additionally, environmental variation can be correlated among populations. This correlation was also set to 0.5 as it is assumed, given the geographic proximity of the subpopulations, that environmental conditions determining survival would typically be somewhat, but not entirely independent among subpopulations.

Catastrophic events

While the threat assessment indicates that the Carolinian population of gray ratsnake is likely subject to periodic catastrophes by way of inclement weather in either the breeding or non-breeding season, catastrophes were not explicitly modeled. As such, our results are likely conservative.

Table 1. Baseline life history parameters used in Vortex models for the Carolinian population of gray ratsnake, with source(s) of information provided in parentheses. Where values have been revised from a source, the final input parameter value was agreed upon by experts during the technical sessions. Variation in key parameters was explored through sensitivity testing to address uncertainty of estimates.

Parameter	Input Value (Source)
Reproductive system	Polygamy (Blouin-Demers et al. 2005, COSEWIC 2007)
Median age of first reproduction	8 years for both sexes (revised from Row et al. 2007)
Maximum age of reproduction	30 (revised from Row et al. 2007, Blouin-Demers et al. 2002)
Maximum lifespan	30 (Blouin-Demers et al. 2004, COSEWIC 2007)
% adult males in breeding pool	100%
% adult females breeding	41.5 ± 8.5% (Blouin-Demers and Weatherhead 2002)
Maximum number of broods/year	1 per year (Blouin-Demers et al. 2004)
Maximum number of eggs per brood	23 (Blouin-Demers et al. 2004, Blouin-Demers et al. 2005)
Mean # of hatchlings/brood	14 ± 2 (Blouin-Demers et al. 2004, S. Thompson, pers. comm.)
Sex ratio at birth (M:F)	1:1 (Blouin-Demers et al. 2004)
% Mortality neonate (both sexes)	70 ± 5 (Pike et al. 2008, Technical Team discussion)
% Mortality subadult (both sexes)	55 ± 5% Age 1-7 years, decreasing 4% each year (Pike et al. 2008)
% Mortality adult (both sexes)	27 ± 5% (Pike et al. 2008)
Dispersal rate	0 (NRSI, unpub data, Loughheed et al. 1999, Howes et al. 2009)
Inbreeding depression	Yes
Lethal equivalents	6.29, with 50% due to recessive lethal alleles
Catastrophes	No
Correlation of environmental variation (EV) in demographic rates within and among populations	0.5

Scenario structure used in PVA simulations

A total of six models, each with multiple scenarios, were developed. Each model was defined by the same set of input parameters (Table 1), except as needed to assess a trait or traits of interest (e.g. initial population size). Through exploration of a range of possible variables that define a population, managers can be better informed about thresholds for characteristics that may contribute to conservation planning success. A summary of the models and their focus is provided below.

1. **Status Quo: What is the likely future of the six extant subpopulations in the Carolinian population based on best available knowledge? How does model output compare to suggested rates of current population decline based on the COSEWIC threat assessment?** A model was initialized with parameters in Table 1. The parameters were based on a combination of information from peer-reviewed publications, in particular from research on the Great Lakes/St. Lawrence population, and guidance elicited from species experts during technical sessions. The model included six subpopulations in which the only parameters that varied between them was initial population size (N) and carrying capacity (K), reflective of recent research by R. Wolfe in the Carolinian subpopulations.
2. **Minimum Viable Population: What is the minimum population size required to maintain 90% of initial genetic diversity in a subpopulation for 30 years?** Using the demographic rates as per the ‘Status Quo’ model, with 6 subpopulations, each with K=300 but varying in initial population size increasing from N = 40 to 100 in increments of 10, and with N=125 and N=150.
3. **Sensitivity analysis: What are the most important demographic drivers of gray ratsnake population trends? What are target rates required to achieve a stable or increasing population trend?** Starting with the demographic rates as per the ‘Status Quo’ model, each of five key demographic parameters was sequentially varied proportionally across a range of plausible values, between 80% and 120% of baseline estimates, at 5% increments (Table 2).
4. **Management scenario 1: What would be the impact of management focused on decreasing mortality? What are target rates required to achieve a stable or increasing population trend?** Starting with the demographic rates as per the ‘Status Quo’ model, mortality rates were varied between 10-40% of the starting value for all life history classes (Table 3). Based on the results, additional model scenarios were run to determine the level at which population trend would stabilize.
5. **Management scenario 2: What would be the impact of augmenting populations? How would this most effectively be accomplished?** Using the demographic rates from the ‘Status Quo’ model as the starting point, the Big Creek (N=100, K=545) and Ruthven (N=20, K=350) subpopulations were modelled under varying augmentation rates. Augmentation was simulated using individuals from three different age classes: 1, 2 and 3 years. These two subpopulations were chosen as being representative of the 2 larger subpopulations, and the 4 smaller subpopulations, respectively. The age classes were chosen based on expert guidance as being the most likely to reflect how augmentation would be undertaken in reality (i.e. headstarting eggs or neonates in human care). A ‘high’ (50%) and a ‘low’ (25%) post-release survival rate was implemented for each age class, based on results of translocations of Massasauga Rattlesnake (Choquette 2024).

Augmentation was implemented for 10 years and then stopped. Models were allowed to run for 30 years.

6. **Management scenario 3: What would be the impact of both decreasing mortality and augmenting subpopulations?** Two sets of additional scenarios were developed using a combination of input from Management Scenarios 1 and 2. As with Management Scenario 2, the Big Creek (N=100, K=545) and Ruthven (N=20, K=350) subpopulations were augmented using individuals 3 years of age, with two different degrees of augmentation and survival, as per Scenario 2. Mortality rates reflected a 10% and 20% decrease from the Status Quo scenario over 30 years.

Model Limitations – Uncertainty

We were unable to create a Status Quo model that reflected the demographics of gray ratsnake in the Carolinian population with certainty, other than population size and carrying capacity, as demographic information is a knowledge gap for this population. However, species experts believe that the majority of life history parameters are likely similar to the well-studied Great Lakes/St. Lawrence population – thus model parameterization was guided by results of research from this population. We attempted to address this large degree of uncertainty in our modelling approach through use of sensitivity analysis, and by assessing results of simulations from both large and small subpopulations. While we do not know what threats have caused the population decline of the Carolinian population, experts believed that the population trend was likely driven by high annual rates of mortality. For this reason, we developed simulations to set goals for conservation management, which could also be used to help assess the success of the conservation actions.

Results of PVA Simulations

A table of the full output of PVA simulations for the following output metrics is provided as an Appendix to this report.

Output Metric Definitions

The presentation of PVA models results in the subsequent sections focus on the following output parameters:

- **Mean population growth rate:** The growth rate can be expressed as either an annual finite rate of change $\lambda = N_{t+1} / N_t$, or as the instantaneous per capita growth rate $r = \ln(\lambda)$. A population with a mean $\lambda > 1.0$ ($r > 0.0$) will grow in abundance over time, while $\lambda < 1.0$ ($r < 0.0$) indicates a declining population.
- **Mean abundance of extant populations:** Figures report both total population abundance and the subset of the total that is comprised of adults. In both cases, abundance reported here for a given model scenario – known as N(Extant) – specifically tallies only those iterations in which the population persists, as defined above. This metric was chosen from Vortex output instead of an alternate metric known as N(All) in which iterations that become extinct (effectively, an abundance of 0) are included in the calculation of the mean abundance. While informative in its own right, relying on N(All) does not accurately report the abundance of a population that escapes the destabilizing forces that can drive a small population to extinction (the “extinction vortex” of Gilpin and Soulé (1986)). That said, it is important to remember that the stated value of N(Extant) may represent a

relatively small proportion of iterations in a given scenario that survive throughout the 50-year projection and, consequently, are likely to represent the relatively “lucky” set of circumstances that led to its persistence through time. Both persistence probability and $N(\text{Extant})$ are perhaps best interpreted using a statement such as “The likelihood of a population persisting over the time period of analysis might be relatively small but IF it persists, the abundance could be substantial”. It is important when reviewing results from any PVA analysis to fully understand the precise definitions of the output metrics and to interpret the output within the appropriate context.

- **Probability of population survival:** The proportion of iterations in a given scenario that do not decline to an abundance of zero, or that result in only one sex remaining in the population, at the end of the 50-year projection. Therefore, this is equivalent to $1 - (\text{probability of population extinction})$.
- **Mean proportion of initial gene diversity (GD) retained:** Also known as expected heterozygosity, this metric tracks the proportion of gene diversity that is retained across the 50 years of the population simulation. Gene diversity is lost more rapidly in smaller populations that experience higher rates of inbreeding, which may lead to long-term declines in a population’s capacity to adapt to changing environmental conditions.

Simulation Results

Status Quo

Questions Addressed:

- **What does the future of the six extant subpopulations in the Carolinian population look like based on best available knowledge?**
- **How does model output compare to suggested rates of population decline based on the COSEWIC threat assessment?**

Results of the simulations indicated a continual declining population trend for all subpopulations over the next 30 years (Figure 2). The probability of subpopulation persistence falls below 50% within 10 years for small subpopulations and below 50% within 20 years for large subpopulations (Figure 3). The greater risk of extirpation in small populations is a result of their small size – small populations are at risk due to stochasticity in reproduction and survival.

Genetic diversity, as well as population size, must also be examined as a component of population sustainability. When genetic diversity of the models for each subpopulation is examined, all 6 subpopulations demonstrate a trend of declining genetic diversity over the next 30 years (Figure 4). While there are few studies on the impact of loss of genetic diversity required to realize inbreeding depression in snakes, a goal of population management for species held in zoological facilities is to retain 90% of starting genetic diversity over 100 years. All four small subpopulations will experience a decline below 90% of starting diversity within 5 years and below 80% in 10 years. The larger subpopulations, Big Creek and Otter Creek, will experience a decline of genetic diversity below 90% of starting levels within 10 years and 80% within 20 years (Figure 4).

Summary:

- COSEWIC threat assessment results suggesting a 10-100% population decline in the next 30 years is supported by the PVA.

- Small subpopulations are not likely to persist for more than 10 years.
- The Sixteen Mile Creek subpopulation would be unable to support the minimum population size required to maintain more than 80% of the starting genetic diversity.
- Small subpopulations are likely to fall below 90% of starting genetic diversity in 5 years and 80% of starting genetic diversity in the next decade. Given the small population size, and optimistic starting point used in Vortex (i.e. all individuals are equally genetically diverse and unrelated), these subpopulations may already be at levels at which inbreeding depression is expressed.
- Large subpopulations are not likely to persist for more than 20 years.
- Large subpopulations are likely to fall below 90% starting genetic diversity in 10 years and 80% of starting genetic diversity in 20 years

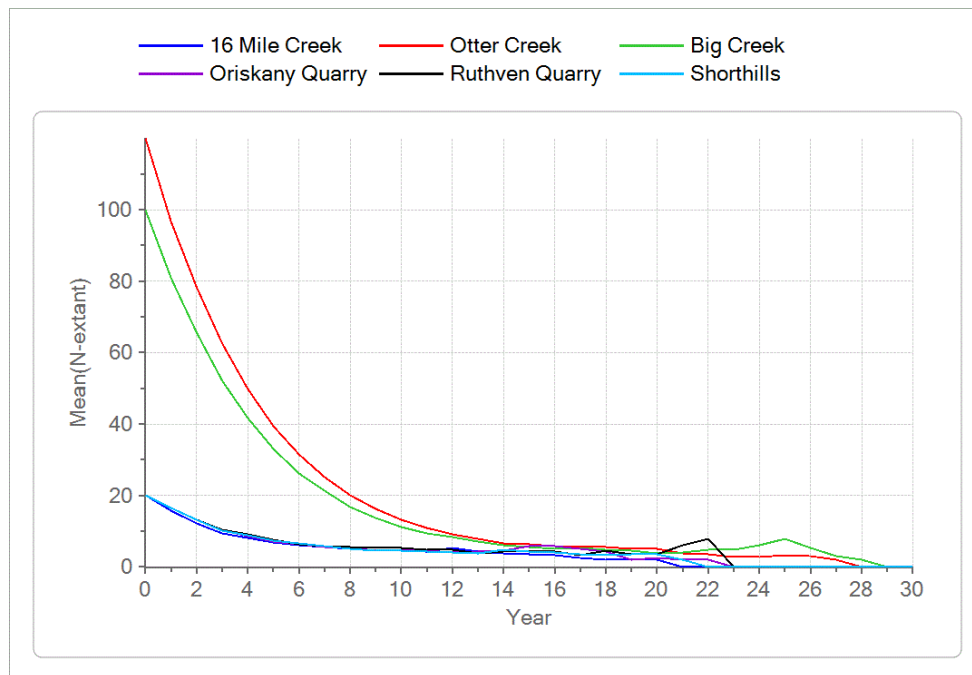


Figure 2. Mean abundance projections for six subpopulations of gray ratsnake in the Carolinian population with demographic rates as described in Table 1. Initial population size and carrying capacity are as noted in subpopulation descriptions in the text. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

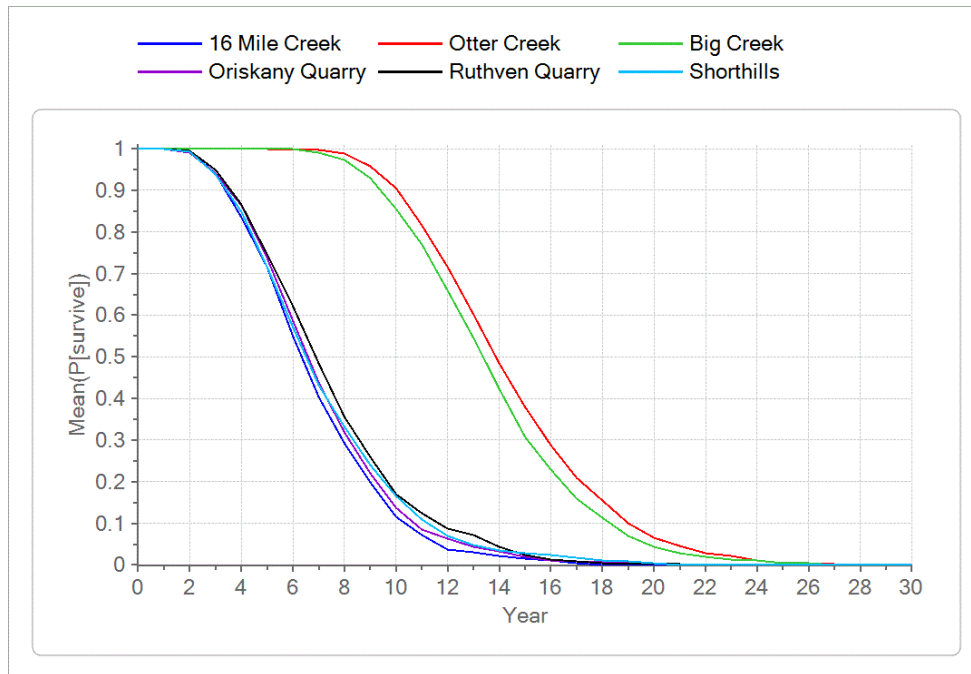


Figure 3. Mean probability of survival for six subpopulations of gray ratsnake in the Carolinian population with demographic rates as described in Table 1. Initial population size and carrying capacity are as noted in the text.

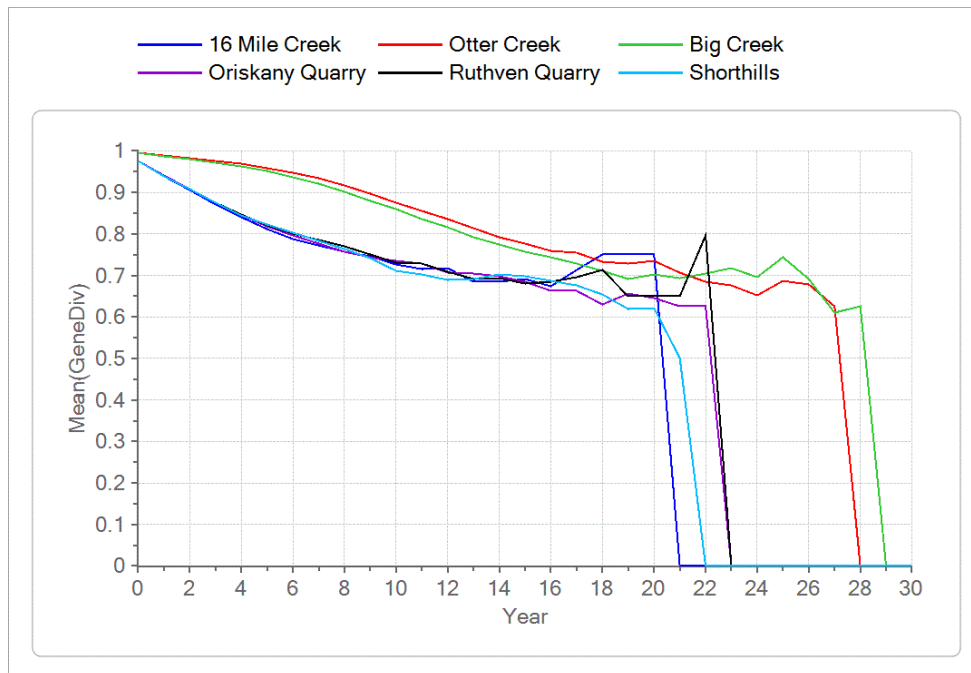


Figure 4. Mean genetic diversity projections for six subpopulations of gray ratsnake in the Carolinian population under demographic parameters described in Table 1. After Year 15 the annual instability is the result of including results from only the iterations in which a subpopulation is extant, given that few iterations result in an extant subpopulation. The rapid decline to GD=0 after Year 20 is a result of all iterations declining to extinction by that time for that subpopulation.

Minimum Viable Population Size

Question Addressed:

- **What is the minimum population size required to maintain 80% or 90% of initial genetic diversity in a subpopulation for 30 years?**

Scenarios were developed to assess loss of genetic diversity over time, and to provide guidance on minimum viable population size required to meet genetic diversity retention goals. Using the Status Quo demographic parameters, scenarios were developed in which initial population size was varied from $N=20$ to $N=200$, in increasing increments of 10 individuals. Results of these simulations suggest that an initial population size equal or greater to 160 adults is required to maintain 90% of initial genetic diversity over 30 years (Figure 7).

Summary:

- Breeding populations require at least 50 individuals to maintain 80% of genetic diversity over 10 years and 160 individuals to maintain 90% of starting genetic diversity over 10 years. In reality, this may be an optimistic view, as Vortex starts simulations under the assumption that all individuals are equally genetically diverse and unrelated. Given the rapid decline and small population size of existing subpopulations, these assumptions are likely being violated in the wild Carolinian population of gray ratsnake.

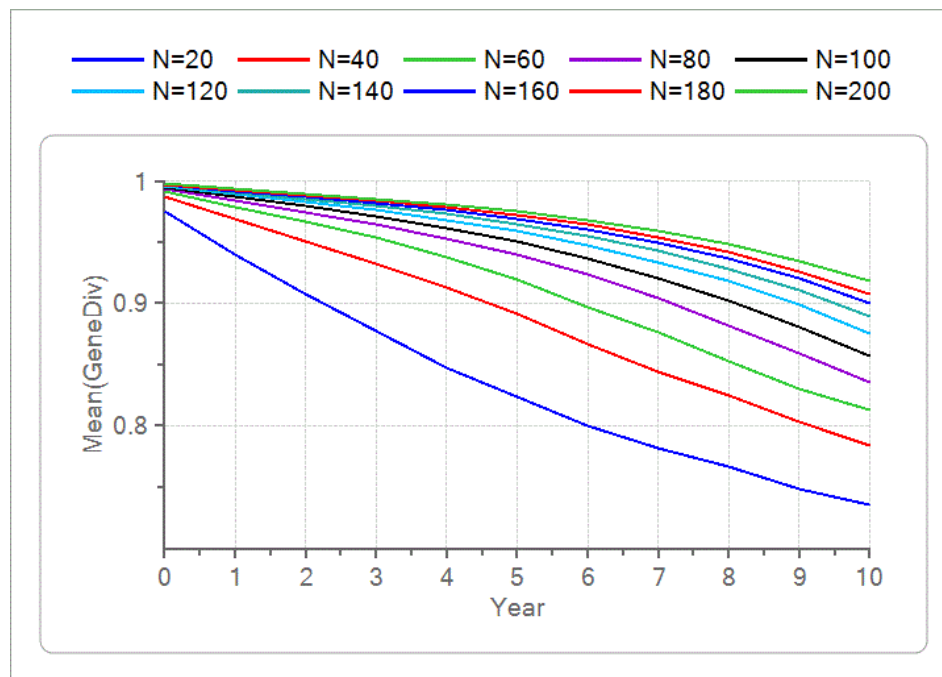


Figure 5. Mean genetic diversity projections for six subpopulations characterized by demographic parameters described in Table 1, but with $K = 300$ and N varying from 20 to 200.

Sensitivity Analysis

Question Addressed:

- **What are the most important demographic drivers of gray ratsnake population trends**

Many of the demographic parameters for this PVA were estimated with at least a moderate level of uncertainty. This uncertainty is qualitatively different in character than the measures of variability included in mean rates of reproduction and survival, which represent annual fluctuations in environmental conditions that influence wildlife population demography. Understanding the impacts of our collective uncertainty in gray ratsnake population dynamics can be important to identify key knowledge gaps, and thus for prioritizing future data collection, and targeted management activities.

In order to evaluate which demographic parameters have the largest impact on population growth, this PVA includes a demographic sensitivity analysis. Based on discussions with the technical team, we chose to focus on mortality rates of each life history class, percentage of females breeding and number of hatchlings. Each of the parameters were sequentially varied proportionally across a range of plausible values, between 80% and 120% of the baseline estimates, at 5% increments. Stochastic population growth rate (stochastic r) was chosen as the output metric for analysis. The output was analyzed graphically by generating a “spider plot” showing stochastic λ for each scenario. In addition, the simplified method of Crowder et al. (1994) was used for calculating the elasticity or proportional sensitivity of each demographic parameter. This method, based on more detailed methods for analyzing population matrix models (Caswell 2001), provides an additional numerical comparison of the relative change in population growth given a perturbation in each of the input parameters of interest. Elasticity for a given parameter x is calculated as:

$$E_x = \frac{\lambda_{(x+0.2x)} - \lambda_{(x-0.2x)}}{0.4 \times \lambda_x}$$

where λ_x is the growth rate estimated when parameter x is at the best baseline value.

Results of this sensitivity analysis are given in Table 2 and Figure 8. Numerically, the normalized elasticity values confirm what can be seen in the figure. Subadult mortality emerges as the parameter with the largest relative elasticity, followed by adult mortality, then percentage of females breeding, number of hatchlings and neonate mortality. Graphically, parameters with the largest relative sensitivity are those with the steepest slope across the range of tested values (Figure 9). A negative slope indicates an inverse relationship between a parameter like mortality, and population growth rate, while a parameter like size shows a positive relationship with stochastic r .

Table 2. Elasticity (proportional sensitivity) $E(x)$ of selected population demographic input parameters for the gray ratsnake PVA. Values presented are stochastic population growth rates λ derived from independent scenarios with the input parameter in the column heading being varied across the range shown in the far left of the table. Abbreviations for input parameters align with those defined in Table 1.

Value	%Fbreed	#Offspring	NeonMort	SubAdult Mort	AdultMort
0.8	0.7704	0.7685	0.7891	0.8446	0.8063
0.85	0.7701	0.7746	0.7848	1.0000	0.7982
0.9	0.7762	0.7731	0.7828	0.8138	0.7925
0.95	0.7751	0.7748	0.7806	1.0000	0.7847
1	0.7761	0.7797	0.7763	0.7771	0.7794
1.05	0.7783	0.7800	0.7724		0.7697
1.1	0.7759	0.7800	0.7684	0.7420	0.7646
1.15	0.7779	0.7786	0.7665		0.7553
1.2	0.7819	0.7738	0.7593	0.7158	0.7535
$E(x)$	0.0370	0.0170	-0.0960	-0.4144	-0.1694
$E(x)$ -Norm	-0.0894	-0.0410	0.2316	1.0000	0.4087

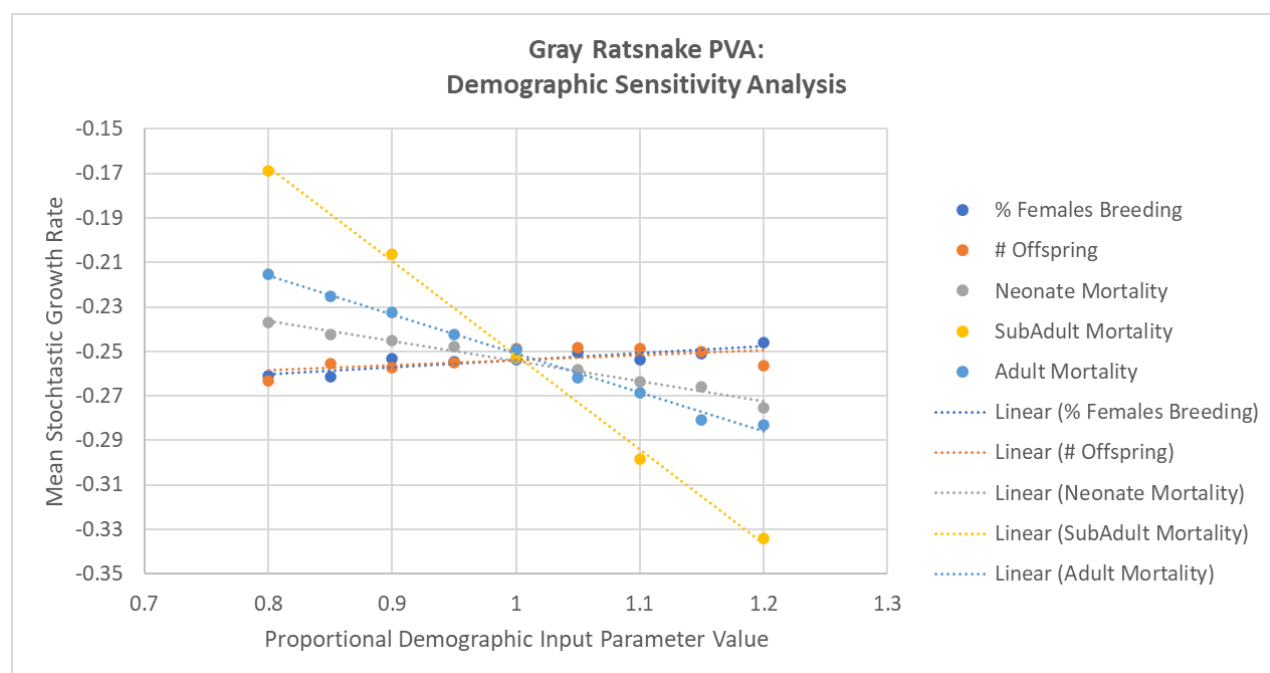


Figure 6. Results of demographic sensitivity analysis in the gray ratsnake PVA. The central ‘body’ of the spider plot gives the stochastic growth rate of the baseline scenario with each parameter at its baseline estimate. Each data point represents an independent scenario featuring a specific parameter that varies proportionally across a plausible range of values. See text for more details.

Summary:

- Significant knowledge gaps exist regarding baseline demographic data, with data on survival/mortality being of particular importance.
- Sub-adult mortality and adult mortality are, respectively, the most important drivers of population trends.

Management Scenario 1:**Question Addressed:**

- **What would the impact be of management focused on decreasing mortality for various life history stages?**
- **What target rates for survival are required to achieve a stable or increasing population trend?**

The threat assessment and status quo model developed for gray ratsnake in the Carolinian population suggests that the subpopulations will likely experience drastic declines in the next 30 years under the current management regime. We do not know the level of impact of specific threats on specific life history traits or life history stages. However, simulations to reflect improvement of demographic rates, as would be expected following effective management action, can help to guide planning by identifying the degree of change required to meet goals. Based on the results of the sensitivity analysis, and discussion with experts during technical sessions, we simulated improved rates of mortality in neonate, subadult and adult snakes. We decreased mortality incrementally by 10% from baseline (Status Quo model, Table 1) for each age class (Table 3). Simulations were run for neonates, subadults and adults independently, for both a large population (Big Creek) and a small population (Ruthven), neither of which would be limited by carrying capacity. Based on results of simulations, we adjusted mortality rates to determine the rate of improvement that would be required across all age classes collectively to achieve a stable to slightly increasing population trend, indicative of a viable population (i.e. the goal for conservation planning for this population).

Table 3. Revised mortality rates for simulations of improved survival/reduced mortality for all age classes of gray ratsnake.

Age Class	Decrease in Mortality				
	Baseline	10%	20%	30%	40%
Neonate	70	63	56	49	42
Age 1-2	55	49.5	44	38.5	33
Age 2-3	51	45.5	40	34.5	29
Age 3-4	47	41.5	36	30.5	27
Age 4-5	43	37.5	32	27	27
Age 5-6	39	33.5	28	27	27
Age 6-7	35	29.5	27	27	27
Age 7-8	31	27	27	27	27
Age 8+	27	24.3	21.6	18.9	16.2

Results of changes to one life history class at a time were the same for both large and small populations. In both, varying of mortality rates for neonate, subadults, or adults did not result in significant projections of population abundance, probability of survival or retention of genetic

diversity. This is likely due to the protracted subadult phase. Results are shown for the Big Creek subpopulation (Figures 7 to 12) but were similar for the simulated small (Ruthven) subpopulation. Simulations suggested that a rate of improvement between 30-40% in all age classes was required to achieve population stability/growth for both large and small populations (not shown). Further simulations were developed to refine this estimate – results suggest that a 34% improvement in mortality rates for all age classes would lead to slight population growth for a large subpopulation and a stabilizing effect for a small population (Figure 13).

Summary

- A decrease in mortality rate is required at all age classes/stages in order to realize positive population growth.
- Large and small populations may be able to achieve positive population growth when mortality is reduced by 34% for all age classes.

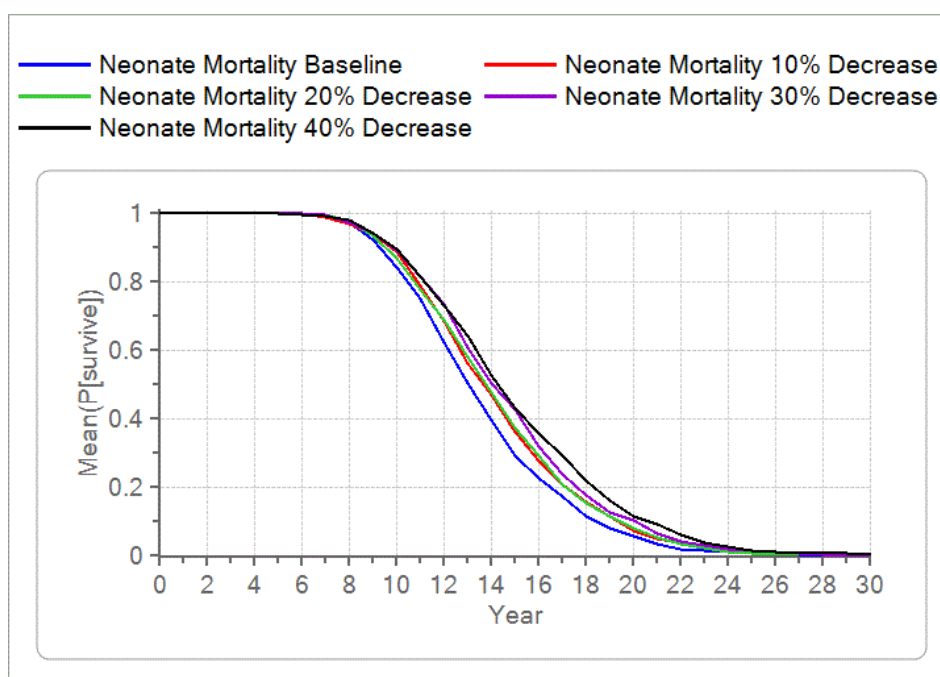


Figure 7. Mean abundance projections for Big Creek subpopulation under scenarios in which neonate mortality rates were improved as described in Table 3. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

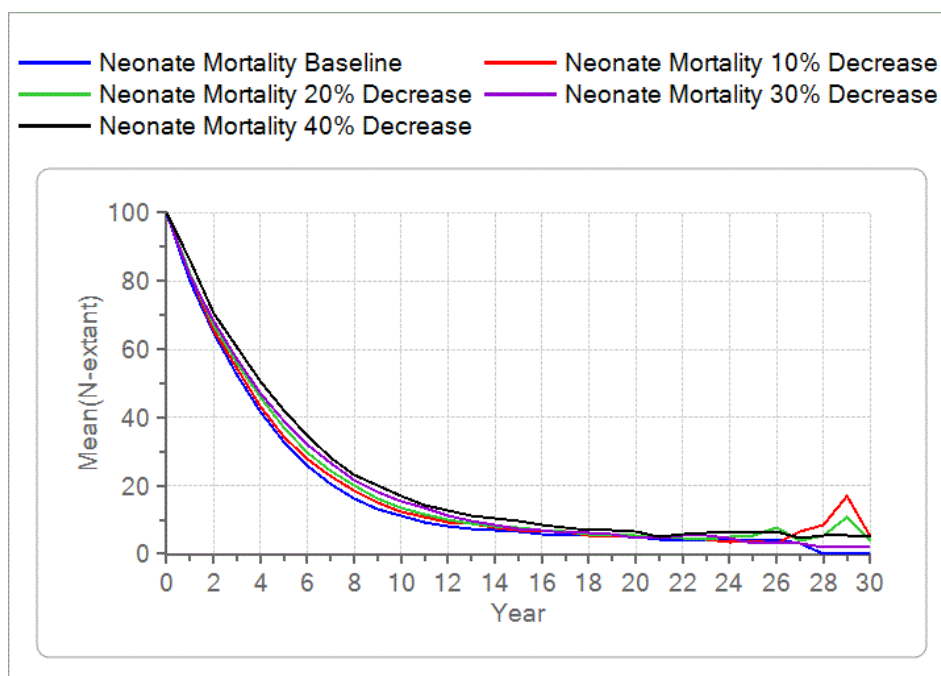


Figure 8. Mean percent survival for the Big Creek subpopulation under scenarios in which neonate mortality rates were improved as described in Table 3.

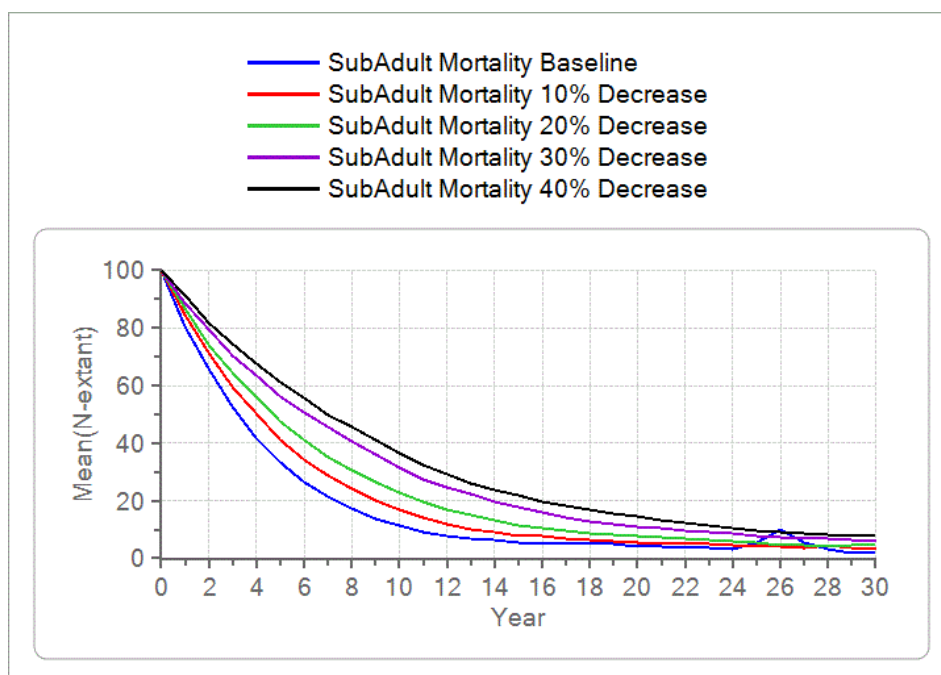


Figure 9. Mean abundance projections for Big Creek subpopulation under scenarios in which subadult rates were varied as described in Table 3 and the text. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

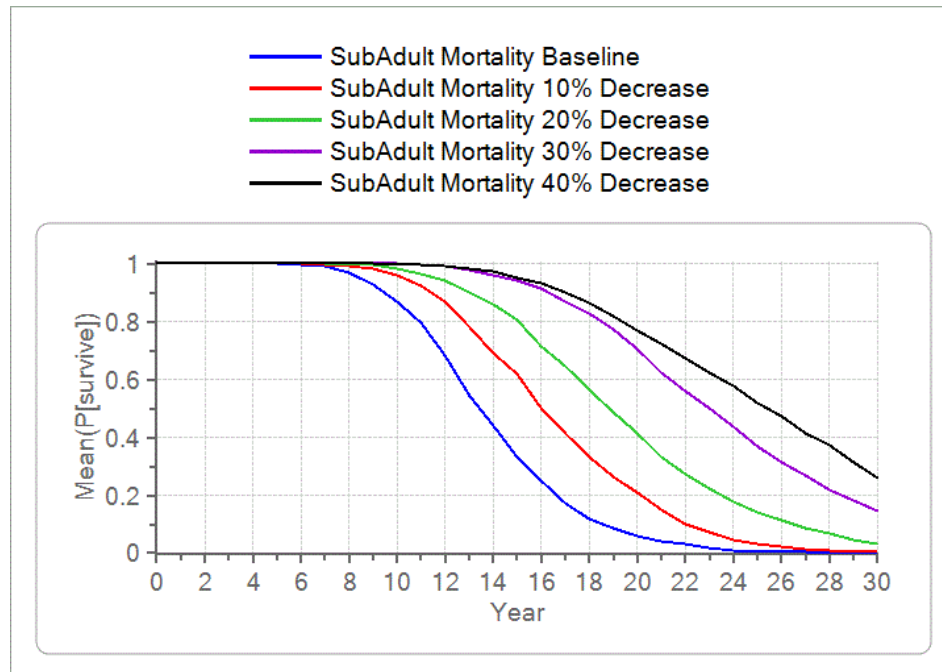


Figure 10. Mean percent survival for the Big Creek subpopulation under scenarios in which subadult mortality rates were varied as described in Table 3.

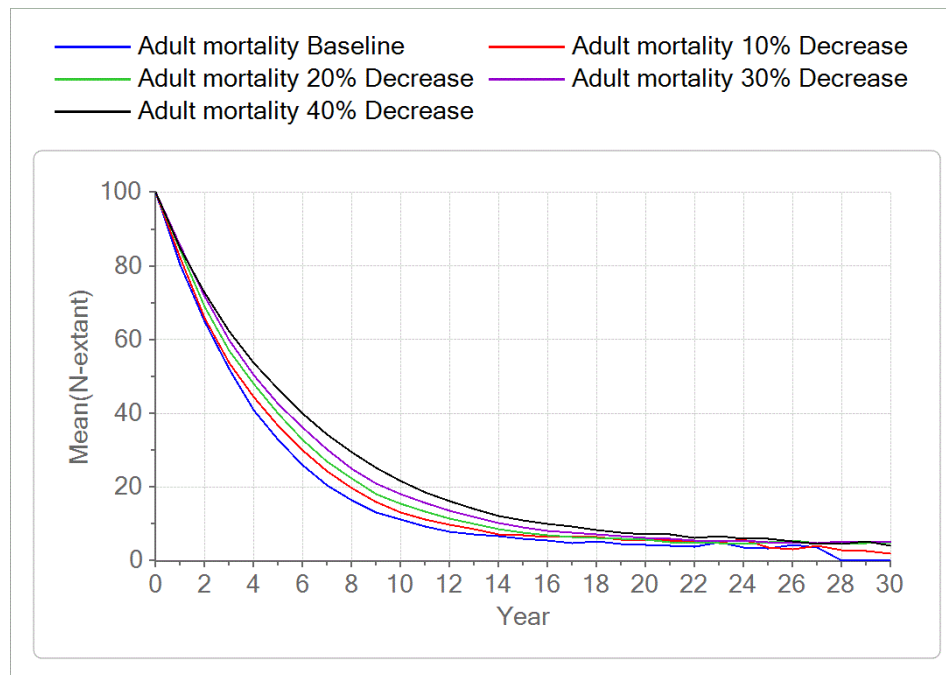


Figure 11. Mean abundance projections for Big Creek subpopulation under scenarios in which adult rates were varied as described in Table 3. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

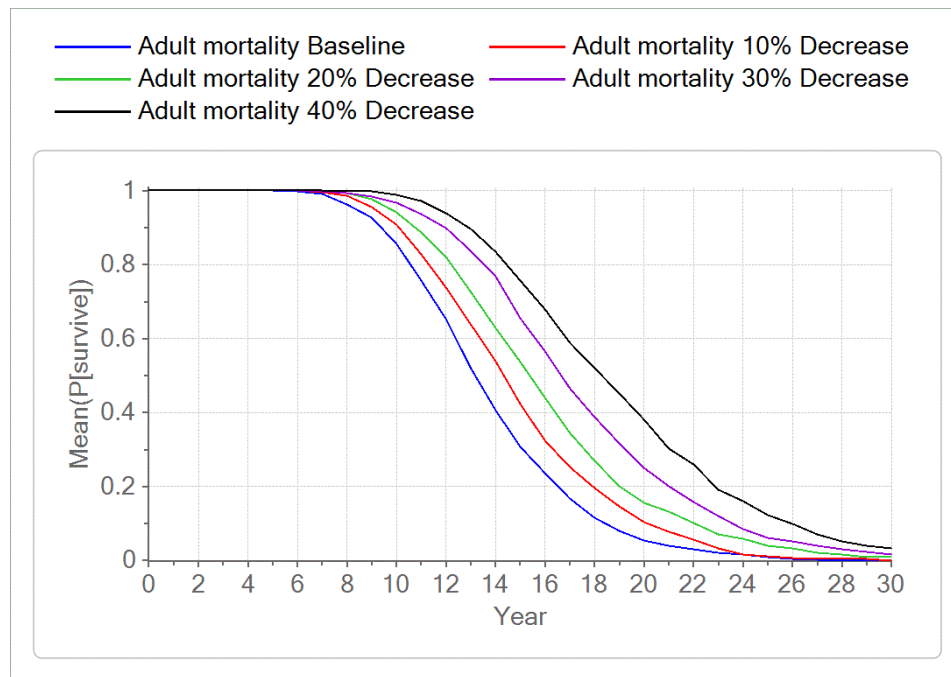


Figure 12. Mean percent survival for the Big Creek subpopulation under scenarios in which adult mortality rates were varied as described in Table 3.

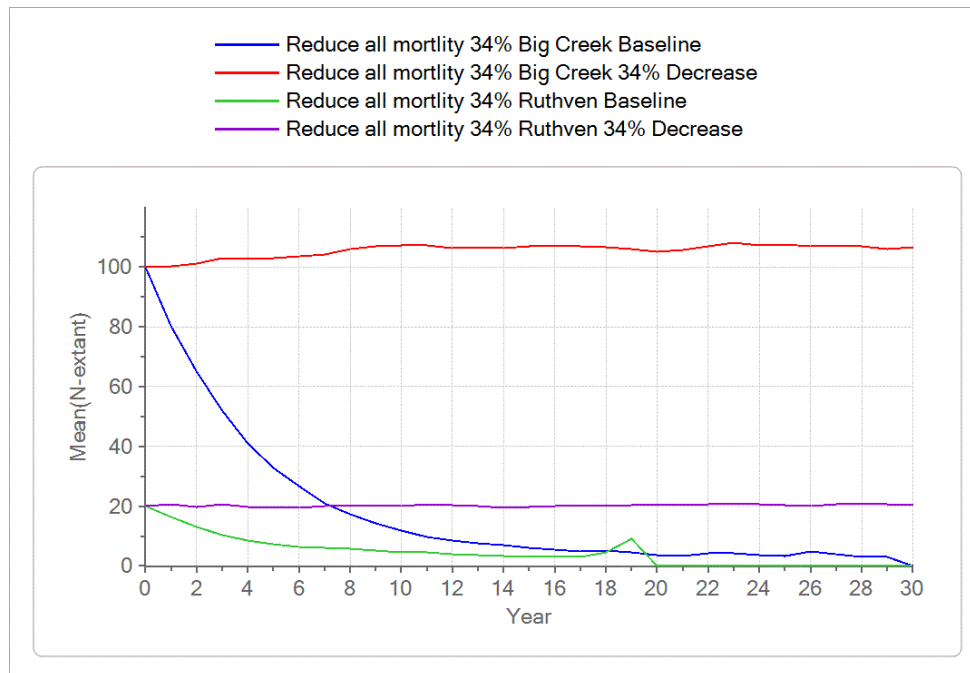


Figure 13. Mean abundance projections for the Big Creek and Ruthven subpopulations under scenarios in which mortality rates were decreased to 34% for all age classes. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

Management Scenario 2:**Question Addressed:**

- **What would the impact be of increasing population size via augmentation?**
- **What is the impact of augmentation based on age of released individuals and post-release survival rates?**

Simulation results presented in prior sections suggest that small population sizes and ongoing threats require urgent management to ensure that the Carolinian population of gray ratsnake does not become extirpated within the next 20-30 years. All but one subpopulation (Sixteen Mile Creek) has room for population growth, a necessary requirement for long-term stability. Further, four of the subpopulations are located in close proximity to another population (Big Creek and Otter Creek, Ruthven and Quarry) – thus theoretically, population size could be increased simply by increasing the connectivity between subpopulations. The question ‘what would be the impact of increasing dispersal?’ was posed during the initial on-line session, following presentation of simulation results on population persistence and genetic diversity decline. Since so little is known of dispersal dynamics, and since the workshop participants believed it was highly unlikely that dispersal could occur among subpopulations, we chose instead to address this question by creating some additional scenarios in which a small (N=20) and large (N=100) subpopulation, similar to Ruthven and Big Creek, respectively were augmented (i.e. new individuals were added to the populations). In doing so, we also address management needs suggested during the ICAP for Canadian snakes (Winton et al. 2021), and the Ontario Government Response Statement for the population.

While we wanted the additional simulations to be simple, due to the existing uncertainty regarding demographic rates, we also wanted them to reflect the reality of mortality following release, which would also be likely during dispersal. We chose to start with the model reflecting a 20% decline, and focused on the Big Creek and Ruthven subpopulations (i.e. a large and a small subpopulation). The model representing a 20% population decline was considered a reasonable low-to-moderate level of decline based on the threat assessment, while also recognizing the uncertainty surrounding population demographic parameters. We developed scenarios in which an equal number of male and female snakes were added to each population. Vortex also requires that the age, as well as sex, of the individuals being added be specified. Based on discussions with the technical team, and in particular those from the zoo community, we chose to focus on addition of younger snakes (i.e. held for no more than 1 year), as would likely be most feasible under a headstarting management approach. We also considered a literature review of translocations of Eastern Massasauga rattlesnake (Choquette 2024), which summarized the results of releasing with individuals varying in age, but with no consideration of sex. The review indicated mortality rates of translocated neonate, sub-adult (age 2-3) and adult (age 3) Eastern Massasauga Rattlesnake were 75-100%, 47-80%, and 100%, respectively. The two subpopulations were supplemented for 10 years, but simulations continued to run for 30 years.

In order to reflect the likely mortality of translocated snakes, scenarios included post-release mortality. Few snake headstarting or translocation programs have been initiated, and we had limited information to draw upon. Thus, we again used estimates based on previous research on the Eastern Massasauga rattlesnakes, we developed scenarios with both a high mortality rate (25% post-release survival) and a low mortality rate (50% post-release survival).

Supplementation rates ranged from N=16, N=24, N=32 and N=40 in cases of low survival, equivalent to the addition of 4, 6, 8 and 10 new individuals to the population (split even between sexes), and N=16, N=24, N=32, N=40 in cases of high survival, equivalent to the addition of 8, 12, 16 and 20 individuals (split evenly between sexes), after mortality had been imposed in the simulation.

Simulation results were first examined to determine which supplemented age class was most effective at increasing population size across both subpopulation sizes, all supplementation rates, and post-release survival scenarios. Across the scenarios evaluated, the release of 7-year-old snakes were most effective at increasing population size (results not shown). This is likely due to the fact that older individuals are closest to breeding age and thus a larger number of snakes released will reproduce than if younger snakes are released as these individuals will still be subject to one or more annual mortality events before reaching sexual maturity.

However, it was determined that head-starting was likely the most realistic option for an augmentation program, using either eggs or neonates obtained from the wild. Experts believed that hatchlings or neonates could be held up to three years before being released. This management action would address the high neonate mortality rate. Further, snakes held in professional care will usually grow more quickly than wild counterparts due to husbandry care (e.g. food is provided more regularly and for potentially a longer active period). Thus, released individuals may reach sexual maturity at an earlier age.

Simulations were run to test the impact of augmenting with age 1-, 2- and 3-year-old snakes, using the same levels of augmentation and mortality rates as previously noted. Unlike previous simulations, the potential benefit was only slightly greater when using older animals, as illustrated for Big Creek (Figure 14). Population augmentation with 3-year-old snakes did not lead to improved population trend for either the small or large subpopulation (Figures 15 and 16). Without threat abatement measures to address the population decline, the modest benefits were not sustained after supplementation was terminated. However, in both size subpopulations, augmentation helped to maintain genetic diversity beyond the time when releases were terminated (Figures 17 and 18).

Summary:

- Augmentation will be most successful with older sub-adult age classes. It will not lead to population growth for any subpopulation without threat abatement, but will help retain genetic diversity.

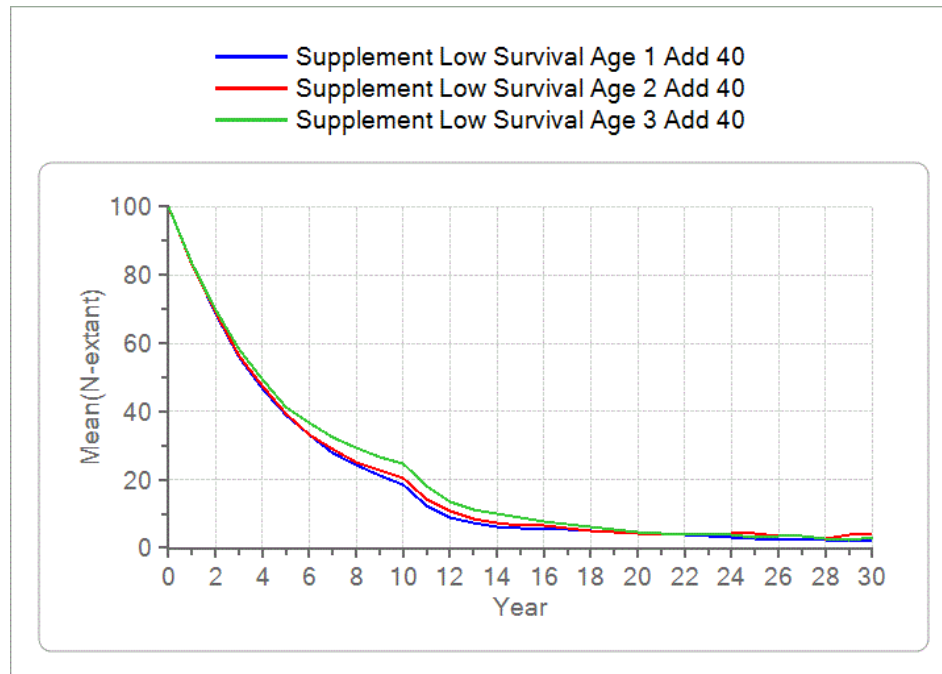


Figure 14. Mean abundance projections for the Big Creek subpopulation with demographic rates as described in Table 1, augmented with 40, 1-, 2- or 3-year-old snakes with (low) 25% post-release survival. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

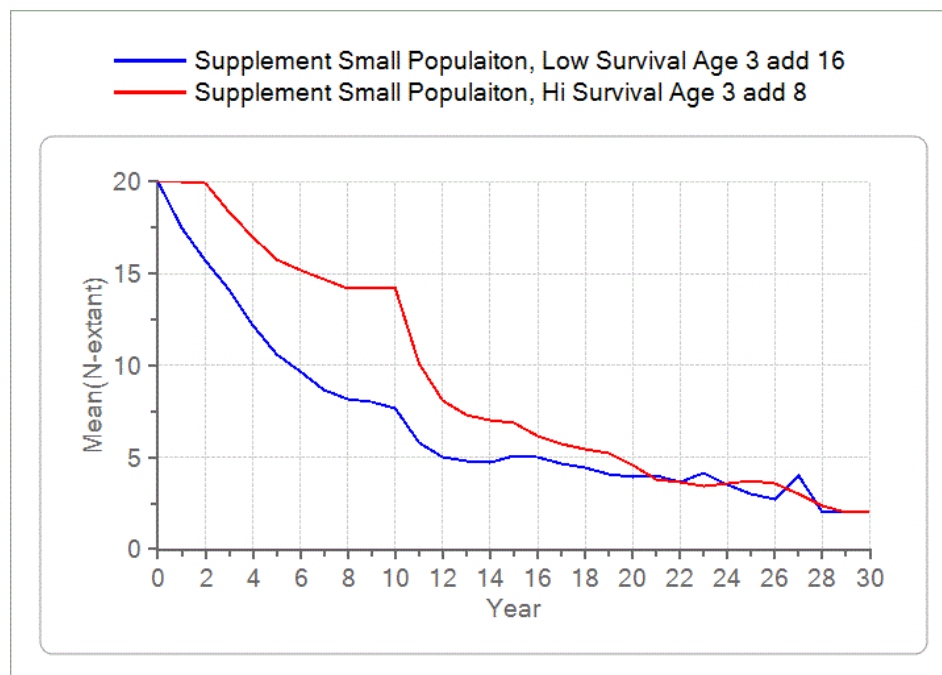


Figure 15. Mean abundance projections for the Ruthven subpopulation with demographic rates as described in Table 1, augmented with 16, or 8 snakes, 3 years of age with low (25%) or high (50%) post-release survival, respectively. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

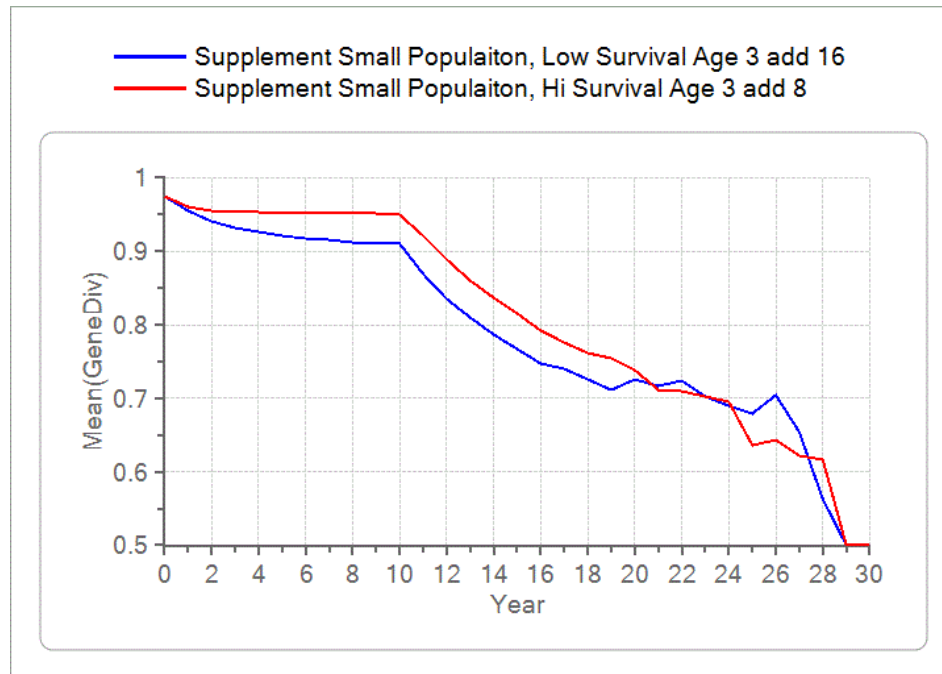


Figure 16. Mean genetic diversity projections for the Ruthven subpopulation of gray ratsnake in the Carolinian population under demographic parameters described in Table 1 and supplementation of 3-year-old snakes.

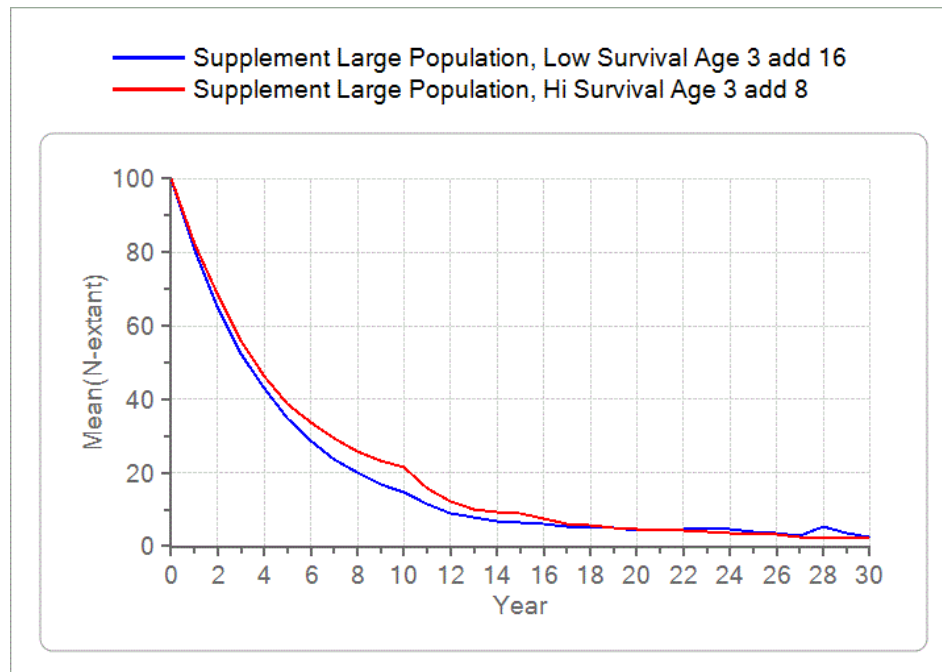


Figure 17. Mean abundance projections for the Big Creek subpopulation of gray ratsnake in the Carolinian population with demographic rates as described in Table 1 augmented with 16, 3-year-old snakes, with 25% post-release survival. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

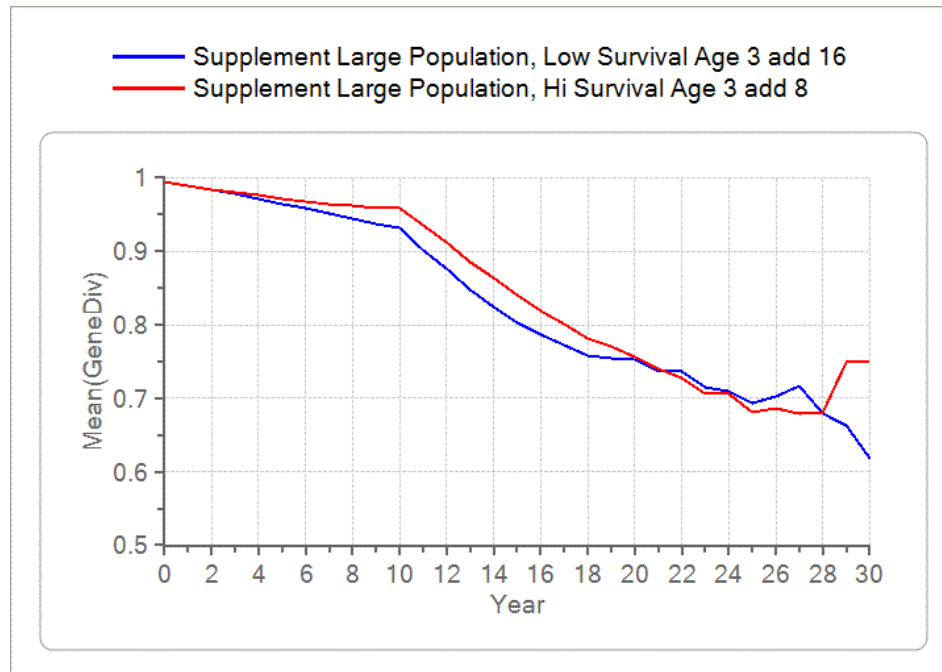


Figure 18. Mean genetic diversity projections for the Big Creek subpopulation of gray ratsnake in the Carolinian population under demographic parameters described in Table 1 and supplementation of 3-year-old snakes, with both high and low post-release survival.

Management Scenario 3:

Question Addressed:

- What would the impact be of **both** decreasing mortality and augmenting subpopulations?

A final set of scenarios was developed that combined input parameters from the first and second management scenarios (i.e. both showing improvement in survival rates and with population augmentation). For both a large and small population, mortality rates were abated by 20% for all age classes (i.e. as in Scenario 2), which was believed to be a realistic outcome from management to address threats (e.g. decrease road mortality). The populations were augmented at low rates, with post-release survival set to 25% (the lower of the two rates modelled in Management Scenario 2). Results suggested that for the large population, decreasing mortality by 20% for neonate, subadult and adult classes, in addition to augmentation with 12, 3-year-old snakes, with 25% post-release survival would result in an overall stabilizing effect in the short-term (Figure 19). Probability of extinction was decreased to less than 20% (Figure 20), and genetic diversity was maintained above 80% over 30 years (Figure 21). However, the realized benefit disappeared after augmentation ceased, suggesting greater improvement in survival was needed. For small populations, augmentation at this same rate but with only a 10% decrease in mortality rates resulted in population growth (Figure 22). Even after augmentation ceases, the probability of survival improved to nearly 60% at the end of 30 years (Figure 23) and genetic diversity was retained above 80% over 30 years (Figure 24).

Summary:

- Augmentation at even low levels for 10 years coupled with a 20% decrease in mortality of all age classes will likely result in stabilized population trend for large populations and positive population growth for small populations, but only during the period of augmentation.
- Low levels of augmentation for 10 years will likely ensure genetic diversity is maintained above 80% of starting levels for both small and large populations over 30 years.
- Low levels of augmentation for 10 years will increase probability of survival to over 90% for large populations and 60% for small populations over 30 years.

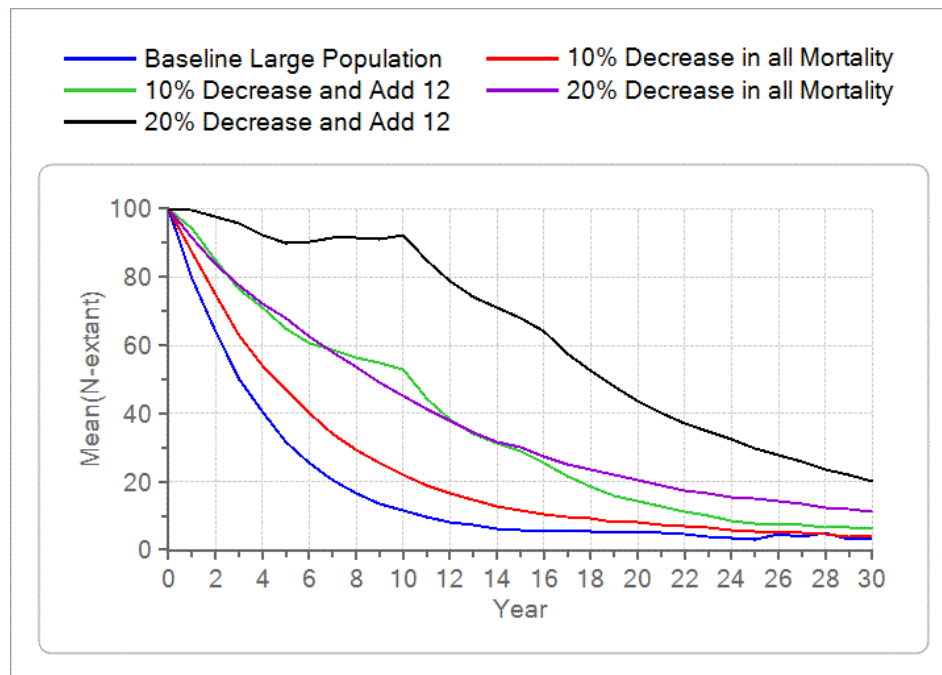


Figure 19. Mean abundance projections for the Big Creek subpopulation in scenarios where mortality rates were improved by 10 or 20%, with and without augmentation of 12, 3-year-old snakes with 25% post-release survival. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists.

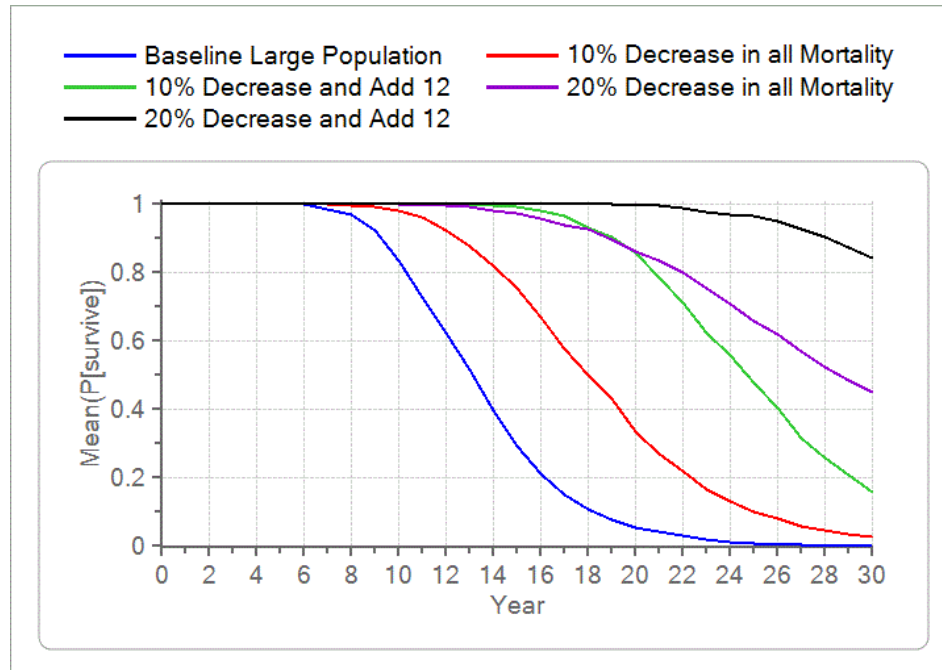


Figure 20. Mean probability of survival for the Big Creek subpopulation in scenarios where mortality rates were improved by 10 or 20%, with and without augmentation of 12, 3-year-old snakes with 25% post-release survival

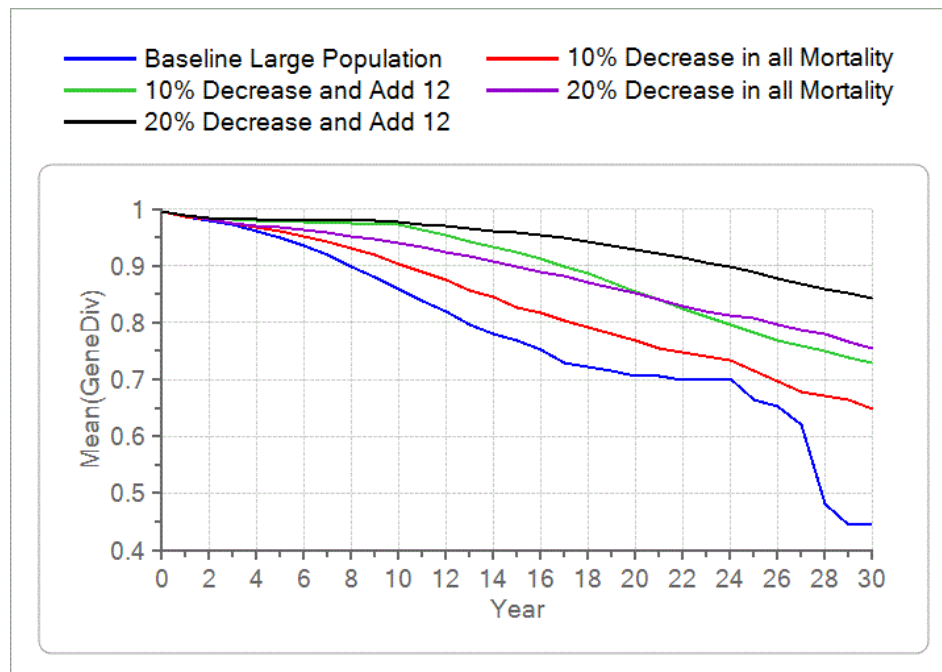


Figure 21. Mean genetic diversity for the Big Creek subpopulation in scenarios where mortality rates were improved by 10 or 20%, with and without augmentation of 12, 3-year-old snakes with 25% post-release survival.

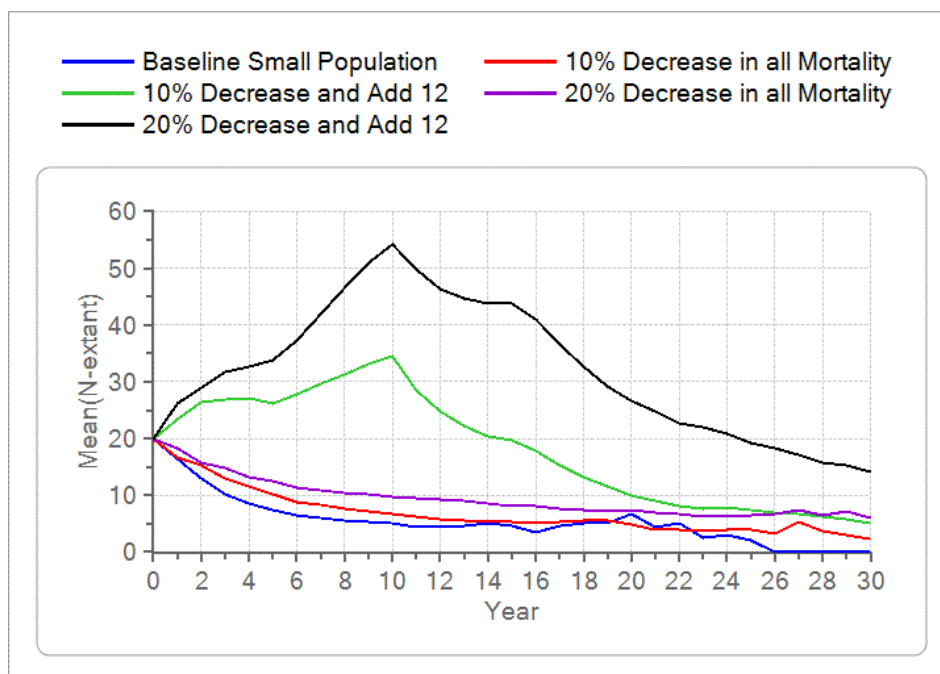


Figure 22. Mean abundance projections for the Ruthven subpopulation in scenarios where mortality rates were improved by 10 or 20%, with and without augmentation of 12, 3-year-old snakes with 25% post-release survival. Calculations of mean abundance include only those simulation iterations in which the subpopulation persists

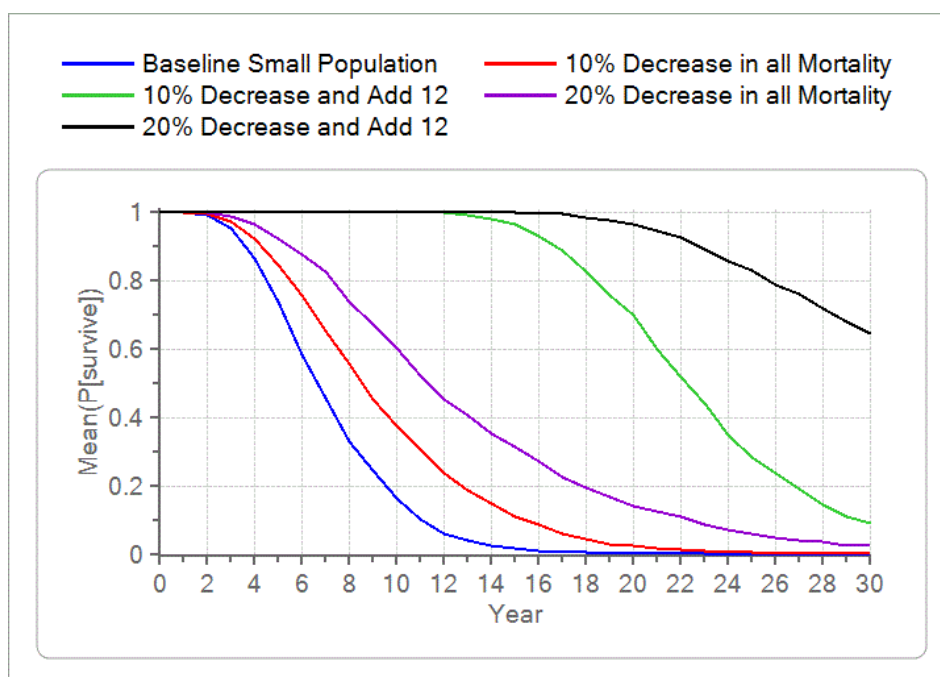


Figure 23. Mean probability of survival for the Ruthven subpopulation in scenarios where mortality rates were improved by 10 or 20%, with and without augmentation of 12, 3-year-old snakes with 25% post-release survival.

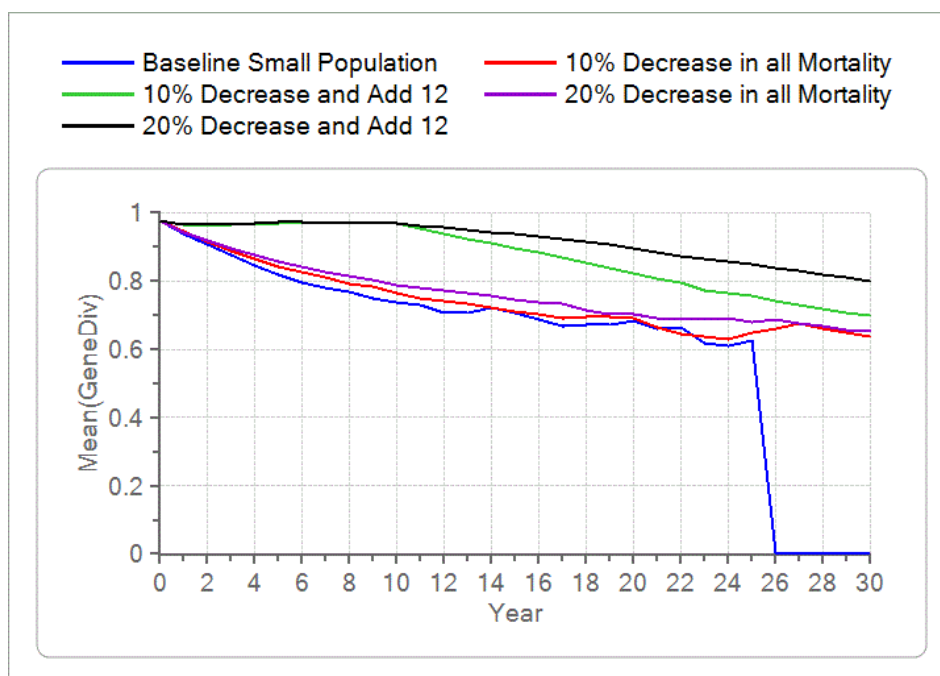


Figure 24. Mean genetic diversity for the Ruthven subpopulation in scenarios where mortality rates were improved by 10 or 20%, with and without augmentation of 12, 3-year-old snakes with 25% post-release survival

Summary of output of PVA simulations (see report for explanation of output metrics):

Scenario	Population	stoch-r	SD(r)	PE	N-extant	SD(N-ext)	GeneDiv	SD(GD)
Status Quo all pops	16 Mile Creek	-0.2589	0.44	1.00	0	0.00	0.0000	0.00
Status Quo all pops	Otter Creek	-0.2505	0.34	1.00	0	0.00	0.0000	0.00
Status Quo all pops	Big Creek	-0.2498	0.36	1.00	0	0.00	0.0000	0.00
Status Quo all pops	Quarry	-0.2571	0.43	1.00	0	0.00	0.0000	0.00
Status Quo all pops	Ruthven	-0.2511	0.43	1.00	0	0.00	0.0000	0.00
Status Quo all pops	Shorthills	-0.2546	0.43	1.00	0	0.00	0.0000	0.00
Status Quo all pops	Meta	-0.2700	0.28	1.00	0	0.00	0.0000	0.00
N versus GD	N=20	-0.2588	0.43	0.83	5	3.36	0.7351	0.07
N versus GD	N=30	-0.2642	0.40	0.70	5	3.09	0.7603	0.08
N versus GD	N=40	-0.2654	0.38	0.60	6	4.31	0.7839	0.07
N versus GD	N=50	-0.2621	0.36	0.47	7	4.71	0.7939	0.07
N versus GD	N=60	-0.2622	0.35	0.40	8	5.31	0.8125	0.07
N versus GD	N=70	-0.2570	0.34	0.28	9	6.16	0.8245	0.07
N versus GD	N=80	-0.2556	0.33	0.22	9	6.48	0.8357	0.07
N versus GD	N=90	-0.2603	0.32	0.20	10	7.26	0.8465	0.06
N versus GD	N=100	-0.2525	0.31	0.14	11	7.51	0.8575	0.06
N versus GD	N=110	-0.2491	0.30	0.11	12	7.77	0.8694	0.05
N versus GD	N=120	-0.2502	0.30	0.09	13	8.55	0.8751	0.05
N versus GD	N=130	-0.2445	0.28	0.07	15	9.09	0.8842	0.05
N versus GD	N=140	-0.2460	0.28	0.04	15	9.57	0.8896	0.05
N versus GD	N=150	-0.2430	0.27	0.04	17	9.86	0.8953	0.04
N versus GD	N=160	-0.2443	0.27	0.03	17	10.17	0.9001	0.04
N versus GD	N=170	-0.2446	0.27	0.03	18	10.27	0.9041	0.04
N versus GD	N=180	-0.2413	0.26	0.02	19	11.21	0.9076	0.04
N versus GD	N=190	-0.2416	0.26	0.02	21	11.67	0.9126	0.04
N versus GD	N=200	-0.2404	0.25	0.01	22	12.20	0.9182	0.03
N versus GD	Meta	-0.2329	0.13	0.00	212	61.60	0.9916	0.00
Females Breeding	Decrease 20%	-0.2608	0.35	1.00	2	0.00	0.7500	0.00
Females Breeding	Decrease 15%	-0.2612	0.36	1.00	0	0.00	0.0000	0.00
Females Breeding	Decrease 10%	-0.2533	0.36	1.00	0	0.00	0.0000	0.00
Females Breeding	Decrease 5%	-0.2547	0.35	1.00	0	0.00	0.0000	0.00
Females Breeding	Baseline Breeding Age	-0.2535	0.35	1.00	2	0.00	0.6250	0.00
Females Breeding	Increase 5%	-0.2507	0.36	1.00	0	0.00	0.0000	0.00
Females Breeding	Increase 10%	-0.2537	0.35	1.00	0	0.00	0.0000	0.00
Females Breeding	Increase 15%	-0.2511	0.35	1.00	0	0.00	0.0000	0.00
Females Breeding	Increase 20%	-0.2460	0.35	1.00	5	0.00	0.6600	0.00
Females Breeding	Meta	-0.2759	0.23	0.99	2	1.13	0.7193	0.05
Neonate Mortality	Decrease 20%	-0.2369	0.39	1.00	3	0.00	0.6111	0.00
Neonate Mortality	Decrease 15%	-0.2423	0.39	1.00	0	0.00	0.0000	0.00

Scenario	Population	stoch-r	SD(r)	PE	N-extant	SD(N-ext)	GeneDiv	SD(GD)
Neonate Mortality	Decrease 10%	-0.2449	0.38	1.00	0	0.00	0.0000	0.00
Neonate Mortality	Decrease 5%	-0.2477	0.36	1.00	4	0.00	0.7188	0.00
Neonate Mortality	Baseline Breeding Age	-0.2532	0.35	1.00	0	0.00	0.0000	0.00
Neonate Mortality	Increase 5%	-0.2583	0.35	1.00	0	0.00	0.0000	0.00
Neonate Mortality	Increase 10%	-0.2634	0.33	1.00	0	0.00	0.0000	0.00
Neonate Mortality	Increase 15%	-0.2659	0.33	1.00	3	0.00	0.7778	0.00
Neonate Mortality	Increase 20%	-0.2753	0.32	1.00	0	0.00	0.0000	0.00
Neonate Mortality	Meta	-0.2726	0.24	0.99	3	0.79	0.7618	0.07
SubAdult Mortality	Decrease 20%	-0.3343	0.41	1.00	0	0.00	0.0000	0.00
SubAdult Mortality	Decrease 10%	-0.2984	0.37	1.00	0	0.00	0.0000	0.00
SubAdult Mortality	Baseline	-0.2522	0.36	1.00	0	0.00	0.0000	0.00
SubAdult Mortality	Increase 10%	-0.2061	0.33	0.99	4	2.00	0.6319	0.11
SubAdult Mortality	Increase 20%	-0.1689	0.30	0.95	5	3.46	0.6795	0.13
SubAdult Mortality	Meta	-0.2280	0.26	0.94	5	3.35	0.6960	0.13
Adult Mortality	Decrease 20%	-0.2153	0.35	1.00	3	2.00	0.6493	0.11
Adult Mortality	Decrease 15%	-0.2254	0.35	1.00	4	1.29	0.6737	0.07
Adult Mortality	Decrease 10%	-0.2326	0.35	1.00	2	0.00	0.5833	0.07
Adult Mortality	Decrease 5%	-0.2425	0.35	1.00	0	0.00	0.0000	0.00
Adult Mortality	Baseline Breeding Age	-0.2492	0.35	1.00	2	0.00	0.5000	0.00
Adult Mortality	Increase 5%	-0.2618	0.35	1.00	0	0.00	0.0000	0.00
Adult Mortality	Increase 10%	-0.2684	0.35	1.00	0	0.00	0.0000	0.00
Adult Mortality	Increase 15%	-0.2807	0.35	1.00	0	0.00	0.0000	0.00
Adult Mortality	Increase 20%	-0.2830	0.36	1.00	0	0.00	0.0000	0.00
Adult Mortality	Meta	-0.2638	0.24	0.98	3	1.24	0.6736	0.11
Hatchlings Increase 5%	Stable Baseline	-0.2564	0.35	1.00	0	0.00	0.0000	0.00
Hatchlings Increase 10%	Stable Baseline	-0.2502	0.36	1.00	0	0.00	0.0000	0.00
Hatchlings Increase 15%	Stable Baseline	-0.2485	0.37	1.00	7	0.00	0.7245	0.00
Hatchlings Increase 20%	Stable Baseline	-0.2484	0.37	1.00	0	0.00	0.0000	0.00
Hatchlings Baseline	Stable Baseline	-0.2488	0.35	1.00	0	0.00	0.0000	0.00
Hatchlings Decrease 5%	Stable Baseline	-0.2552	0.34	1.00	0	0.00	0.0000	0.00
Hatchlings Decrease 10%	Stable Baseline	-0.2573	0.34	1.00	3	0.00	0.7222	0.00
Hatchlings Decrease 15%	Stable Baseline	-0.2554	0.34	1.00	0	0.00	0.0000	0.00
Hatchlings Decrease 20%	Stable Baseline	-0.2633	0.33	1.00	7	0.00	0.6837	0.00
Supplement Low Survival Age 1	Big Creek add 16	-0.2413	0.31	1.00	5	2.12	0.7292	0.07
Supplement Low Survival Age 1	Big Creek add 24	-0.2349	0.31	1.00	3	0.00	0.6111	0.00
Supplement Low Survival Age 1	Big Creek add 32	-0.2266	0.31	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 1	Big Creek ad 40	-0.2185	0.31	1.00	2	0.00	0.6250	0.00
Supplement Low Survival Age 1	Ruthven add 16	-0.1514	0.37	1.00	0	0.00	0.0000	0.00

Scenario	Population	stoch-r	SD(r)	PE	N-extant	SD(N-ext)	GeneDiv	SD(GD)
Supplement Low Survival Age 1	Ruthven add 24	-0.1432	0.35	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 1	Ruthven add 32	-0.1207	0.32	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 1	Ruthven add 40	-0.1308	0.34	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 1	Meta	-0.2433	0.23	0.99	3	1.55	0.6991	0.07
Supplement Low Survival Age 2	Big Creek add 16	-0.2310	0.31	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 2	Big Creek add 24	-0.2214	0.31	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 2	Big Creek add 32	-0.2116	0.31	1.00	3	0.71	0.6736	0.07
Supplement Low Survival Age 2	Big Creek at 40	-0.2066	0.31	1.00	4	0.00	0.7500	0.00
Supplement Low Survival Age 2	Ruthven add 16	-0.1416	0.36	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 2	Ruthven add 24	-0.1256	0.32	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 2	Ruthven add 32	-0.1238	0.34	1.00	0	0.00	0.0000	0.00
Supplement Low Survival Age 2	Ruthven add 40	-0.1202	0.34	1.00	3	0.00	0.6667	0.00
Supplement Low Survival Age 2	Meta	-0.2323	0.23	0.99	3	0.69	0.7386	0.05
Supplement Low Survival Age 3	Big Creek add 16	-0.2180	0.31	1.00	3	0.71	0.6181	0.01
Supplement Low Survival Age 3	Big Creek add 24	-0.2082	0.31	1.00	2	0.00	0.7500	0.00
Supplement Low Survival Age 3	Big Creek add 32	-0.1970	0.31	1.00	3	0.58	0.6157	0.01
Supplement Low Survival Age 3	Big Creek at 40	-0.1907	0.31	1.00	3	1.41	0.6719	0.07
Supplement Low Survival Age 3	Ruthven add 16	-0.1301	0.36	1.00	2	0.00	0.5000	0.00
Supplement Low Survival Age 3	Ruthven add 24	-0.1171	0.35	1.00	7	2.12	0.7133	0.02
Supplement Low Survival Age 3	Ruthven add 32	-0.1091	0.34	1.00	2	0.00	0.6250	0.00
Supplement Low Survival Age 3	Ruthven add 40	-0.0937	0.46	0.99	4	2.48	0.6694	0.10
Supplement Low Survival Age 3	Meta	-0.2141	0.25	0.97	3	1.65	0.7065	0.08
Supplement Hi Survival Age 1	Big Creek add 8	-0.2273	0.30	1.00	0	0.00	0.0000	0.00
Supplement Hi Survival Age 1	Big Creek add 12	-0.2159	0.30	1.00	0	0.00	0.0000	0.00
Supplement Hi Survival Age 1	Big Creek add 16	-0.2066	0.30	1.00	5	2.12	0.7361	0.02
Supplement Hi Survival Age 1	Big Creek at 20	-0.2030	0.31	1.00	5	0.71	0.6625	0.05
Supplement Hi Survival Age 1	Ruthven add 8	-0.1355	0.34	1.00	0	0.00	0.0000	0.00
Supplement Hi Survival Age 1	Ruthven add 12	-0.1283	0.34	1.00	2	0.00	0.5000	0.00
Supplement Hi Survival Age 1	Ruthven add 16	-0.1194	0.34	1.00	0	0.00	0.0000	0.00

Scenario	Population	stoch-r	SD(r)	PE	N-extant	SD(N-ext)	GeneDiv	SD(GD)
Supplement Hi Survival Age 1	Ruthven add 20	-0.1136	0.35	1.00	8	0.00	0.7266	0.00
Supplement Hi Survival Age 1	Meta	-0.2289	0.24	0.99	4	2.26	0.7223	0.10
Supplement Hi Survival Age 2	Big Creek add 8	-0.2153	0.31	1.00	3	0.00	0.7222	0.08
Supplement Hi Survival Age 2	Big Creek add 12	-0.2017	0.31	1.00	6	0.00	0.6944	0.00
Supplement Hi Survival Age 2	Big Creek add 16	-0.1916	0.30	1.00	2	0.58	0.6204	0.01
Supplement Hi Survival Age 2	Big Creek at 20	-0.1844	0.31	0.99	6	2.80	0.7275	0.05
Supplement Hi Survival Age 2	Ruthven add 8	-0.1241	0.34	1.00	3	0.00	0.6111	0.00
Supplement Hi Survival Age 2	Ruthven add 12	-0.1126	0.34	1.00	2	0.00	0.6250	0.00
Supplement Hi Survival Age 2	Ruthven add 16	-0.1019	0.34	1.00	2	0.00	0.6875	0.09
Supplement Hi Survival Age 2	Ruthven add 20	-0.0989	0.34	1.00	2	0.00	0.5000	0.00
Supplement Hi Survival Age 2	Meta	-0.2123	0.23	0.97	3	2.17	0.7300	0.06
Supplement Hi Survival Age 3	Big Creek add 8	-0.1952	0.31	1.00	2	0.00	0.7500	0.00
Supplement Hi Survival Age 3	Big Creek add 1	-0.1831	0.31	1.00	3	1.30	0.6153	0.14
Supplement Hi Survival Age 3	Big Creek add 16	-0.1759	0.30	0.99	2	0.44	0.6620	0.09
Supplement Hi Survival Age 3	Big Creek at 20	-0.1703	0.30	0.99	4	2.15	0.7022	0.05
Supplement Hi Survival Age 3	Ruthven add 8	-0.1108	0.34	1.00	2	0.00	0.5000	0.00
Supplement Hi Survival Age 3	Ruthven add 12	-0.0990	0.34	1.00	0	0.00	0.0000	0.00
Supplement Hi Survival Age 3	Ruthven add 16	-0.0940	0.33	1.00	3	1.41	0.5156	0.15
Supplement Hi Survival Age 3	Ruthven add 20	-0.0882	0.33	1.00	2	0.50	0.7083	0.10
Supplement Hi Survival Age 3	Meta	-0.1997	0.23	0.93	3	1.32	0.7408	0.08
SubAdult Mortality Goal	Big Creek Baseline	-0.2464	0.36	1.00	2	0.00	0.5000	0.00
SubAdult Mortality Goal	Big Creek 10% Decrease	-0.2083	0.33	1.00	3	1.26	0.6913	0.07
SubAdult Mortality Goal	Big Creek 20% Decrease	-0.1769	0.30	0.97	4	2.21	0.6725	0.11
SubAdult Mortality Goal	Big Creek Decrease 30%	-0.1455	0.28	0.85	6	4.13	0.7143	0.10
SubAdult Mortality Goal	Big Creek Decrease 40%	-0.1305	0.26	0.74	8	5.94	0.7390	0.10
SubAdult Mortality Goal	Ruthven Baseline	-0.2576	0.43	1.00	0	0.00	0.0000	0.00
SubAdult Mortality Goal	Ruthven 10% Decrease	-0.2148	0.40	1.00	0	0.00	0.0000	0.00
SubAdult Mortality Goal	Ruthven 20% Decrease	-0.1820	0.38	1.00	3	0.71	0.6181	0.01
SubAdult Mortality Goal	Ruthven Decrease 30%	-0.1610	0.35	0.99	4	2.22	0.6155	0.10

Scenario	Population	stoch-r	SD(r)	PE	N-extant	SD(N-ext)	GeneDiv	SD(GD)
SubAdult Mortality Goal	Ruthven Decrease 40%	-0.1507	0.34	0.99	6	6.02	0.6430	0.12
SubAdult Mortality Goal	Meta	-0.1767	0.20	0.56	8	6.46	0.7826	0.09
Adult mortality Goal	Big Creek Baseline	-0.2512	0.36	1.00	0	0.00	0.0000	0.00
Adult mortality Goal	Big Creek Decrease 10%	-0.2295	0.35	1.00	2	0.00	0.6250	0.00
Adult mortality Goal	Big Creek Decrease 20%	-0.2133	0.35	0.99	5	3.77	0.7013	0.06
Adult mortality Goal	Big Creek Decrease 30%	-0.1936	0.35	0.98	5	3.64	0.6926	0.06
Adult mortality Goal	Big Creek Decrease 40%	-0.1780	0.35	0.97	4	2.27	0.6792	0.10
Adult mortality Goal	Ruthven Baseline	-0.2575	0.44	1.00	0	0.00	0.0000	0.00
Adult mortality Goal	Ruthven 10% Decrease	-0.2403	0.44	1.00	0	0.00	0.0000	0.00
Adult mortality Goal	Ruthven Decrease 20%	-0.2228	0.43	1.00	0	0.00	0.0000	0.00
Adult mortality Goal	Ruthven Decrease 30%	-0.2015	0.43	1.00	0	0.00	0.0000	0.00
Adult mortality Goal	Ruthven Decrease 40%	-0.1892	0.43	1.00	10	0.00	0.7300	0.00
Adult mortality Goal	Meta	-0.2224	0.25	0.92	4	3.17	0.7311	0.09
Neonate Mortality Goal	Big Creek Baseline	-0.2513	0.35	1.00	0	0.00	0.0000	0.00
Neonate Mortality Goal	Big Creek Decrease 10%	-0.2405	0.37	1.00	5	0.00	0.7000	0.00
Neonate Mortality Goal	Big Creek Decrease 20%	-0.2406	0.39	1.00	4	0.00	0.7188	0.00
Neonate Mortality Goal	Big Creek Decrease 30%	-0.2333	0.40	1.00	2	0.00	0.3750	0.00
Neonate Mortality Goal	Big Creek Decrease 40%	-0.2273	0.42	1.00	5	2.58	0.5779	0.14
Neonate Mortality Goal	Ruthven Baseline	-0.2605	0.43	1.00	0	0.00	0.0000	0.00
Neonate Mortality Goal	Ruthven 10% Decrease	-0.2546	0.45	1.00	0	0.00	0.0000	0.00
Neonate Mortality Goal	Ruthven 20% Decrease	-0.2597	0.48	1.00	0	0.00	0.0000	0.00
Neonate Mortality Goal	Ruthven Decrease 30%	-0.2410	0.50	1.00	0	0.00	0.0000	0.00
Neonate Mortality Goal	Ruthven Decrease 40%	-0.2476	0.52	1.00	0	0.00	0.0000	0.00
Neonate Mortality Goal	Meta	-0.2599	0.27	0.99	4	2.15	0.6228	0.15
Reduce all mortality 10%	Big Creek Baseline	-0.2492	0.36	1.00	0	0.00	0.0000	0.00
Reduce all mortality 10%	Big Creek 10% Decrease	-0.1812	0.34	0.97	5	2.83	0.6768	0.11
Reduce all mortality 10%	Ruthven Baseline	-0.2571	0.43	1.00	0	0.00	0.0000	0.00
Reduce all mortality 10%	Ruthven 10% Decrease	-0.1945	0.43	1.00	4	0.00	0.6250	0.00
Reduce all mortality 10%	Meta	-0.2146	0.30	0.97	5	2.71	0.6913	0.09
Reduce all mortality 20%	Big Creek Baseline	-0.2541	0.35	1.00	2	0.00	0.5000	0.00
Reduce all mortality 20%	Big Creek 20% Decrease	-0.1100	0.31	0.55	12	10.13	0.7715	0.10

Scenario	Population	stoch-r	SD(r)	PE	N-extant	SD(N-ext)	GeneDiv	SD(GD)
Reduce all mortality 20%	Ruthven Baseline	-0.2569	0.44	1.00	0	0.00	0.0000	0.00
Reduce all mortality 20%	Ruthven 20% Decrease	-0.1341	0.42	0.98	5	4.25	0.6083	0.18
Reduce all mortality 20%	Meta	-0.1411	0.28	0.53	12	10.11	0.7724	0.10
Reduce all mortality 34%	Big Creek Baseline	-0.2488	0.35	1.00	0	0.00	0.0000	0.00
Reduce all mortality 34%	Big Creek 34% Decrease	-0.0067	0.20	0.01	107	69.84	0.9186	0.04
Reduce all mortality 34%	Ruthven Baseline	-0.2608	0.43	1.00	0	0.00	0.0000	0.00
Reduce all mortality 34%	Ruthven 34% Decrease	-0.0440	0.36	0.52	20	18.94	0.7359	0.11
Reduce all mortality 34%	Meta	-0.0321	0.18	0.01	116	73.08	0.9268	0.04
Reduce all mortality 40%	Big Creek Baseline	-0.2526	0.35	1.00	5	0.00	0.7200	0.00
Reduce all mortality 40%	Big Creek 40% Decrease	0.0011	0.20	0.00	131	87.34	0.9258	0.04
Reduce all mortality 40%	Ruthven Baseline	-0.2615	0.43	1.00	0	0.00	0.0000	0.00
Reduce all mortality 40%	Ruthven 40% Decrease	-0.0350	0.37	0.45	24	21.68	0.7421	0.10
Reduce all mortality 40%	Meta	-0.0242	0.18	0.00	144	93.93	0.9334	0.04
Reduce all mortality 20% and Augment 8 after mort	Big Creek Baseline	-0.2536	0.35	1.00	0	0.00	0.0000	0.00
Reduce all mortality 20% and Augment 8 after mort	Big Creek 20% Decrease	-0.1102	0.31	0.54	12	10.60	0.7644	0.10
Reduce all mortality 20% and Augment 8 after mort	Big Creek 20% Decline Add 12	-0.0836	0.28	0.28	17	14.06	0.8207	0.08
Reduce all mortality 20% and Augment 8 after mort	Ruthven Baseline	-0.2612	0.43	1.00	0	0.00	0.0000	0.00
Reduce all mortality 20% and Augment 8 after mort	Ruthven 20% Decrease	-0.1309	0.42	0.97	7	7.65	0.6265	0.13
Reduce all mortality 20% and Augment 8 after mort	Ruthven 20% Decline Add 12	-0.0356	0.31	0.37	14	10.36	0.8024	0.08
Reduce all mortality 20% and Augment 8 after mort	Meta	-0.0964	0.20	0.05	29	20.76	0.8916	0.05
Reduce all mortality 20% and Augment 6 after mort	Big Creek Baseline	-0.2506	0.35	1.00	0	0.00	0.0000	0.00
Reduce all mortality 20% and Augment 6 after mort	Big Creek 20% Decrease	-0.1091	0.31	0.54	12	9.89	0.7699	0.09
Reduce all mortality 20% and Augment 6 after mort	Big Creek 20% Decline Add 12	-0.0860	0.28	0.29	16	13.24	0.8119	0.08
Reduce all mortality 20% and Augment 6 after mort	Ruthven Baseline	-0.2567	0.43	1.00	0	0.00	0.0000	0.00
Reduce all mortality 20% and Augment 6 after mort	Ruthven 20% Decrease	-0.1379	0.42	0.97	6	4.80	0.6337	0.14
Reduce all mortality 20% and Augment 6 after mort	Ruthven 20% Decline Add 12	-0.0350	0.31	0.37	14	10.97	0.8048	0.08
Reduce all mortality 20% and Augment 6 after mort	Meta	-0.0982	0.20	0.06	29	20.78	0.8889	0.06

Appendix F. Ontario government led and government supported actions.

The Committee on the Status of Species at Risk in Ontario (COSSARO) conducted a status assessment in 2009, with a subsequent reassessment in 2020, which resulted in the Carolinian population of gray ratsnake being listed as endangered in Ontario. A provincial Recovery Strategy for the species was developed in 2010, which provide generalized advice to the Province of Ontario on the recommended approach to recovery for both populations.

Subsequently, the province released a Government Response Statement (GRS) that listed a fairly comprehensive set of government-led and government-supported actions for the species in Ontario to achieve their goal for the recovery of the gray ratsnake, which is to halt the decline of the Carolinian population. A summary of government led and government supported actions as specified in the GRS is as follows:

To help protect and recover the gray ratsnake, the Ontario provincial government has committed to directly undertake the following actions:

1. Develop a protocol to protect hibernating gray ratsnakes if they are accidentally unearthed.
2. Develop a survey protocol to be used by proponents and partners to detect the presence or absence of gray ratsnakes.
3. Conduct a monitoring program for gray ratsnake at priority Ontario Parks locations to determine species presence, distribution and habitat use.
4. Educate other agencies and authorities involved in planning and environmental assessment processes on the protection requirements under the Endangered Species Act (ESA).
5. Encourage the submission of gray ratsnake data to the Ministry's central repository at the Natural Heritage Information Centre or to the Ontario Reptile and Amphibian Atlas. Ensure appropriate data sensitivity guidelines are put in place.
6. Undertake communications and outreach to increase public awareness of species at risk in Ontario.
7. Protect the gray ratsnake and its habitat through the ESA
8. Develop and enforce a regulation prescribing the habitat of the species.
9. Support conservation, agency, municipal and industry partners in undertaking activities to protect and recover the gray ratsnake. Support will be provided through funding, agreements, permits (including conditions) and advisory services.
10. Establish and communicate annual priority actions for government support in order to encourage collaboration and reduce duplication of efforts.

The provincial government endorses the following actions for the protection and recovery of the gray ratsnake. Actions identified as “high” will be given priority consideration for funding or for authorizations under the ESA. The government will focus its support on these high-priority actions over the next five years.

Protection and Management: Protect and manage the habitat of the species and mitigate priority threats.

Actions:

1. **(HIGH)** Research and implement methods for reducing significant threats, including road mortality and human persecution, and evaluate their effectiveness.
2. **(HIGH)** Refine and promote best management practices for landowners and land managers.
3. Assess the tolerance of habitat features to disturbance.
4. As opportunities arise, support the securement of lands that contain gray ratsnake subpopulations through existing land securement and stewardship programs.

Monitoring: Develop and implement a coordinated monitoring program focused on population indices and distribution, habitat stresses and the effectiveness of recovery actions.

Actions:

5. **(HIGH)** Identify locations of hibernacula, other habitat features and the extent of the species' distribution.
6. **(HIGH)** Maintain current monitoring activities (e.g., of hibernacula) and develop and implement a plan to extend monitoring activities, including establishing additional monitoring stations in the Carolinian Zone.
7. Develop a process for analyzing monitoring data to determine the effectiveness of recovery actions and to inform future management activities.

Awareness: Reduce human persecution of gray ratsnakes and promote stewardship.

Actions:

8. Evaluate existing communications and outreach approaches and develop new strategies that will have a positive impact on people's behaviour.
9. Deliver effective communications and outreach to key stakeholders, including landowners, cottage associations and schools within the range of Gray ratsnake.

Research: Address knowledge gaps, including ecological studies of habitat and genetic connectivity.

Actions:

10. Undertake a population and habitat viability assessment (PHVA): determine data needs and use of PHVA, conduct research required for the PHVA and carry out the assessment once the information is available.
11. Research and collate data on habitat restoration practices, and evaluate their potential for widespread use.
12. Determine how genetic connectivity among sub-populations is maintained, including the relative importance of different mechanisms, such as juvenile dispersal, adult dispersal and multiple paternity.
13. Conduct additional studies into the habitat use and movement patterns of the Carolinian population of Gray ratsnake.
14. Investigate potential approaches to augmenting the Carolinian population of Gray ratsnake.