Understanding and Enhancing Connectivity of Michigan's Eastern Massasauga Rattlesnake Population to Guide Management and Conservation



Prepared By: Yu Man Lee Michigan Natural Features Inventory Michigan State University Extension P.O. Box 13036 Lansing, MI 48901-3036

Prepared For: U.S. Geological Survey Grant/Cooperative Agreement No. G17AC00408

12/30/2020

MNFI Report No. 2020-31



MICHIGAN STATE

Suggested Citation:

Lee, Y. 2020. Understanding and Enhancing Connectivity of Michigan's Eastern Massasauga Rattlesnake Population to Guide Management and Conservation. Michigan Natural Features Inventory, Report No. 2020-31, Lansing, MI.

Copyright 2020 Michigan State University Board of Trustees. MSU Extension programs and materials are open to all without regard to race, color, national origin, gender, religion, age, disability, political beliefs, sexual orientation, marital status or family status.

The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Geological Survey. Mention of trade names or commercial products does not constitute their endorsement by the U.S. Geological Survey.

Cover:

Background Photo: Eastern massasauga occupied habitat in population in Livingston County in southeast Michigan. Photo by Yu Man Lee.

Snake Photo: Eastern massasauga rattlesnake (Sistrurus catenatus). Photo by Joseph Sage.

Table of Contents

Introduction Project Objectives	1 3
Methods Study Areas Landscape Genetic Analysis	4 4 4
Multi-temporal Habitat Analysis	5
Results Landscape Genetic Analysis Multi-temporal Habitat Analysis	7 7 8
Discussion	10
Acknowledgements	13
Literature Cited	14

List of Figures

Figure 1. Map of eastern massasauga study regions/populations	6
Figure 2. Photos of eastern massasaugas found during this project	9
Figure 3. Photos of eastern massasauga habitat at occupied and surveyed sites	11
Figure 4. Photo of eastern massasauga habitat experiencing woody encroachment	12

List of Appendices

Appendix 1. Eastern Massasauga Search Effort Data Sheet	.16
Appendix 2. Eastern Massasauga Observation Data Sheet	17

Introduction

The Eastern Massasauga Rattlesnake (EMR, *Sistrurus catenatus*) has declined throughout much of its range and was officially listed as threatened by the U.S. Fish and Wildlife Service (USFWS) on 29 Sept 2016 (USFWS 2016). Michigan is a relative stronghold for this species with more historical and extant populations than any other state or province (Szymanski 1998, Szymanski et al. 2015). Therefore, ensuring that Michigan populations are managed effectively is essential for recovery of this species. Habitat loss and fragmentation are the most significant threats to EMR viability (Szymanski et al. 2015). Maintaining or restoring population connectivity will be crucial for counteracting the negative effects of habitat fragmentation (e.g., negative population growth rates, inbreeding depression). Understanding how landscape features influence connectivity among populations in this species is important for effective will allow for tailored management prescriptions by providing detail on preferable spatial scale and frequency of management actions that will enhance connectivity and ensure population viability while minimizing incidental mortality.

To sustain the species in Michigan and contribute to conservation and recovery of this species rangewide, the Michigan Department of Natural Resources (MDNR) has initiated efforts to develop an EMR conservation plan that identifies priority populations and the management actions needed to maintain those populations. These efforts have included identifying and delineating extant EMR populations and assessing the condition and viability of these populations (Lee and Enander 2015). These recent population delineations suggest that some EMR populations are isolated from each other and potentially subject to loss of gene flow and increased genetic drift in these smaller, fragmented subpopulations. The landscape perspective employed by Lee and Enander (2015) also provided land managers with important information on the extent of suitable habitat within their management areas and potential corridors for snake movement. For areas with multiple element occurrences (EOs), these landscape analyses represent different hypotheses of the level of connectivity between EOs. However, the barriers to gene flow are not adequately described and the nature and level of landscape connectivity are poorly understood and require EMR movement data to be formally tested.

One way to further assess EMR population structure and condition, including landscape connectivity, in Michigan is to use spatially explicit, high resolution genetic data to infer movement and dispersal within and among populations across the state. Our current understanding of EMR population structure and gene flow is largely inferred from research studies conducted outside of Michigan (Chiucchi and Gibbs 2010, Dileo et al. 2013, Gibbs et al. 1997) where EMRs are more sparsely distributed and have experienced more severe population declines (Szymanski 1998, Szymanski et al. 2015). Therefore, conclusions that snakes do not move between populations drawn from these genetic studies do not necessarily reflect patterns of genetic diversity or gene flow in Michigan. Larger EMR populations in Michigan may allow us to understand EMR population genetic structure on a fine scale in a more intact landscape. Fine-scale landscape genetic approaches have been successfully applied to a variety of taxa (Laurence et al. 2013, Peterman et al. 2014, Row et al. 2010), providing important information on functional connectivity across the landscape. Movement data also could come from radio telemetry documenting the presence/absence of movement of individuals between EOs but the genetic option is a less invasive (Lentini et al. 2011) and more cost-effective means of acquiring these data (DeYoung and Honeycutt 2005, Millspaugh and Thompson III 2009).

Effective landscape conservation design is critically dependent on adequate characterization of actual and needed connectivity. Describing functional connectivity is at the core of this proposal. Gene flow and dispersal were largely dismissed as important evolutionary forces affecting EMR populations in the most recent species status assessment (Szymanski et al. 2015); however, this was based on previous studies of EMR gene flow that have not included Michigan populations. Incorrectly inferring that population isolation represents the norm for EMRs may preclude proactive management to maintain or enhance population connectivity, thus negatively affecting viability. Results from this project will provide a better understanding of the importance of dispersal and gene flow, the scale at which they are occurring, and their effects on long-term population viability (Szymanski et al. 2015).

Project results also will help resolve appropriate units and areas for management and collaboratively identify landscape-scale management solutions that are essential for long-term species recovery. Fine-scale genetic data could be readily incorporated into current conservation planning and management decisions to determine if populations comprised of multiple sites should be treated as genetically isolated units or as a single population with multiple core areas. Without detailed information on the genetic structuring of EMR populations, movement corridors could be negatively affected by management practices based on the incorrect assumption that no gene flow is occurring. Evidence for a lack of gene flow would help management efforts prioritize either corridor creation or expansion of available habitat for existing sites. Conversely, detecting gene flow would allow for further landscape analyses to determine the landscape features associated with successful EMR dispersal between occupied sites. Management activities could then be focused on maintaining these features.

The U.S. Geological Survey (USGS), USFWS, MDNR, and other conservation partners will use results from this research to develop conservation actions to maintain and improve connectivity among EMR populations. Michigan's Eastern Massasauga Candidate Conservation Agreement with Assurances (CCAA) is a programmatic agreement between the U.S. Fish and Wildlife Service, the MDNR, and the MI Department of Military and Veterans Affairs (DVMA) to further the conservation of the EMR on non-Federal lands. Most viable populations of EMR occur on land managed by the MDNR and the DMVA. The CCAA framework is based in part on management strategies designed to protect EMR populations while also creating and restoring (e.g., through prescribed fire) suitable habitat needed to sustain its populations. The CCAA is based on adaptive management principles that address the uncertainty associated with how some management techniques may affect population viability and for which an understanding of population structure is essential. This work will help to inform the adaptive management approach of the CCAA. The information gained on EMR population structure and gene flow may further help inform management strategies on National Wildlife Refuge lands as well on other conservation lands through the Midwest.

This project represented a collaborative effort between the USGS, USFWS, Grand Valley State University (GVSU), and the Michigan Natural Features Inventory (MNFI), a program of Michigan State University Extension (MSUE). Grand Valley State University led the research efforts to collect samples from EMR study populations, conduct analyses to examine dispersal and gene flow to infer connectivity and identify key landscape features and historical processes responsible for observed patterns of gene flow, and produce the final project report and other associated products. Michigan Natural Features Inventory provided assistance with these efforts. This report summarizes MNFI's contributions to project activities and results.

Project Objectives:

The goal of this project was to collaboratively investigate functional connectivity among EMR populations in Michigan.

To achieve this goal, we addressed the following overall objectives:

- 1. Examine dispersal and gene flow to infer connectivity in areas with multiple populations in close proximity (<5 km) within multiple habitat types in both northern and southern Michigan. Because Michigan supports higher densities of EMR populations than any other state or province, we hypothesized that Michigan populations are better connected across broader landscape scales than populations throughout the rest of the range.
- 2. Identify key landscape features and historical processes responsible for observed patterns of fine-scale gene flow. We hypothesized that wetland complexes and riparian habitat connect nearby populations while roads and vegetative succession disrupt connectivity, and that these features differ between northern and southern populations. Habitat configurations are more easily defined for the southern populations which are typically associated with discrete patches of open canopy habitat whereas the northern populations are situated in a more forested landscape with less discernible population/habitat boundaries.

To help achieve the project's overall goal and objectives, the Michigan Natural Features Inventory (MNFI) addressed the following specific objectives:

- 1. Help collect EMR tissue samples from approximately six locations across Michigan
- 2. Assist with conducting GIS/landscape analyses of EMR habitat distribution
- 3. Participate in writing of scientific publications linking EMR gene flow and landscape connectivity

Methods

Study Areas

This study focused on EMR populations throughout the Lower Peninsula of Michigan, which is the center of the range and the stronghold for EMRs. We initially identified six regions (three in northern Michigan and three in southern Michigan) where EMR populations/sites occur in close proximity. These included EMR populations/sites in Barry, Washtenaw, Livingston, and Oakland counties in southern Michigan, and in Alcona, Iosco, Crawford, and Kalkaska counties and Bois Blanc Island in northern Michigan (Figure 1). We included Bois Blanc Island as one of the northern MI regions because the island presents a rare opportunity to sample snakes in a low fragmentation setting reminiscent of pre-European settlement landscapes. Northern and southern populations potentially differ in habitat suitability and their response to the differing degree of habitat loss and fragmentation that has occurred. However, we were able to obtain sufficient samples from only three to four of the six study populations/regions, and only these populations were included in the landscape genetic and habitat/connectivity analyses (Figure 1).

Landscape Genetic Analysis

Our approach was to generate habitat suitability models that informed landscape resistance surfaces (hypotheses of landscape connectivity) and to use landscape genetic techniques to correlate gene flow with landscape connectivity. Genetic data also were used to validate previous population delineations which were based on habitat features and proximity of documented EMR element occurrences (EOs) or sites.

To address Objective 1, GVSU and MNFI staff collected genetic samples from at least 5-10 discrete habitat patches from EOs from each of the study regions/populations. We generated genetic baselines for northern and southern MI populations by sampling across the state including Bois Blanc Island in northern MI. We were particularly interested in determining if EMR populations are still highly structured in this type of environment with few anthropogenic barriers to hinder movement. Surveys were conducted during spring, summer, and/or early fall (i.e., April – early October) to locate, capture, and collect blood and/or tissue samples from EMRs for genetic analysis. Visual encounter surveys and/or coverboard surveys were utilized to locate EMRs in the field. Surveys were conducted by at least two trained surveyors, and all surveyors were trained on the survey and sampling protocol. Survey and capture locations were recorded in the field using GPS. Data on the condition of the animals (i.e., body and tail lengths, weight, number of rattles, sex, age class, dorsal pattern, and evidence of snake fungal disease) and habitat also were recorded on data forms (Appendices 1 and 2) or on tablets.

GVSU staff utilized samples collected in the field to conduct landscape genetic analysis using the following approach. Individuals were genotyped using a set of 19 polymorphic microsatellite loci developed for EMR (Anderson et al. 2010) to calculate pairwise genetic distances. Microsatellite data were analyzed to determine whether the genetic structuring of EMR populations is most likely due to isolation by distance (IBD) or the presence of landscape barriers (anthropogenic or natural) that impede gene flow. Multiple genetic methods were compared for identifying potential barriers including boundary detection (e.g., MONMONIER) and Bayesian genetic clustering methods (e.g., GENELAND, STRUCTURE) (Blair et al. 2012). A landscape genetic analysis to evaluate functional connectivity for EMR populations at each study site was conducted using pairwise individual-based genetic distance estimates. The individual genetic distances were paired with resistance surfaces to assess fine scale gene flow among habitat patches within each site. This allowed for comparisons in various landscape contexts such as varying distances separating patches and different landscape matrix features (e.g., forest, rivers, agriculture). Clustered sites in northern and southern MI were compared to existing samples from isolated MI sites to investigate influences of varying habitat types and levels of fragmentation on genetic diversity. Least-cost paths (LCPs) and resistance surfaces were generated from Circuitscape for southern Michigan. LCPs and Circuitscape resistance surfaces were related to individual genetic distances using Mantel tests. Mantel test-based landscape associations have been criticized (Zeller et al. 2016) but using this approach in conjunction with genetic clustering methods has been proposed as a beneficial way to identify certain recent barriers to gene flow (Blair et al. 2012). A new approach implemented in the R package ResistanceGA that optimized resistance surfaces using pairwise genetic distance information also was used (Peterman et al. 2014).

Multi-temporal Habitat Analysis

To address Objective 2, species distribution models (SDMs) (one for northern Michigan and one for southern Michigan due to habitat differences) were used to evaluate the distribution of suitable habitat and identify key areas for habitat management, road mitigation, and corridor creation. GVSU has generated a preliminary species distribution model (SDM) for EMR populations in northern Michigan using the MAXENT program. This model was improved by testing additional environmental predictor variables that could refine our habitat suitability (HSM) and evaluating models that subdivide northern Michigan based on unique habitat preferences. Prior to this project, we had already generated some least cost path and resistance layers representing different hypotheses for how EMR traverse the landscape (see Lee and Enander 2015, McCluskey 2016). Genetic data allowed us to formally test these hypotheses and potentially identify functionally important snake movement corridors. A landscape genetic approach was used to correlate resistance models with gene flow to identify landscape features that affect connectivity. This information could be matched to recovery goals aimed at enabling natural recolonization or dispersal by improving habitat corridors.

Historical aerial photographs also were analyzed to make long term habitat assessments in terms of quantifying changes in available habitat and fragmentation, which can be related to current genetic data (Epps and Keyghobadi 2015). We classified 1938 aerial photographs that were available for our sampling sites using object-based classification and manual digitizing of landscape features. These historical land cover maps were matched with the northern and southern Michigan HSMs based on current conditions to estimate changes in available habitat and landscape changes (e.g. new roads, expanding forested areas, reduced agriculture) that may better explain the observed genetic structuring of populations than present day landscape configurations. We wanted to compare areas that have experienced high historical fragmentation or connectivity from the 1930s to the present and investigate whether there are resulting genetic consequences for the snakes inhabiting either type of landscape. This approach also could be useful for identifying recolonization patterns in former agricultural fields and assessing how much habitat has been lost via succession (McCluskey 2016).

Michigan Eastern Massasauga Landscape Genetics/Connectivity Study Regions & Populations

Populations included in Landscape Genetic/Connectivity Analyses

Populations not included in Landscape Genetic/Connectivity Analyses - Surveyed/Insufficient Samples

Populations not included in Landscape Genetic/Connectivity Analyses - Not Surveyed

Counties



Figure 1. Map of eastern massasauga rattlesnake (EMR) study regions and populations selected and/or included in the landscape genetic and/or habitat/connectivity analyses.

Results

Landscape Genetic Analysis

In 2018, MNFI focused on conducting surveys and collecting blood and/or tissue samples from EMRs in one population in the study region in Livingston and Washtenaw counties in southeast Michigan (Figure 1). Surveys were conducted at seven different locations within this population but mainly focused on three locations separated by 0.1 km to 4.7 km. Surveys were conducted by two to three field staff from 1 May to 25 July. A total of 10 EMRs was observed during the surveys, of which 7 were captured and processed. Of the seven snakes captured, six were adult females and one was a juvenile/yearling (young from previous year) (Figure 2). Blood or tissue samples were collected from all seven snakes captured. All three snakes that were not captured were juveniles or yearlings (young from the previous year). All 10 snakes were observed in two of the three locations surveyed, but the 7 snakes that were captured were from one location. Blood and tissue samples and associated capture data were provided to GVSU. However, because all the samples were collected essentially from only one location, this population could not be included in the landscape genetic analysis.

In 2018, MNFI staff also conducted EMR surveys at two additional populations. One population was located in the Livingston/Washtenaw County study region, but no EMRs were found at this site. The other population was located in the study region in Kalkaska and Crawford counties in northern Michigan (Figure 1). MNFI and GVSU staff assisted with conducting surveys and collecting blood samples from EMRs in this population as part of this project and a different project. A total of 61 blood samples was collected from this population in 2018, which were provided to GVSU for this study.

In May and June 2019 and July and August 2020, MNFI staff assisted GVSU with conducting surveys and collecting blood samples from EMRs at multiple locations within two populations in the study region in Barry County in southwest Michigan. We had originally planned to conduct additional surveys in populations in the study regions in Livingston, Washtenaw, and Oakland counties in southeast Michigan. But we decided to focus our surveys on the Barry County study region/populations to collect additional samples to ensure sufficient numbers and distribution of samples from this region for the landscape genetic analysis. The surveys with which MNFI assisted GVSU documented and captured at least one EMR in 2019 and three EMRs in 2020 at two locations within the Barry County study region/population (Figure 2). Results of these surveys will be provided by GVSU in their final report. In May 2019, MNFI staff also assisted with conducting surveys and collecting blood samples from EMRs within a population in the study region in Kalkaska and Crawford counties in northern Michigan as part of a different project. A total of 14 additional blood samples was collected during these surveys and provided to GVSU for the landscape genetic analysis. GVSU conducted surveys and collected blood samples from EMRs at one additional location within this population/study region in 2019.

MNFI's surveys from 2018-2020 were conducted in addition to EMR surveys and sample collection conducted by GVSU in 2018-2019. GVSU researchers conducted surveys and collected at least 78 blood samples from five EMR populations in study regions in Barry, Oakland, Alcona, and Iosco counties and on Bois Blanc Island as part of this project and other EMR projects (Moore and McCluskey pers. comm.). Additional blood samples from study

populations/regions collected by GVSU during other EMR projects prior to this study also were included in the landscape genetic and connectivity analyses where appropriate.

Multi-temporal Habitat Analysis

To identify key landscape features and historical processes responsible for observed patterns of fine-scale gene flow, GVSU used species distribution models (SDMs) to evaluate the distribution of suitable habitat and analyzed historical aerial imagery to quantify changes in available habitat and habitat fragmentation and relate these changes to current genetic data and population structure/connectivity. MNFI assisted GVSU with these analysis by reviewing and providing technical consultation on the SDMs. MNFI also acquired, geo-rectified and provided mosaics of 1938 aerial photographs/imagery that were available for five sampling sites/populations associated with three study regions located in Livingston, Washtenaw, Kalkaska, and Crawford counties and on Bois Blanc Island. MNFI also provided geo-rectified mosaics of 1938 aerial photographs of the EMR populations in the Barry County study region.

Grand Valley State University is in the process of completing the landscape genetic analysis and multi-temporal habitat analysis. Results of these analyses will be provided in GVSU's final project report. MNFI will assist GVSU with development and/or review of scientific publications and presentations generated as part of or after completion of this project. Additionally, MNFI updated information on the status, distribution and/or extent of EMR populations or element occurrences (EOs) within the Michigan Natural Heritage Database (NHD) with results from MNFI and GVSU's surveys from 2018-2020. These results updated and/or expanded the status, distribution and/or extent of nine previously documented EMR populations or EOs in the NHD (MNFI 2020). This information is critical for continuing to assess and monitor the status and distribution of EMRs and guiding management and conservation efforts for this species within these populations.



Figure 2. Photos of eastern massasaugas documented and processed during field surveys in study populations in Livingston/Washtenaw (top left and right photos) and Barry counties (bottom left and right photos) from 2018-2020.

Discussion

Obtaining sufficient numbers of samples (i.e., >5) from at least 5-10 discrete habitat patches from EOs in close proximity (<5 km) from each of the study regions/populations proved to be more challenging than anticipated, particularly in southern Michigan. Although intensive surveys for EMRs were conducted by MNFI and/or GVSU at study populations in Barry, Livingston, and Washtenaw counties from 2018-2020, sufficient numbers of EMR samples were collected from only one or a few (<5) locations/ habitat patches within these study regions/populations, despite multiple surveys at multiple locations. These locations included sites at which EMRs had been documented during previous surveys. Some of these locations still appeared to contain suitable habitat for EMRs, despite the species not being documented during the surveys (Figure 3). However, habitat appeared to be degraded and/or less suitable for EMRs at a few of the locations due to vegetative succession or woody encroachment by trees, shrubs, and/or invasive species (Figure 4) or due to flooding or increased water levels in the wetlands compared to habitat conditions during previous surveys. Additional surveys should be conducted in the study regions/populations in the future, especially those that were not surveyed during this study and in locations at which EMRs were not documented to determine if the species still occurs there and in what density. Some of these locations also may warrant habitat management and restoration efforts, particularly if the landscape genetic analysis indicate gene flow has occurred and is significant between habitat patches within study regions/populations. Project results will provide critical information which will help guide the development and implementation of effective conservation and recovery efforts for the eastern massasauga in Michigan and rangewide.



Figure 3. Photos of habitat in which eastern massasaugas (EMRs) have been found (top photo) and suitable habitat for EMRs at a site at which EMRs have not been found during surveys conducted during and prior to this project (bottom photo).



Figure 4. Photo of eastern massasauga habitat surveyed as part of this project that is experiencing woody encroachment from shrubs.

Acknowledgements

Funding for this project was provided by the U.S. Geological Survey (USGS) under Grant/Cooperative Agreement No. G17AC00408. I would like to gratefully acknowledge and thank Ralph Grundel with USGS for serving as the project sponsor and principal investigator, and providing support, assistance, and guidance to help ensure the success of this project. I also would like to acknowledge and thank Jack Dingledine, formerly with the U.S. Fish and Wildlife Service (USFWS) East Lansing Ecological Services Field Office, for serving as the USFWS project officer for this project. I would like to recognize and thank Dr. Jennifer Moore and Dr. Eric McCluskey with Grand Valley State University (GVSU) for collaborating and providing the opportunity for MNFI to be involved in the design and implementation of this project. I also would like to recognize and thank Helen Enander, MNFI GIS Specialist, for her contributions to the multi-temporal habitat analysis component of the project. I would like to express my sincere appreciation to MNFI Zoologist, Ashley Cole-Wick; MNFI seasonal technicians, Daniel Earl and Kailyn Atkinson; Huron Pines AmeriCorps members serving with MNFI, Courtney Ross, Frank Schroyer and Zack Pitman; and GVSU researchers, students, and field assistants, Dr. Jennifer Moore, Dr. Eric McCluskey, Joe Altobelli, Danielle Bradke, Tyler DeVos, Justine Fox, Jenny Kovach, Caleb Krueger, Nathan Kudla, Diane Methner, Alvssa Swinehart, Arin Thacker, and Jake Vaughn, for providing invaluable assistance with the field surveys and/or sample collection. I also would like to recognize and thank the Michigan Department of Military and Veterans Affairs and all the conservation partners and volunteers for providing funding and/or assistance with the massasauga field surveys and sample collection at the study population/region in Crawford and Kalkaska counties. Finally, I would like to thank the following individuals for providing administrative support and assistance with this project: Brian Klatt, Michael Monfils, Nancy Toben and Ashley Adkins, MNFI; and Michigan State University Office of Sponsored Programs and Contract and Grant Administration, Michigan State University Extension Grant Services, and USGS' budget and contracting offices and/or staff. This project would not have been possible without your assistance.

Literature Cited

- Anderson, C. S., H. L. Gibbs, and J. Chiucchi. 2010. Nineteen polymorphic microsatellite loci isolated from the eastern massasauga rattlesnake, *Sistrurus c. catenatus*. Conservation Genetics Resources 2(1):243-245.
- Blair, C., D. E. Weigel, M. Balazik, A. T. Keeley, F. M. Walker, E. Landguth, S. Cushman, M. Murphy, L. Waits, and N. Balkenhol. 2012. A simulation-based evaluation of methods for inferring linear barriers to gene flow. Molecular Ecology Resources 12:822–833.
- Chiucchi, J. E. and H. L. Gibbs. 2010. Similarity of contemporary and historical gene flow among highly fragmented populations of an endangered rattlesnake. Molecular Ecology 19:5345–5358.
- Derosier, A. L., S. K. Hanshue, K. E. Wehrly, J. K. Farkas, and M. J. Nichols. 2015. Michigan's Wildlife Action Plan. Michigan Department of Natural Resources, Lansing, MI.
- DeYoung, R.W. and R. L. Honeycutt. 2005. The molecular toolbox: genetic techniques in wildlife ecology and management. Journal of Wildlife Management 69:1362–1384.
- Dileo, M. F., J. D. Rouse, J. A. Davila, and S. C. Lougheed. 2013. The influence of landscape on gene flow in the EMR rattlesnake (*Sistrurus c. catenatus*): Insight from computer simulations. Molecular Ecology 22:4483–4498.
- Epps, C. W. and N. Keyghobadi. 2015. Landscape genetics in a changing world: disentangling historical and contemporary influences and inferring change. Molecular Ecology 24:6021–6040.
- Gibbs, H. L., K. A. Prior, P. J. Weatherhead, and G. Johnson. 1997. Genetic structure of populations of the threatened EMR rattlesnake, *Sistrurus c catenatus*: Evidence from microsatellite DNA markers. Molecular Ecology 6:1123–1132.
- Laurence, S., M. J. Smith, and A. I. Schulte-Hostedde. 2013. Effects of structural connectivity on fine scale population genetic structure of muskrat, *Ondatra zibethicus*. Ecology and Evolution 3:3524–3535
- Lee, Y. and H. D. Enander. 2015. Developing an EMR Conservation Plan for Michigan Phase I. Michigan Natural Features Inventory Report No. 2015–10, Lansing, MI. 51 pp.
- Lentini, A. M., G. J. Cramshaw, L. E. Licht, and D. J. McLelland. 2011. Pathologic and hematologic responses to surgically implanted transmitters in EMR rattlesnakes (*Sistrurus catenatus catenatus*). Journal of Wildlife Diseases 47:107–125.
- McCluskey, E. M. 2016. Landscape ecology approaches to EMR Rattlesnake conservation. Dissertation, Ohio State University, Columbus, USA.
- Michigan Natural Features Inventory (MNFI). 2020. Michigan Natural Heritage Database, Lansing, MI.
- Millspaugh, J. J. and F. R. Thompson III. 2009. Models for planning wildlife conservation in large landscapes. Elsevier/Academic Press, Boston, Massachusetts, USA
- Moore, J. A. and E. M. McCluskey. 2020. Personal communication. Grand Valley State University, Allendale, MI.

- Peterman, W. E., G. M. Connette, R. D. Semlitsch, and L. S. Eggert. 2014. Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander. Molecular Ecology 23:2402–2413.
- Row, J. R., G. Blouin-Demers, and S. C. Lougheed. 2010. Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (*Mintonius gloydi*) across a fragmented landscape. Molecular Ecology 19:5157–5171.
- Szymanski, J. 1998. Status Assessment for the Eastern Massasauga Rattlesnake, U.S. Fish and Wildlife Service, USA.
- Szymanski, J., C. Pollack, L. Ragan, M. Redmer, L. Clemency, K. Voorhies, and J. JaKa. 2015. Special status assessment for the Eastern Massasauga Rattlesnake (*Sistrurus catenatus*), U.S. Fish and Wildlife Service, USA.
- U. S. Fish and Wildlife Service (USFWS). 2016. Endangered and threatened wildlife and plants: threatened species status for the Eastern Massasauga Rattlesnake. Federal Register 80:58688-58701.
- Zeller, K. A., T. G. Creech, K. L. Millette, R. S. Crowhurst, R.A. Long, H. H. Wagner, N. Balkenhol, and E. L. Landguth. 2016. Using simulations to evaluate Mantel-based methods for assessing landscape resistance to gene flow. Ecology and Evolution 6(12):4115-4128.

Appendices

Appendix 1. Eastern Massasauga Search Effort Data Sheet

Site/Unit Name or Number	Massasauga Popula	tion								
Starting Location Description & GPS Waypoint Date Latitude/UTM Northing	Site/Unit Name or I	Number								
Date Latitude/UTM Northing Team Leader Longitude/UTM Easting +/n Time START STOP Shaded Air Temp	Starting Location De	escription & G	PS Waypo	oint						
Team Leader LongitudeUTM Easting +/n Time START STOP Shaded Air Temp	Date		Latitu	de/UTM Nor	thing					
START STOP Shaded Air Temp	Team Leader		Longi	tudeUTM Eas	ting			+/	_m/ft	
Time			STAR	г			STOP			
Shaded Air Temp	Time				_					
Substrate lemp	Shaded Air Temp			•F / (C			_•F/C		
wind speed mpn mpn Humidity % Cloud Cover 0% 25% 50% 75% 100% Precipitation none drizzle mod. rain none drizzle mod. rain	Substrate Temp			•F / 0	C			_•F/C		
Humidity % % % Cloud Cover 0% 25% 50% 75% 100% Precipitation 0 none 0 drizzle 0 mod. rain 0 none 0 drizzle 0 mod. rain Deserver Name Time In Time Out Penalties Total Min.	wind Speed			mpr	ו			mpn		
Cloud Cover Downell 25% Blows Downell 25%	Humidity	- 00 - 050	- 500/	%				%		
Precipitation Index I drizzle Index I driz Index I drizzle Index	Cloud Cover		0 50%	0 75% 0 100%	6	0%		□ 75% □ 100%		
Observer Name Time In Time Out Penalties Total Min.	Precipitation	□ none □ dr □ heavy rain	izzle □n □fog	nod. rain		□ non □ hea	ne □drizzle □r ivy rain □fog	nod. rain		
Image: Sistering of the second size of the second se	Observer Name			Time In	Time (Dut	Penalties	Total Min.		
Image: Second										
Image: State of the second										
Image: Starturus catenatus catenatus catenatus catenatus minimum record for 1 observer; if more GPS available, record individually obs 1						_				
Image: State of the system						-				
Image: Second State Sta						_				
Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure Image: Second Structure						-				
Image: Second system of the						-				
Image: State of the state						-				
Image: Sister of the system						_				
Transect length (by GPS) (at minimum record for 1 observer; if more GPS available, record individually obs 1 obs 2 obs 3 obs 4 Total Snakes Found (#) EMR Sistrurus catenatus catenatusN. Ribbon Snake Thamnophis sauritus catenatusN. Ribbon Snake Thamnophis sirtalisBlue Racer Coluber constrictorBrown snake Storeria dekayiN. water snake Nerodia sipedonBlack Ratsnake Elaphe obsoletaBlack Ratsnake Elaphe obsoletaUnknown species Number of Other Herp Species Found: Comments (use back as needed)						-				
Transect length (by GPS) (at minimum record for 1 observer; if more GPS available, record individually obs 1 obs 2 obs 3 obs 4 Total Snakes Found (#) EMR Sistrurus catenatus catenatus N. Ribbon Snake Thamnophis sauritus EMR Sistrurus catenatus catenatus N. Ribbon Snake Thamnophis sauritus EMR Sistrurus catenatus catenatus N. Ribbon Snake Thamnophis sirtalis EMR Sistrurus catenatus catenatus N. Ribbon Snake Thamnophis sirtalis Kirtland's Clonophis kirtlandi Eastern Gartersnake Thamnophis sirtalis Blue Racer Coluber constrictor Brown snake Storeria dekayi N. water snake Nerodia sipedon Black Ratsnake Elaphe obsoleta Eastern milksnake Lampropeltis triangulum Unknown species Number of Other Herp Species Found:						-				
Transect length (by GPS) (at minimum record for 1 observer; if more GPS available, record individually obs 1 obs 2 obs 3 obs 4 Total Snakes Found (#) EMR Sistrurus catenatus catenatusN. Ribbon Snake Thamnophis sauritus						– Tota	I Survey Time_			
Transect length (by GPS) (at minimum record for 1 observer; if more GPS available, record individually obs 1										
Obs 2 Obs 3 Obs 4 Total Snakes Found (#) EMR Sistrurus catenatus catenatus N. Ribbon Snake Thamnophis sauritus Kirtland's Clonophis kirtlandi Eastern Gartersnake Thamnophis sirtalis Blue Racer Coluber constrictor Brown snake Storeria dekayi N. water snake Nerodia sipedon Black Ratsnake Elaphe obsoleta Eastern milksnake Lampropeltis triangulum Unknown species Number of Other Herp Species Found:	Transect length (by	GPS) (at mini	mum rec	ord for 1 obs	erver; if n	ore G	iPS available, ro 4	ecord individua	ally:	
Total Snakes Found (#) EMR Sistrurus catenatus catenatus N. Ribbon Snake Thamnophis sauritus Kirtland's Clonophis kirtlandi Eastern Gartersnake Thamnophis sirtalis Blue Racer Coluber constrictor Brown snake Storeria dekayi N. water snake Nerodia sipedon Black Ratsnake Elaphe obsoleta Eastern milksnake Lampropeltis triangulum Unknown species Number of Other Herp Species Found:	0051	005 2		005 5		0054	•			
EMR Sistrurus catenatus catenatus N. Ribbon Snake Thamnophis sauritus Kirtland's Clonophis kirtlandi Eastern Gartersnake Thamnophis sirtalis Blue Racer Coluber constrictor Brown snake Storeria dekayi N. water snake Nerodia sipedon Black Ratsnake Elaphe obsoleta Eastern milksnake Lampropeltis triangulum Unknown species Number of Other Herp Species Found: Comments (use back as needed)			٦	Fotal Snakes	Found (#)					
Kirtland's Clonophis kirtlandi Eastern Gartersnake Thamnophis sirtalis Blue Racer Coluber constrictor Brown snake Storeria dekayi N. water snake Nerodia sipedon Black Ratsnake Elaphe obsoleta Eastern milksnake Lampropeltis triangulum Unknown species Number of Other Herp Species Found: Comments (use back as needed)	EMR Sistrurus catenatus catenatus				N. Ribbon Snake <i>Thamnophis sauritus</i>					
Blue Racer Coluber constrictor Brown snake Storeria dekayi N. water snake Nerodia sipedon Black Ratsnake Elaphe obsoleta Eastern milksnake Lampropeltis triangulum Unknown species Number of Other Herp Species Found: Comments (use back as needed)	Kirtland's <i>Clonophis kirtlandi</i> Ea				Eastern Gartersnake Thamnophis sirtalis					
N. water snake Nerodia sipedonBlack Ratsnake Elaphe obsoletaBastern milksnake Lampropeltis triangulumUnknown species Number of Other Herp Species Found:Comments (use back as needed)	Blue Racer Coluber constrictorBrow				Brown	Brown snake Storeria dekayi				
Eastern milksnake Lampropeltis triangulumUnknown species Number of Other Herp Species Found: Comments (use back as needed)	N. water snake Nerodia sipedonBlac				Black F	Black Ratsnake Elaphe obsoleta				
Number of Other Herp Species Found: Comments (use back as needed)	Eastern mill	ksnake <i>Lampro</i>	peltis tri	angulum	Unkno	wn sp	ecies			
Comments (use back as needed)	Number of Other H	erp Species Fo	ound:							
			Comi	ments (use ba	ack as nee	ded)				
						,				

MASSASAUGA SEARCH EFFORT SHEET

Each observer should keep track of his/her own time on a small notepad. Use a watch and keep track of many minutes you spend NOT searching for massasaugas (i.e. minutes spent tying your shoe or talking to each other or chasing a turtle). This effort should be recorded to the whole minute.

Appendix 2. Eastern Massasauga Observation Data Sheet

Version 5.5.2011		SNAKE	STATUS: Captured	D Processe	d at Ecolab 🗆 Habitat Assessmer	nt Complete 🗆 Released 🗆	
General Location:		MA Filled	SSASAUGA DAT	A SHEET	Encounter Date		
Site/Unit Name:	Surve	y Team:					
GPS waypoint:	rded in GPS)	EMR	Observer:		Snake Identification	n # ssigned at processing	
1. Thermal Data	(Infrared 1	Temps): He	ead°C	Body	°C Tail	°C	
□ in full sun		2.	Behavioral Data		□ in rodent burrow	If in Burrow:	
□ in full shade □	⊐ lving still		piled tight (stacked)		□ in cravfish burrow	Burrow diameter	
□ in part sun 0	⊐ moving		piled loose (single laye	er)		cm	
	Ū	🗆 lo	oped, touching once		visible at entrance		
□ fully exposed [⊐ rattling	🗆 lo	oped, not touching		🗆 head out	Burrow temp	
□ completely covered [⊐ not rattling	g ⊡st	raight		head and some body ou	t°C	
partially covered (grass, etc.	.)				body out, head in		
	3. PH	OTOGRAPH SN	AKE IN SITU; TH	EN, CAPT	URE SNAKE !!!!!		
4. Environmental Da	ata			5. Cap	oture Data		
Precipitation		Time of sighting					
□none □drizzle □mod.r	rain	General Location	1		UTM Northir	Ig	
🗆 heavy rain 🛛 🗆 fog		Color of flag left	at site		UTM Easting		
Cloud cover						+/m/ft	
□0% □25% □50% □75	% □100%	Capture Type:	Capture	□ Sighting	Only		
Humidity9	%		Resighting – rat	tle color	•		
Wind r	n/sec		Photograph 🗆 Y	es □No			
Shaded Air Temp °	°C		0.				
Substrate Temp	Ϋ́F	Method of Find:	□ Funnel trap/fence #	□ Metal (#	Coverboard □Carpet Coverbo #	ard □Visual □Sound	
		6.	. Habitat and Spati	al Data			
Within 1 meter radius centered on Percent Cover Types @ flag	snake site - U S	lse "Not Visible" if need	# ¼ squares of	ccupied			
					(96 total quarter squares)		
			Detrestivitely	4			
Grass/Leaf Litter			Retreat within	1 meter		т туре	
# Woody stems			Was area burr	ned recently	y? □ Yes □ No		
AT ECOLAB: Morphologica	l and Ident	ification Data	Filled out by:		Date:		
	Cantura 🗖		ent Recenture		Photo Numbers:		
Say (from probing):			ent Recapture		Blood Samples		
Subcaudal Count		*If famala			Gapatics sample		
Total Longth		grouid?			Chamistry sample		
Total Length	-111	graviu :			Chemistry sample		
		****			Classel Such		
		recent mea	II [
iviass	grams				ID Photograph		
					Fecal Sample	LI Yes LI NO	
PIT Tag #		Cautery	ID # and Descriptio	n:			
Rattle Description	+	(<u>BorT</u> +	+ <u>#ofrattles</u>) Rattle	e Color			
Saddle description							
Release Data Date: (MM			Time				
Release Location, if differer	nt from GPS	above:					
Other Notes (use back if ne	eeded):						
Natural Community Descri	ption:						