United States Department of the Interior
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In Reply Refer To:
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March 28, 2017

Memorandum

To: Assistant Regional Director, Ecological Services

From: Field Supervisor, Chicago Ecological Services Field Office, Illinois

LOUISE CLEMENCY Digitally signed by LOUISE CLEMENCY Date: 2017.03.28 16:40:14 -05'00'

Subject: Biological Opinion. Section 7 Programmatic Consultation on Issuance of Section 10(a)(1(A) Scientific Take Permits and Providing Funding Pursuant to Endangered Species Grants for the Eastern Massasauga Rattlesnake (*Sistrurus catenatus*)

Introduction

This Biological Opinion is prepared pursuant to Section 7 of the Endangered Species Act of 1973, as amended (16 U.S.C.1531 *et seq.*) (Act). At issue is the effects of three actions with respect to the eastern massasauga rattlesnake (EMR; *Sistrurus catenatus*), including: (1) issuance of Section 10(a)(1)(A) permits for scientific and conservation purposes, (2) the granting of funds for non-habitat related recovery and enhancement of the species, and (3) controlled propagation and management of the captive population of this species. These actions would be authorized by the U.S. Fish and Wildlife Service's (Service) Regional Director, Region 3 and his/her employees and agents, through issuance of permits and subpermits by the Regional Office, pursuant to the ESA section 10(a)(1)(A), and the granting of funds for recovery or enhancement of survival related activities.

This programmatic Biological Opinion evaluates the impacts of proposed Service actions that are likely to result in adverse effects to the EMR. These actions are intended to promote long-term recovery, but may result in short-term adverse effects such as harm and harassment. Mortality may also occur on a small scale as a result of these activities. Although these actions may adversely affect individual EMR, they would enhance survival of the species and thus provide a net benefit to the species. Subpermits for take under section 10(a)(1)(A) would be issued under the authority of the Service's Region 3 Endangered Species Blanket Permit (TE-697830). These subpermits would be issued to qualified individuals to benefit the recovery of the EMR.

It is our opinion that proposed actions carried out pursuant to the Service's recovery grant and section 10(a)(1)(A) programs are not likely to jeopardize the continued existence of the EMR.

This opinion is based on information provided in the Species Status Assessment for the EMR (Szymanski *et al.*2016), Federal Register/Vol. 80, No. 189, 2015 Proposed Rules, East Lansing Field Office EMR Conference Opinion (June 2016), and contact with species experts, including at meetings, telephone conversations, conference calls, briefings, email correspondences, field investigations, literature, and other sources of information. A complete record of this decision is on file at the Service's Chicago Field Office, Chicago, Illinois.

Consultation History

Because the EMR is recently listed (U.S. Fish and Wildlife Service 2016), there are no previous consultations related to its enhancement of survival as a listed species. However, the species has been of high profile, and has an extensive prior record of research or other activities that may enhance its survival while leading to short term adverse effects. This biological opinion was thus prepared in anticipation that such activities will continue to be needed.

Biological Opinion

Activities Proposed

The activities included in this Biological Opinion are restricted to (1) scientific studies and field surveys, (2) the granting of funds for recovery and enhancement of survival of a listed species, and (3) controlled propagation and management of a captive population. This Biological Opinion is effective range wide for the EMR (Figure 1) for the issuance of permits or granting of funds for scientific research and surveys that promote the conservation of the EMR.

Scientific studies and field surveys include the gathering of scientific information important to inform conservation efforts. Population surveys, protocols for handling, and data collection are to follow Service guidelines outlined for the Service (Casper et al. 2001), unless otherwise described herein, or proposed and justified by a permit holder and agreed to by the Ecological Services Field Office(s) that service(s) the state(s) where these activities will occur. Specific activities to be considered for permit issuance ate detailed below.

Controlled propagation and management of the captive population includes propagating rearing, placing individuals into the captive population, and releasing captive individuals and other conservation related actions that involve handling and management of such individuals. All activities related to controlled propagation and management of captive populations of the EMR will follow the Service's guidelines for controlled propagation (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000), and annual planning and management as reported by the Association of Zoos and Aquariums Species Survival Plan for the EMR (most recent example, Jundt et al. 2016).

Species Affected

Eastern massasauga rattlesnake (*Sistrurus catenatus*). Listed under the Endangered Species Act of 1973 on October 31, 2016 (U.S. Fish and Wildlife Service 2016). Critical habitat for this species has not been designated.

Programmatic Consultation Approach

Pursuant to section 7 of the ESA, the Service must ensure that its proposed actions do not jeopardize the continued existence of any federally listed species or result in the adverse modification or destruction of designated critical habitat. In accordance with this mandate, each proposed action must undergo a section 7(a)(2) review. In the context of the potential proposed actions described here, all section 10(a)(l)(A) permits and all future Service recovery grants are subject to section 7 consultation. We anticipate that activities similar to those described below will be authorized, conducted, or funded by the Service. This section 7 programmatic consultation for Region 3's section 10(a)(l)(A) permits and Service recovery grant programs is conducted to address our section 7(a)(2) requirements with respect to the EMR. This programmatic Biological Opinion excludes land management measures for the EMR which will be addressed in a separate Biological Opinion.

This document analyzes the potential effects of actions we anticipate permitting, funding, or carrying out for research and conservation of the EMR, excluding habitat and land management actions. We also recommend actions to avoid take that we will incorporate into proposed section 10(a)(l)(A) recovery permits or into the project designs. Each section 10(a)(l)(A) permit and funding proposal will be reviewed to ensure that (1) the actions to be permitted or funded were contemplated in this programmatic Biological Opinion, (2) the appropriate recommended actions have been incorporated into the permit or project design, (3) the anticipated effect of the permit or grant is commensurate to what was anticipated in this programmatic Biological Opinion, (4) the level of take permitted or expected does not exceed the level anticipated in this programmatic Biological Opinion, and (5) the appropriate terms and conditions from this programmatic Biological Opinion have been incorporated into the permit or project design. Projects that are funded through the Service's recovery grant programs or require a section 10(a)(l)(A) permit that have potential effects not directly considered in this programmatic Biological Opinion will require an additional section 7 review, and may necessitate an amendment to this programmatic Biological Opinion.

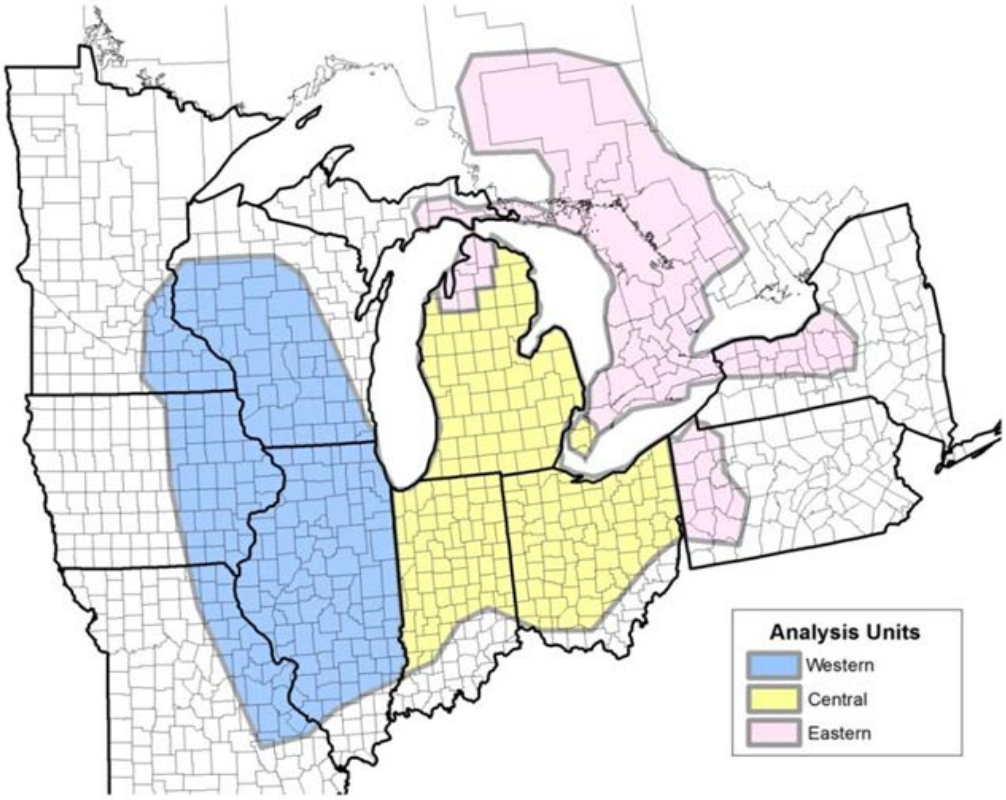

Figure 1. The historic and current range of the EMR includes the blue, yellow, and pink shaded areas as one (*in* Szymanski *et al.* 2016). Confirmed historical occurrences are not documented from all counties shaded in this map.

Description of Proposed Action

There are two general types of proposed actions: (1) issuance of 10(a)(1)(A) permits and (2) granting funds for projects that promote the conservation of EMR.

1) 10(a)(1)(A) permits

The Services proposes to issue section 10(a)(1)(A) permits to purposefully take the EMR for recovery and scientific purposes. Pursuant to section 10 of the ESA, we may authorize otherwise prohibited actions for scientific purposes or to enhance the propagation or survival of listed species. All activities authorized by the Service under section 10(a)(1)(A) must meet permit issuance criteria at 50 CFR 17.22, 17.32, or 17.52. The ESA and its implementing regulations mandate that such activities be for the purposes of enhancement of survival and recovery of listed species.

In determining whether to issue a section 10(a)(1)(A) permit, the Service must consider the following criteria: (1) whether the purpose for which the permit is required is adequate to justify removing from the wild or otherwise changing the status of the wildlife sought to be covered by the permit; (2) the probable direct and indirect effects that issuing the permit would have on the

wild populations of the wildlife sought to be covered by the permit; (3) whether the permit, if issued, would in any way, directly or indirectly conflict with any known program intended to enhance the survival probabilities of the population from which the wildlife sought to be covered by the permit was or would be removed; (4) whether the purpose for which the permit is required would be likely to reduce the threat of extinction facing the species of wildlife sought to be covered by the permit; (5) the opinions or views of scientists or other persons or organizations having expertise concerning the wildlife or other matters germane to the application; and (6) whether the expertise, facilities, or other resources available to the applicant appear adequate to successfully accomplish the objectives stated in the application.

As with other federally listed species, the section 10(a)(1)(A) process for the EMR is initiated with an application for a permit. The Service processes and evaluates the permit in accordance with general permit procedures and permit issuance criteria. The Regional Permits Coordinator consults with the Chicago Field Office to determine whether (1) the proposed activities have been considered in this biological opinion, (2) the appropriate conservation measures have been incorporated into the permit, (3) the effect of the permit is commensurate with what was anticipated in this biological opinion, (4) the type and amount of take will not exceed the level identified in this biological opinion, and (5) the appropriate terms and conditions have been incorporated into the permit. The Regional Permits Coordinator and the Chicago Field Office also ensure the appropriate conservation measures identified in this biological opinion (see "Conservation Measures") are incorporated into the permit as enforceable terms and conditions. If the Service issues a permit, the Service may incorporate special terms and conditions to minimize incidental take. Under the section 10(a)(l)(A) permit process, take will be permitted for only those activities that promote the conservation and recovery of the EMR. Only qualified individuals, as determined by the Service, will be issued permits to take the EMR. All data collected as a result of these actions are reported to the Service on an annual basis to facilitate recovery monitoring and planning. Upon receipt of the required annual report, the Service records the amount of take that occurred.

2) Granting of funds to enhance conservation of EMR

The Service also may provide funding for research projects and other studies that promote the conservation of the EMR. Several grant sources have been established to benefit listed species, including the Service's recovery grants, as discussed below. Additional grants that are not explicitly discussed here may be covered under this programmatic biological opinion, provided they are for the purpose of EMR conservation and the effects of the project are considered in this programmatic biological opinion.

The authority for several of the funding sources is provided pursuant to ESA and the Land and Water Conservation Fund Act of 1965 (LWCP). Some of these funding sources include the Cooperative Endangered Species Conservation Fund (also known as section 6) and the Land and Water Conservation Fund. In addition to section 6 grants, we are also evaluating projects funded by Region 3's appropriated program funds (such ESA Recovery funds) and direct expenditure of those funds by the Service. Grants from the Service's State Wildlife Grants, Wildlife and Sport Fish Restoration (previously known as Federal Aid), and other

Service recovery-oriented grants are also evaluated here provided they meet the criteria previously described.

Section 6

Section 6 authorizes the Service to enter into cooperative agreements with any State which establishes and maintains an adequate and active program for the conservation of endangered and threatened species. Under this authority, we may provide financial assistance to any such State or Territory (hereafter, "States") for the purposes of developing conservation programs for listed species or for monitoring the status of candidate or recovered species. In order for a State program to be deemed an adequate and active program, the Service must find and reconfirm, on an annual basis, that the State program, among other things, has: (1) the authority to conserve resident endangered or threatened species of fish and wild life or plants; (2) established an acceptable conservation program, consistent with the purposes and policies of the ESA, for all resident species of fish and wildlife or plants in the State which are endangered or threatened; and has furnished a copy of such program together with all pertinent details, information, and data requested to the Service; and (3) authorization to conduct investigations to determine the status and requirement s for survival of resident species of fish and wildlife or plants.

The section 6 grant review and approval process for EMR related projects is as follows. Project proposals are developed by the States in coordination with their field office(s), or the Regional Office, depending upon the project's location, and are evaluated based on the merits, benefits, and risks. Once proposals are selected for funding, an Intra-Service Section 7 Biological Evaluation form is completed prior to project commencement. For projects that are likely to adversely affect EMR, the lead field office or the Regional Office will follow the same review and consultation process as the section 10(a)(l)(A).

Recovery Programs

The Service's Recovery Program (Recovery Program) works with Federal, State, tribal and non-government entities to take action to prevent the extinction of species, prepare recovery plans to ensure coordinated, effective recovery actions, and implement actions to reverse the decline of listed species and expedite those species' recovery. Recovery Program funds may be used to implement on-the-ground conservation actions.

Other Grant Programs

Other grant programs are available that could benefit listed species. Some of these programs are ephemeral and new programs are sometimes implemented. The grant programs listed in this biological opinion are not meant to be all inclusive, but to give a general sense of the types of programs available. For example, the Service's Wildlife and Sport Fish Restoration Division transfers and administers federal grants through a variety of programs. The grant dollars are often derived from State hunting and fishing excise tax. These monies are transferred to State fish and wildlife agencies for projects that enhance fish, wildlife, and habitat resources for present and future public benefit. The States use these grants, which typically account for one-fourth to one-third of their respective fish and wildlife management budgets, for work across the full spectrum of their management responsibilities. Some of these programs could directly

benefit listed species including the EMR.

In addition, the Land and Water Conservation Fund provide money to Federal State and local governments to acquire land, water and conservation easements on land and water for the benefit of all Americans.

In this Biological Opinion, Region 3's section 6, recovery grant programs, applicable Wildlife and Sports Fish Restoration, and other grant programs will be collectively referred to as the Service's Recovery Grant programs. The Service's staff may also conduct independently or cooperatively (e.g., with State agencies or other stakeholders) direct activities (e.g., research, captive population management, including propagation, and other handling by qualified permit holders) that benefit the conservation of EMR. These activities are covered under this consultation, provided the effects of that action have been considered. For other projects that may affect this or other listed species, a separate section 7 consultation may be required.

To provide additional scientific information on EMR, proposals should specify the purposes and objectives of the research project, describe how the project will benefit and contribute to the recovery of EMR, and whether other research of the type proposed has been conducted in the past and include those past study results. The study design and techniques should be described in specific detail. Specific activities that may be funded or permitted by the Service's Recovery Grant programs and section 10 (a)(l)(A) permits are described in the following sections:

Field Surveys and Scientific Studies

Field surveys and scientific studies of listed species such as the EMR can be crucial to furthering the understanding of the needs of wild populations, so that conservation efforts can continue to evolve. For example, several studies using capture-mark-recapture of EMR in the wild have provided an excellent baseline for how populations are structured demographically, and have provided key vital rates so that extinction risk in small populations can be modelled. For example, in 2008 the Service convened a workshop of EMR experts who combined knowledge of the species' literature and their own experiences to define a suite of population vital rates. These were in turn used to develop two related extinction risk models (Faust et al., 2011, Szymanski et al. 2016). Continuation or expansion of monitoring efforts using these techniques will be important for the Service so we can evaluate recovery efforts for the species, and when qualified individuals apply for permits to conduct such surveys, this should be encouraged. Most of the Service's standardized survey protocols (outlined by Casper et al. 2001) still provide an adequate tool for planning EMR surveys, though we are aware of work by several researchers who are studying detection probability and occupancy modeling for the EMR. When published, those studies may suggest significant changes in the way these surveys are planned and conducted. The Service's qualifications for potential EMR surveyors (Casper et al. 2001) also still apply.

Similarly, studies of disease, and snake fungal disease in particular, in wild EMR have rapidly helped our understanding of how this pathogen functions. Several different approaches may be used to conduct field studies that can be informative to the conservation of the EMR. These approaches may take place in the field, or during temporary holding of wild-caught EMR in ex-

situ (e.g., in the laboratory) situations, and thus may involve take through capture, and even temporary holding of animals before they are returned to their point of capture. In viable populations of EMR, it is possible to capture large numbers of individuals, thus ensuring adequate sample size for scientific analyses. While capture is a form of take that must be quantified, if best practices are followed, including the terms and conditions to be included in 10(a)(1)(A) permits as listed later in this document, lethal take should be minimal. The potential benefits of these approaches to enhance the survival of the species are described below.

Visual encounter surveys
Visual encounter surveys (VES) are often used to determine presence of the EMR in an area, and may be used to estimate relative abundance of the EMR in an area, accumulate baseline information on populations, or track long term trends of populations, and may thus be beneficial to the enhancement of the species. VES usually are performed by individuals or teams who walk through known occupied or presumed occupied habitat searching for EMR to confirm the species presence/absence, count individual EMR, or collect a suite of data that do not require handling (Casper et al. 2001, page 5).

VES, when used in conjunction with capturing and marking individual EMR (both topics discussed further below) prior to release at the point of capture has also proven to be a reliable way to build baseline data on large portions of EMR populations. Those data can be used for population estimates and other studies of demography and population structuring in this species.

Road cruising surveys
Roads are attractive to EMR for basking, thermoregulation, and mate seeking. While roads are a potential cause of habitat fragmentation that can transect habitat patches, they may also be traversed by EMR moving between the patches. Road cruising is described as driving in a vehicle on lightly traveled roads that intersect prime EMR habitat until a snake is encountered. This can be done at any time of the day or night and on paved or unpaved roads. Summer and fall are peak EMR activity periods. Road cruising may be a beneficial way to augment sampling of EMR populations. Also, because individual EMR may be killed by cars, detecting, counting, salvaging carcasses of EMR killed on roads may serve as a way to augment data (including this source of mortality, as well as identifying road mortality "hot spots" so that measures to avoid or minimize mortality can be implemented, or use of the carcasses/tissues for genetic studies), on the population.

Cover boards
Snakes often seek shelter under naturally occurring cover objects, and introducing artificial cover objects (usually wooden boards, sheet metal panels, or carpet remnants) into areas with scarce natural cover is a useful method for sampling snakes during inventory or monitoring. Because cover boards may provide attractive sources of cover for snakes and can be systematically or randomly distributed across a habitat patch, they can augment detection of EMR during surveys.

Capture and handling
Capturing EMR is usually necessary for positive identification and collection of data from individuals, and can be useful for monitoring population trends and informing efforts to conserve the species. Capture methods are most commonly used in conjunction with visual encounter

surveys. When encountered, visible EMR are captured with snake tongs, hooks, clear tubing (slid over the snakes heads and anterior portion of body to allow restraint), or by gently coaxing or sweeping a snake into a tall bucket or other container. Trapping or using cover boards to detect EMR may also be used (both methods described below). Examples of data that can be collected from individuals include morphological characteristics, demographic information (gender, age/size class), distribution, habitat use, and (if individuals are captured on multiple occasions) growth and survival. In order to gather these data following capture, EMR would be subjected to a suite of measures to identify them at an individual level related to the time of capture, including determining body condition (length and mass), determining gender (usually by cloacal probing), marking for future recognition in one or more ways (see below), having tissues harvested (see below), general or specific examination for disease, followed by release.

Capture and handling of EMR in the field may be necessary to remove individual snakes from a hazardous situation. As stated in the Final Rule to list the species (U.S. Fish and Wildlife Service 2016), no permit is required for take when a listed species presents a hazard to human safety or property as long as the snake is immediately released on adjacent habitat. Holding nuisance EMRs for data collection, etc., would still require a 10(a)(1)(A) permit.

Drift Fence Trapping

Drift fences using funnel or pitfall trapping is a sampling technique which is commonly used in reptile surveys (Fitzgerald 2012, Foster 2012). This technique involves erecting a short (usually less than 1m/3ft tall), temporary, and impenetrable fence within habitat to be sampled, and placing traps along the sides and at the ends of the fence. Animals moving through the habitat are intercepted by the fence and directed towards one of the traps where they are captured upon entry. This technique is used to study community composition, seasonal occurrence, spatial distribution patterns, and to compare relative abundance in different microhabitats. It has the benefit of being passive, so is less labor intensive than VES (Bartman et al. 2016). A funnel trap usually includes an aluminum or steel hardware cloth or screen barrel, with a funnel (made of plastic or additional hardware cloth funnel at one end. These traps are placed along the drift fence, and when a snake encounters them, they enter via a small hole in the funnel. While snakes are not completely trapped (they can exit if they can find the hole in the funnel), the hole is not easy for them to find and once they have entered they generally remain in the traps. The pitfall trap is the use of a pit in the ground into which a ground-dwelling animal falls in and in most cases, cannot escape. Pitfall traps may be used either alone (placed randomly in the field), or also in conjunction with a drift fence as described above. Since either of the above approaches includes trapping individual EMR, these would require a 10(a)(1)(A) permit.

"Wet" pitfall traps contain a solution designed to trap, kill and preserve an animal or animals. Aqueous solutions used in these traps include; formalin (10% formaldehyde), alcohol, methylated spirits, trisodium phosphate and picric acid) are not considered in this Biological Opinion. "Wet" pitfall traps shall not be used for EMR surveys, nor at sites with documented EMR populations.

Individual Data Collecting

Field studies of EMR biology that use any of the above methods to encounter EMR biology often require collecting data from individual animals. While measuring, weighing, photographing, and

cloacal probing to determine gender of individual snakes can all be done in the field, because the EMR is a venomous species, and presents added hazards to handlers, some consider it to be much safer and more distraction free to conduct such activities in a laboratory or other *ex-situ* setting. Collecting data that requires handling of individual EMR would require a 10(a)(1)(A) permit. Occasionally, "parts" of individual EMR may be collected to study genetics, or other information. For example, these techniques may include blood and/or venom sampling, scale clipping, or the collection of shed snake skins or deceased EMR. These are addressed under the following sections including and related to veterinary care. In some circumstances, gravid female EMR are held so that data can be collected on their litters of young (including litter size, as well as body size and sex of individual neonates).

Veterinary Care

Instances may arise when EMRs held by permit holders require veterinary medical care. In such instances, the permit holder would have discretion to seek veterinary care, and no additional permit would be needed by a licensed veterinarian to carry out medical procedures, including euthanasia using their professional judgement.

Prevention of Disease Transmission

Recent concern over the emergence of snake fungal disease (Allender et al. 2011) has increased awareness for the potential to spread pathogens between populations. Researchers taking personal precautions to reduce the risk of disease transmission by scrubbing footwear or field equipment with bleach solutions may enhance the survival of the species, and is encouraged as a "best practice," but does not require a 10(a)(1)(A) permit. Taking snakes for long-term or permanent quarantine due to them showing signs of disease, or if they are found to carry a potential disease after they are collected would represent take and would require a 10(a)(1)(A) permit.

Nasal-Facial/Bodily Swabbing

Monitoring the health of individual EMRs may be beneficial to informing management decisions and research, and this would enhance survival of the species. In order to determine the range wide extent of snake fungal disease, other external pathogens, or ectoparasites, nasal and/or facial swabbing, or swabbing the skin of an EMRs body may be necessary following capture of individuals.

Tissue collection

Collecting tissues from EMRs can be useful for assessing health and/or exposure to internal diseases, studies of conservation genetics, paternity/maternity, isotopic studies of prey or exposure to environmental contaminants. In snakes, including EMR, tissues are usually sampled by biopsy through scale clipping, drawing blood from the subcaudal vein of the snake, or by salvaging whole carcasses or tissues from dead specimens found in the field. Because collecting blood, tissues, or other types of biopsies requires handling of EMR, these activities would require a 10(a)(1)(A) permit.

Salvage

Depending on their condition when found, whole or partial carcasses of EMR found dead in the field or on roads, or portions of shed skins from EMR can still be useful for inclusion in several

types of studies (for example genetics) that can inform conservation of the species. Because possession of "parts" of listed species is prohibited by the act, a 10(a)(1)(A) permit would be necessary for field workers to salvage these.

Individual Marking
Marking individuals is a common practice in wildlife management, and allows for future recognition so that future encounters of previously encountered and marked snakes can be easily quantified versus individuals that are newly encountered for the first time. This also allows several types of demographic and growth data to be monitored over time. Marking in conjunction with field studies of the EMR can thus be beneficial to enhancement of survival of the EMR. Several types of marking are commonly used (and sometimes together to provide redundancy and reduce the likelihood that individuals are counted twice) to recognize individual EMRs found during field studies. Marking methods considered here include use of PIT (Passive Integrated Transponders) tags (also commonly called "microchips"), painting or marking rattle segments, scale clipping, and dorsal saddle pattern recognition through controlled photography. Because all (including, in most cases photography of detail needed for individual recognition) of these approaches would require individual EMR to be handled, all would require a 10(a)(1)(A) permit, and are considered below under "Effects of the Action."

Radiotelemetry
The capture of EMR and equipping individuals with radio telemetry devices (transmitters) can help obtain detailed information on spatial ecology, including movements, habitat use, movement patterns, distribution throughout the species annual cycle, response to disturbance, and behavior. There have been numerous studies of the spatial ecology and behavior of EMR that have used implanted radio transmitters. Two types of radio telemetry devices can be used, internal or external. Internal transmitters can be surgically implanted into the EMR or external transmitters can be applied to the skin of the snake. In addition, because radio telemetry allows repeated observations of individuals throughout portions of their lifespan, and often determination of fate, this method has also been used to calculate survivorship (Jones, et al. 2012), a demographic vital rate important to risk assessment and conservation modeling. This method thus has value in providing information useful in the enhancement of survival of the species. However, concerns about increased risk of disease or mortality to EMR related to radio telemetry (Lentini et al. 2011) are discussed below in "Effects of the Action." Because radiotelemetry requires handling, including potentially invasive surgery, this technique would require a 10(a)(1)(A) permit.

Controlled Propagation and Management of Captive Population

In this Biological Opinion, controlled propagation and management of captive population refers to several actions that may enhance survival of the species through reproduction of or captive maintenance of EMRs. Individual EMR that were legally held prior to the date of listing are not unlawful to possess. An EMR currently in a display at an interpretive/visitor center, or held in a zoo does not need a permit as long as it wasn't collected post-listing. However, progeny are protected as if born in the wild, therefore captive propagation programs must be permitted by

10(a)(1)(A) permit. Captive maintenance and propagation, as well as research involving captives are considered.

Captive Maintenance and Propagation
This includes maintaining individual EMR, and breeding adults in captivity, managing the genetic variation of a captive population through planned breeding transfers between permitted or accredited facilities (e.g., at a zoo accredited by the Association of Zoos and Aquariums) or other facilities for outreach or display purposes. The purposes of this population would be to maintain a "refugium" or assurance population, and would thus contribute to enhancement of survival of the species.

Placement of Surrendered Captives into Captive Management
Due to reasons that may preclude their release into the wild, legally maintained captive EMRs are occasionally surrendered, and illegally maintained captive EMRs are sometimes confiscated by law enforcement actions and are occasionally placed with conservation agencies, interpretive/visitor centers or accredited zoological facilities. In cases where no documentation showing that the animals were legally held prior to their surrender or confiscation, a 10(a)(1)(A) permit would be required for placement and continued maintenance in the captive population.

Collection and Removal from The Wild For captive Breeding
This includes collecting individuals from the wild to enhance genetic stock of the captive population, and ensuring a viable captive population is available. We anticipate that this would be done only in rare circumstances. For example if best available data, from monitoring of an individual population were shown to have declined to such small size that it is unlikely to recover on its own, attempts to remove the few remaining individuals may be permitted. This type of scenario would be contingent on agreement(s) from state and local land management agencies or landowners, as well as availability of an AZA or other appropriate facility to receive individual EMR into a captive program for propagation or educational purposes.

Population Augmentation and Reintroduction
This includes use of EMR propagated and reared in captivity to augment wild populations, to reestablish populations at sites where the species has been extirpated, or to attempt to establish new wild populations within the species historic range. As with collection and removal from the wild, and because the EMR is a venomous snake, we anticipate any such effort would be controversial, and would be conducted only in rare circumstances. This type of scenario would also be contingent on agreement(s) from the state conservation agency, and from local land management agencies or land owners having authority over sites where this would occur. Previous attempts to release EMR reared in captivity have met with mixed results (King et al. 2004; Harvey et al. 2014), but did provide information on viability of this method. Any additional attempts should continue to follow an adaptive or experimental approach.

Research Involving Captives
This includes the use of captive EMR in research that could benefit or inform recovery of the species. Individual EMR held in captivity could be valuable for studies into the species' biology that that may not require wild individuals, or could be used to augment data gathered from studies of wild individuals. Examples include studies of the physiology, metabolism,

reproductive cycles, or reproductive behavior of captive EMR, or pathology and disease management, all of which could inform conservation of wild EMR populations, and thus contribute to enhancement of the survival of the species.

Status of the Species

This section presents the biological or ecological information relevant to formulating the biological opinion. The purpose is to provide the appropriate information on the species' life history, its habitat, and its range-wide distribution and conservation status for analyses in later sections. This section also considers the effects of all past human and natural activities or events that have led to the current status of the species.

The Final Rule listing the EMR as threatened under the Endangered Species Act was published in the Federal Register on September 30, 2016. A Species Status Assessment (SSA) team prepared, and continues to update, an SSA report for the EMR (Szymanski *et al.* 2016). The SSA team was composed of U.S. Fish and Wildlife Service biologists, who developed the report in consultation with other species experts. The SSA represents a compilation of the best scientific and commercial data available concerning the status of the species, and an assessment of the impacts of past, present, and future factors (both negative and beneficial) affecting the EMR. The SSA and other materials relating to EMR listing proposal can be found on the Midwest Region website at http://www.fws.gov/midwest/Endangered/ and at http://www.regulations.gov under docket number FWS–R3–ES–2015–0145.

General Habitat Requisites

The EMR is active in the spring, summer, and fall and inactive in the winter when it hibernates. Therefore, depending on whether the snake is active or inactive determines what type of habitat is required. Active season habitat consists of thermoregulatory or *basking* sites, *retreat* sites, and *foraging* sites. Inactive season habitat consists of hibernacula, often within wetlands or in cavities that allow snakes to retreat to the upper portion of the water table (Reinert 1978).

EMR have been found in a variety of wetland habitat types across their range, including bogs, fens, shrub swamps, wet meadows, marshes, moist grasslands, wet prairies, peatlands, coniferous forests and floodplain forests (Minton 1972, Seigel 1986, Hallock 1991, Weatherhead and Prior 1992, Johnson 1995, Kingsbury 1996, Harding 1997, Sage 2005). At many locations, individual EMR may also move from wetlands to drier upland sites during certain parts of the year to forage, disperse, gestate, and even hibernate in some cases (Reinert and Kodrich 1982, Seigel 1986, Weatherhead and Prior 1992, Johnson 1995, King 1997, Bissell 2006). Suitable upland habitat types range from forest edges and openings, savannahs and prairies to meadows, old fields and some agricultural lands.

During the active season, EMR need highly intermixed and interspersed opportunities to bask and retreat from sun, hide from predators, attack prey without a chase, find mates, and travel to and from hibernacula seasonally through corridors that lack potentially lethal barriers such as roads.

During the winter months EMR occupy hibernacula. These hibernation sites can occur in wetland, wetland edges, wet prairie, closed canopy forests with mossy substrates (DeGregorio 2008), wet grassland, and sedge meadow (Mauger and Wilson 1999). Across its range, EMRs have been reported to hibernate for up to six months of the year. Crayfish burrows are

commonly used over much of the range, though mammal burrows, rocky crevices, rodent holes, hummocks, old stumps, rotten logs, and tree and shrub root systems are also used, (Wright 1941; Johnson 1995; Mauger and Wilson 1999; McCumber and Hay 2003; Dreslik 2005; Harvey and Weatherhead 2006; Johnson and Leopold 1998; Sage 2005) as may be cavity that reaches the water table (Reinert 1978).

Consistent hydrology at winter hibernacula sites is important in maintaining conditions that support EMR over-winter survival. To survive the winter, each individual EMR requires a suitable hibernation site which is critical to avoid lethally low temperatures and reduce the risk of desiccation (Reinert and Kodrich 1982). Consequently, hibernation sites must provide insulated and moist subterranean spaces below the frost line where individuals can avoid freezing and dehydration (Sage 2005). Most EMRs will either return to the same hibernacula annually (Johnson *et al.* 2000) or to an area within roughly 100 m (328 ft) of their previous hibernation site (Sage 2005; Harvey and Weatherhead 2006).

Requirement for Connectivity Between Microhabitats
EMR need corridors between microhabitats (basking sites, retreat sites, and foraging areas) and between seasonal habitats. EMRs can traverse corridors most successfully (reduced likelihood of mortality) between habitats when there are no barriers such as roads, rivers, or anything that can act as a barrier to snake movement. The absence of roads is an important criterion because roads are a strong barrier to EMR movement due to road mortality (Shepard *et al.*, 2008a, Shepard 2008b; Choquette 2011) or road avoidance behavior.

Connectivity between the active season (summer) habitat and inactive season (winter) habitat is crucial for population sustainability. Similarly, when temperatures shift the snakes must have the unimpeded ability to either access or retreat to a particular (summer or winter) habitat.

Habitat Requirements for Successful Reproduction
Male EMR may exhibit prolonged periods of mate searching, longer daily movements, and defensive female polygyny during the mating season (Jellen 2005; Johnson 2000). Males may use chemical cues to simultaneously trail and pursue individual females during the mating season (Johnson 1989). Because mature male EMRs often occur in higher numbers than receptive females, competition for mates can be intense.

Thermoregulation is so important to gravid female EMR that they spend the majority of the gestation period within open-canopy areas (Reinert and Kodrich 1982). This type of habitat has significantly higher mean soil temperature than early to mid-successional wetlands (Foster *et al.* 2009). Depending on the location of the population, gestation habitat of gravid female EMRs could be rock outcroppings, open grassland, shoreline, sedge meadow, barrens, or any suitable land characteristic that provides the snake the ability to thermoregulate and avoid predators.

Usually gravid females will remain near their winter hibernacula until parturition in late July or early August and then move to other foraging locations (Marshall *et al.* 2006; Johnson 1995). Foster *et al.* (2009) identified "the importance of accessible early/mid-successional upland areas adjacent to wetlands for the reproductive success of *S. c. catenatus*. This vegetation type apparently provides gravid females with favorable thermal conditions, which ultimately may

enhance *S. c. catenatus* productivity." Local gestation sites may be used by several females in a given season and appear to be used by the same individuals in successive breeding years. While at their chosen gestation sites, gravid female snakes generally engage exclusively in basking behavior, forfeiting opportunities for other essential behaviors such as feeding (Keenlyne and Beer 1973; Marshall *et al.* 2006; Weatherhead and Prior 1992). Since gravid females feed very little, if at all, it appears that they maintain themselves on reserved body energy (fat) throughout their pregnancies (Keenlyne and Beer 1973). In the fall, gravid females continue to thermoregulate more than males or non-gravid female snakes, despite giving birth in late summer (Harvey and Weatherhead 2011).

Rangewide distribution and abundance

The EMR historically occupied sections of western New York, western Pennsylvania, southeastern Ontario, the upper and lower peninsulas of Michigan, the northern two thirds of Ohio and Indiana, the northern three quarters of Illinois, the southern half of Wisconsin, extreme southeast Minnesota, east central Missouri, and the eastern third of Iowa. The limits of the current range of the EMR are similar to the historical range; however, the geographic distribution of extant localities has been restricted by the loss of the populations from much of the area within the boundaries of that range (Szymanski *et al.* 2016) (Figure 2).

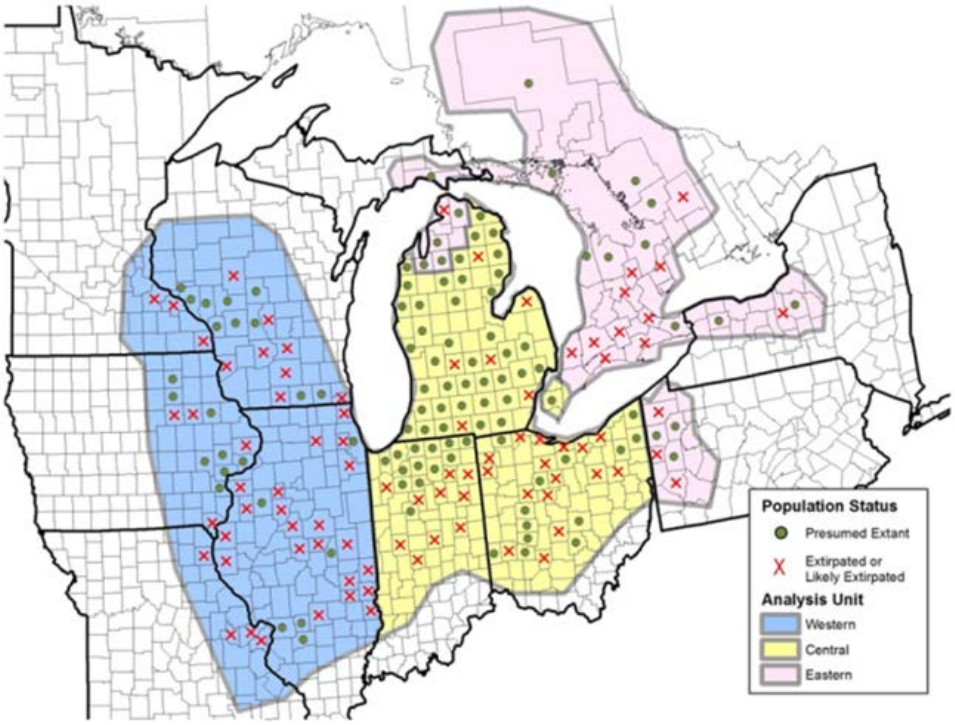

Figure 2. The geographical distribution of presumed extant (extant and unknown status) and extirpated EMR populations over the entire range and within each analysis unit (Szymanski *et al.* 2016). Green dots represent presumed extant EMR populations. Red Xs represents extirpated or likely extirpated EMR populations.

Threats
The most prominent threats include: habitat loss and fragmentation through development and vegetative succession; mortality of individuals as a result of roads, hydrologic alteration resulting in drought or flooding; persecution; collection; and post-emergent prescribed fire, mowing, and disking. Disease is a relatively recent threat with still unknown consequences. The effects of threats on extinction risk to EMR populations were included in model evaluations by Faust et al. (2011), while the Species Status Assessment (Szymanski et al., 2016) considered the total number of sites range wide where specific threats were reported.

Habitat Loss and fragmentation
The effects of past, widespread wetland loss continue to impact EMR populations. Development and agricultural practices continue to cause habitat loss, although to a lesser degree than in the past. Habitat loss increases the distance between populations and can isolate seasonally used habitats within individual populations, can restrict gene flow, and other effects of small population dynamics, as well as increase exposure to sources of mortality.

In addition, urban encroachment has disrupted the natural disturbance processes (such as hydrological cycles and fire frequency), and subsequently, changes in habitat structure and vegetative composition have occurred. Prolonged flood conditions may make wetlands too deep for use by EMR, while prolonged drought conditions may affect crayfish populations and thus reduce the number of suitable hibernacula (crayfish burrows) available for EMR.

Vegetative Succession
Woody succession, especially by introduced species such as Eurasian buckthorn, that results in EMR's preferred gramminoid (grasses, sedges, and rushes) dominated habitat becoming too shaded may reduce or eliminate these sites as suitable places for EMR to bask and thermoregulate. Unmanaged succession is now the most common risk factor, with 75 percent of the sites being impacted range-wide.

Post-emergent land management practices (prescribed fire, mowing, and disking)
The dependence of EMR on early to mid-successional stage gramminoid (grasses, sedges, and rushes) dominated plant communities necessitates that these communities be managed in a manner that controls woody species from dominating them. One of the most commonly used management techniques for this is prescribed fire, since it is a relatively inexpensive technique and mimics the natural fire processes that would have regulated these plant communities prior to European settlement. However, although EMR likely evolved in these fire dependant communities, direct mortality of EMR can result from exposure to fire if burning occurs when the snakes are out of their hibernacula (post-emergent fire) (Cross 2009; Cross *et al.* 2015; Dreslik 2005; Dreslik *et al*. 2011 *in* Szymanski *et al.* 2016)**.** In Missouri, Durbian (2006) observed the mortality of 8 western massasauga rattlesnakes on a 16.6 ha (41 ac) prairie after a burn conducted on April 18, 2000 (*in* Szymanski *et al.* 2016).

Mowing is another strategy often used in conjunction with prescribed burning, to control woody vegetation and invasive species encroachment. Durbian and Lenhoff (2004) postulated that pre-burn mowing may potentially reduce fire related mortality of EMRs and other snake species by negatively modifying the occupied habitat forcing the snakes to leave the area or seek refuge

below ground. Durbian (2006) subsequently found that pre-burn mowing at a height of 20 cm (8 in) resulted in the direct mortality of 3 of 7 radio-marked EMRs (*in* Szymanski *et al.* 2016). After the burn, 3 unmarked individuals in the burned area were killed by the fire itself indicating that a number of EMRs did not leave the site after mowing as hypothesized by Durbian and Lenhoff (2004 *in* Szymanski *et al.* 2016). Durbian (2006) concluded that mowing prior to burning results in additional direct mortality to EMRs beyond that incurred by prescribed burning and advises to conduct burns while EMRs are hibernating until methods that effectively reduce mortality while achieving the treatment objectives are identified (Durbian 2006 *in* Szymanski *et al.* 2016).

Road Mortality

EMR are more sedentary than other snakes and they also move more slowly which increases the probability of being killed while crossing roads (Andrews and Gibbons 2005).

Hydrologic alteration resulting in drought or artificial flooding

Individual populations of EMR often occur in riparian areas, wet prairies, or other places that are prone to fluctuations in hydrology. While EMRs are to a degree adapted to natural hydrological fluctuation, altered flood and drought cycles, or naturally occurring floods and droughts can have effects on EMR or the burrowing crayfish they rely upon for hibernacula. Prolonged flood conditions in a Missouri population of the western massasauga (*Sistrurus tergeminus*) led to changes in population and reproductive characteristics as well as an immediate affect on body condition (Seigel et al., 1999). Conversely, prolonged drought or drawdown conditions may affect water table and burrowing crayfish populations and thus reduce the number of suitable hibernacula (crayfish burrows) available for EMR.

Persecution / Collection

Persecution and collection of EMR are documented threats (Szymanski *et al.* 2016), with several populations having been collected beyond a recoverable threshold. Generally, people have a negative view of snakes and snake encounters frequently result in snake mortality. Poaching and the illegal collection of snakes for the pet trade is also a factor that contributes to the species decline. In Wisconsin, illegal collecting has been documented despite many years of legal protection (Christiansen 1993, Wisconsin Department of Natural Resources 2011). An Indiana Department of Natural Resources law enforcement investigation in 1998 uncovered a well-organized, multi-state effort to launder State protected reptile species (including EMR). The investigation concluded with the indictment of 40 defendants.

Disease

Snake fungal disease (SFD) is an emerging and significant threat to EMR populations (Allender *et al.* 2011). Recently, a growing number of snakes have been found in the U.S. with severe and often fatal fungal infections. The number of species of snakes with documented or suspected cases of the disease, and the geographic area the disease has been found, continues to increase annually. A causative agent, *Ophidiomyces ophidiicola* (formerly *Chrysosporium ophidiicola*) was first described from an Eastern Rat Snake (*Pantherophis obsoletus*) in Georgia (Rajeev *et al.* 2009). Five individuals from three sites in Michigan tested positive for SFD in 2013 and 2014 and two EMR were confirmed to be infected in 2015 in the Grayling area. Both died within a few weeks of capture. In the wake of the devastating impacts on amphibians due to Chytrid beginning in 1996

(caused by the fungus *Batrachochytrium dendrobatidis*) (Longcore *et al.* 1999), and White Nose Syndrome (caused by the fungus *Pseudogymnoascus destructans*) on bats beginning in 2005 (Gargas *et al.* 2009), there may be genuine cause for concern that the emerging fungal disease in snakes could have a significant impact on EMR populations.

Climate change
Climate change is one of several factors believed to be actively leading to declines in reptile populations (Gibbons *et al.* 2000). The EMR scored Highly Vulnerable to climate change in an analysis using the NatureServe Climate Change Vulnerability Index tool (Hoving *et al.* 2013). Poor dispersal ability, landscape barriers, and drought sensitivity all contributed to the highly vulnerable score (Hoving, unpublished). Another assessment modeled demographic rates under past and future climate scenarios. They found that past climate change explained the observed recent range contraction, and suggested that the range contraction would continue. Only populations in northern Michigan and Ontario were likely to persist to mid-century (Pomara *et al.* 2014). While these studies suggest that EMR populations in southern Michigan are not viable, there is some uncertainty about this prediction. Although additional models suggest drying in southern Michigan, nearly as many models suggest a wetter climate.

Conclusion
In assessing the occurrence of these threats or risk factors, Szymanski *et al.* (2016) found that 94% of EMR populations have at least one risk factor currently affecting the site. Habitat loss or modification is the most common risk factor occurring at 55% of the sites with 3 % of these sites at risk of total habitat loss. The second most common risk factor is fragmentation which occurs at 49% of the sites. Unmanaged vegetative succession is the third most common factor occurring at 31% of sites.

Among the other factors considered, road mortality occurs at 20 %, collection of persecution occurs at 17%, water fluctuation at 7 %, and pre or post-emergent fire at less than 1% of sites (Szymanski *et al.* 2016).

The risk factors most likely to push a population to quasi-extirpation within 25 years (high magnitude risk factors) are late-stage vegetative succession, high habitat fragmentation, moderate habitat fragmentation, total habitat loss, and moderate habitat loss or modification.

Environmental Baseline Conditions

The environmental baseline includes past and present impacts of all Federal, State, or private actions in the action area; the anticipated impacts of all proposed Federal actions in the action area that have undergone formal or early section 7 consultation; and the impact of state and private actions that are contemporaneous with the consultation process. The environmental baseline defines the current status of the species and its habitat in the action area to provide a platform to assess the effects of the action.

Historically and rangewide there were 558 known EMR populations. Currently, 266 of these are known to be extant, 211 are extirpated or likely extirpated, and 84 are of uncertain status (Szymanski *et al.* 2016). The EMR Species Status Assessment (Szymanski *et al.* 2016) grouped

the current number of extant rangewide EMR populations (263) with the current number of EMR populations of unknown status (84) and considered these populations in total as currently being "presumed extant". Therefore the total number of currently presumed extant EMR populations is 347 (263 + 84) (Table 1).

Table 1. The number of EMR populations by status within each analysis unit and rangewide. WAU = Western Analysis Unit, CAU = Central Analysis Unit, EAU = Eastern Analysis Unit (from Szymanski et al. 2016)

	Analysis Unit		
Status	WAU	CAU	EAU
Extant	18	189	56
Likely Extirpated	15	19	9
Extirpated	37	75	56
Unknown	2	67	15
Rangewide	72	350	136

The EMR has been extirpated from Minnesota and Missouri and from counties in every state across the range, and the Canadian Province of Ontario (Fig 2). The range of the EMR is divided into three analysis units (western analysis unit (WAU), central analysis unit (CAU), and eastern analysis unit (EAU)) (Figure 1). These three geographic "analysis units" correspond to broad scale genetic differences across the EMR range and represent areas of unique adaptive diversity. The EMR occupied spatial extent rangewide has declined by a northeasterly contraction in the range and by the loss of area occupied within each analysis unit. Overall, there has been more than 41% reduction in the extent of occurrence rangewide. This loss has not been uniform, with most of this decline occurring in the WAU (70% reduction in the extent of occurrence). However, losses of 33% and 26% in the CAU and EAU, respectively, are notable as well (Table 2).

Table 2. The percent of range falling within each analysis unit and the percent reduction in EoO from historical to present day. WAU = Western Analysis Unit, CAU = Central Analysis Unit, EAU = Eastern Analysis Unit, RW = Rangewide (from Szymanski et al. 2016)

	% Range within AU		
Analysis Unit	Historical	Current	% Reduction
WAU	27%	14%	70%
CAU	37%	42%	33%
EAU	35%	44%	26%
RW			41%

Of those range wide populations presumed extant, 139 (40%) are presumed to be quasi-extirpated (see SSA analysis Szymanski *et al.* 2016) while 105 (30%) are presumed to be demographically, genetically, and physiologically (DGP) robust (Redford *et al.* 2011 in Szymanski *et al.* 2016). Of these 105 presumed DGP robust populations, only 53 EMR populations range-wide are considered to be self-sustaining (Szymanski *et al.* 2016).

The greatest decline has occurred in the western analysis unit where only one population is considered to be self-sustaining out of 20 presumed extant populations (see SSA Szymanski *et al.* 2016). In the central analysis, only 47 populations are considered to be self-sustaining (see SSA Szymanski et al. 2016). The eastern analysis unit has only six self-sustaining populations (Szymanski *et al.* 2016).

Effects of the Actions

Effects of the actions refer to direct and indirect effects of an action on the species. Direct effects are those immediate effects of the proposed action on EMR and its habitat (50 CFR 402.02). When considering direct effects, interrelated and interdependent actions must also be considered. Interrelated actions are those that are part of a larger action and depend on the larger action for their justification. Interdependent actions are those that have no independent utility apart from the action under consideration. Indirect effects are those that are caused by the proposed action and are later in time, but are still reasonably certain to occur.

Although the proposed actions (below) are expected to have overall positive effects on the conservation status of the EMR, adverse effects to individuals or temporary adverse impacts to individuals or populations may unavoidably occur. In this Biological Opinion, the Service anticipates issuing permits or grants for EMR research and conservation activities listed in this section. This Biological Opinion excludes analysis on habitat management intended to benefit the EMR. A separate Biological Opinion will address that topic.

Take of federally listed species is differentiated based on its intent, i.e., purposeful or incidental. Purposeful take is take that is intended as part of the action (e.g. capturing EMR). Purposeful take is quantified here, based on the maximum amount that is expected to occur as part of the proposed actions. Incidental take is take that occurs accidentally during the performance of an otherwise lawful activity (e.g., injuring EMR during capture, or EMR that die unintentionally following capture). Incidental take is also quantified here to the extent possible and is further discussed in the incidental take statement.

Purposeful take in the form of pursuit, capture, and harassment is expected to occur for all captured EMR. Several of the types of studies that are most informative to conservation and enhancement of survival of the species rely on large data sets generated by capture, handling and collection of the data from individual snakes. “Harass” is defined by the Service as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering. “Harassment” will occur in all cases where wild EMR encountered during field surveys are then captured and handled for data collection, and in this Biological Opinion, harassment is usually expected to be a part or result of purposeful take. Because of this, we anticipate that a large

number of EMR may be subject to purposeful take (Table 3). Since the species became of conservation concern in the 1980's it has become one of the most studied species of snakes in North America, with well over 100 publications and gray literature titles appearing in that time. Experienced EMR researchers have established a number of successful protocols for surveys, including capture and handling techniques, that minimize stress and other risk of injury to individual EMRs (discussed below). Thus, purposeful take, where best practices and terms and conditions outlined below are followed, is not anticipated to result directly in injury or mortality to large numbers of EMRs. Nevertheless, some incidental take that does result in injury or mortality to EMR is anticipated, and both types of take are analyzed separately below.

Incidental take, in the form of injury or mortality, could occur as a result of capturing and handling individuals or accidentally crushing individual EMR for capture. Since the Act also stipulates that "parts" (including whole carcasses, skin, or tissues) of individuals of listed species are also prohibited from take, salvaging specimens (e.g., carcasses found on roads or in the wild) would also constitute purposeful take. Incidental take may also occur in the form of harm or harassment. "Harm," as defined by the Service includes significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering.

Field Surveys and Scientific Studies

Visual encounter surveys
VES result in no take, and thus do not require a 10(a)(1)(A) permit as long as EMR that are encountered are not captured touched, disturbed, nor their escape impeded in any way. Simply documenting (e.g., by photography) an individual EMR encountered during a VES does not represent purposeful take and would not require a permit as long as there are no additional efforts to draw or remove the snake from overlying cover, pose it for identifying or "better" photos, etc. Examples of the types of data that can be collected when EMR are not to be handled (Casper et al., 2001) include:

- Exact location (e.g., from GPS coordinates)
- Photographs of the animal without effort to pose it
- Size estimate, count of rattle segments
- General notes about health, distinctive scars, etc.
- Animal about to shed (evidenced by opaque eyes)
- Description of habitat
- Weather conditions, temperature
- Nearness to crayfish burrow or other potential retreat(s)
- Behavioral notes
- Detection methods used

For visual encounter surveys that do not require handling, no 10(a)(1)(A) permit is required unless additional more invasive procedures to document the animal or collect data (as described in the following subsections) are anticipated. Similarly, quantifiable take (either purposeful or incidental) is not likely by simply walking through occupied habitats. While we anticipate purposeful take in the form of capturing and temporarily holding (for data collection, etc.,)

animals found using VES, if animals are held and released in a manner as prescribed below, we anticipate low or discountable risk of mortality. However, we anticipate that incidental take (resulting from accidental injury or mortality) during capture, handling or data gathering, following all forms of encounter (see below) may total five snakes over a five-year period, rangewide (WAU = 1, EAU = 1, CAU = 3).

Road cruising surveys

Road mortality has been documented as a significant source of mortality for EMR in some areas (Shepard et al., 2008; Baker et al, 2016). We do not anticipate that this will be a widely used method for study of EMR, but rather that it would be used to augment data when researchers or managers drive on roads between or within sites that have EMR populations. Conducting surveys from the road could result in incidental (lethal) take of an EMR if the automobile involved in the survey drives over a snake on the road. However, because surveyors using this technique are more likely to be alert for snakes on the road, the likelihood of this is still far less than incidental take by motorists/cars already using public roads, and is thus considered to be of low risk. Take resulting from road surveys can be further minimized if multiple observers occupy the vehicle and if driving speed is appropriate to allow avoiding snake strikes. Handling snakes to immediately move them off of the road (and thus remove them from places where they could present a hazard to human health), was addressed in the Service's Final Rule to list the EMR (U.S. Fish & Wildlife Service 2016). In such situations, removing individual EMR from hazardous situations does not constitute take as long as the snakes are immediately moved to the adjacent habitat. Thus, road cruising surveys that do not require handling EMR beyond moving them from a road do not require a 10(a)(1)(A) permit. This would have an added benefit to the enhancement of survival of the species by helping to reduce or mitigate immediate risk of road mortality to the encountered animals. However, purposeful take would occur if EMR encountered on roads by researchers, resource managers, etc., are then held for any period of time (e.g., for data collection, marking, photographs, etc.) more than required to release them to adjacent habitat, or to measure or collect data from these animals. In these cases a 10(a)(1)(A) permit would be required. While we anticipate purposeful take in the form of capturing and temporarily holding (for data collection, etc.,) animals found using road surveys, we anticipate that these would result in fewer than 100 individual EMR being captured rangewide over a five-year period. If animals are held and released in a manner as prescribed below, we anticipate low or discountable risk of mortality. We anticipate that incidental take as a direct result of injury or mortality from researchers using road cruising surveys will total 10 rangewide (WAU = 2, EAU = 2, CAU = 6) over a five-year period.

Finally, whole carcasses, tissues, or other portions of individual EMR found dead on the road ("DOR") may be valuable for conservation purposes (for example for genetic samples, or for laboratory examination to confirm if individuals were marked as part of other study). However, possession of whole carcasses or parts of the animals salvaged from roads is prohibited by the Act, and thus would require a 10(a)(1)(A) permit (for more information, see "Salvage" below). Carcasses or parts of EMR that are salvaged by permit holders, but killed by other motorists are not considered in the incidental take estimates made directly as a result of actions considered in this Biological Opinion.

Coverboards

Use of coverboards to attract reptiles for later detection when they use the space underneath as a retreat is a standard survey technique (Fitgerald, 2012, Foster, 2012). When compared to other methodologies, cover board sampling is a relatively nonintrusive procedure. It is comparable to and can be used to effectively augment results of VES. When EMR are inventoried or monitored using the cover board technique, it is not necessary to handle individual snakes unless some other method that requires sampling from individuals is also part of the survey. Thus, like VES, cover board surveys do not result in take, as long as EMRs that are encountered are not captured, touched, disturbed, nor their escape impeded in any way. Simply using and lifting cover boards (whether they were present as pre-existing litter in EMR habitat, or deployed specifically to aid snake surveys) to survey for snakes, including EMR, does not constitute take and does not require a 10(a)(1)(A) permit. Similarly, documenting an individual EMR found under a cover board would not require a permit as long as there are no additional efforts to pose it for photography, etc. that result in touching the snake or impeding its escape. We anticipate no take, or discountable take by researchers who only use this method to detect EMR. However, any action by the field researcher subsequent to discovery of the snake that results in capture, handling, restraint, or impeding escape of an EMR can result in disturbance and harassment to the animals, and would thus be considered purposeful take requiring a 10(a)(1)(A) permit. While we anticipate purposeful take in the form of capturing and temporarily holding (for data collection, etc.,) animals found using coverboard surveys, if animals are held and released in a manner as prescribed below, we anticipate low or discountable risk of mortality. We anticipate that incidental take (resulting from accidental injury or mortality during capture, handling or data gathering, following all forms of encounter, see below) may total five snakes over a five-year period, rangewide (WAU = 1, EAU = 1, CAU = 3).

There also is some risk that surveyors or land occupants can step on cover boards and crush snakes and other animals, which could result in injury or mortality. A pole, trail blazing tape, or some other marker could be placed next to the cover boards to prevent accidentally stepping on it. An additional concern is that cover boards are sometimes placed in the field by potential poachers, or cover boards placed in the field by researchers may be found by poachers and animals could then be collected. Collecting or any unauthorized disturbance of individual EMR as described above would still be subject to prosecution as provided by the act. Thus, while using cover boards as a survey technique to augment detection of EMRs would not itself require permits, those using this method should inform the appropriate land owners or managers, and if necessary obtain local permits. Boards used in areas where EMR are known to occur should be placed away from trails or other places of high traffic by humans and from which they may be easily detected by curious people. Boards should be visited regularly, and removed if the person(s) responsible has reason to suspect that poachers may be visiting the boards, and appropriate conservation law enforcement agencies informed. Boards should be removed promptly upon conclusion of a survey period.

Capture and handling

Purposeful take in the form of capture and handling of wild EMR has been an essential aspect of most studies to date on the species. Capture and handling allows individual snakes to be marked, gender identified, measured/weighed, sampled for disease, have blood drawn or tissues collected for genetic studies, and in some cases determination of reproductive condition (Casper et al. 2001). When these data are gathered on individual snakes and they are marked (see “Individual

marking" below) and released for potential recapture at a later date, capture and handling allows estimates of survivorship, growth, and other vital rates that can be used to assess population structure, size and viability. Thus capture and handling for individual data collection is crucial for informing the continued conservation and enhancement of survival of the EMR. Capture and handling for scientific studies is likely to represent the most significant source of purposeful take of the EMR. We are currently aware of at least three long term studies that involve capture-mark-release-recapture of EMR. Following the Final Rule and current listing, additional such studies should be encouraged to ensure adequate monitoring at sites in each of the analysis units. Two ongoing studies have documented a rate of injury or mortality to be 0.001 or less directly resulting from capture and handling (Dr. Eric Hileman, Trent University, and Michael Dreslik, Illinois Natural History Survey, personal communications to M. Redmer, February 22, 2017).

Because EMR are venomous, and bites can be of medical significance, capture and handling of individuals presents additional risk (beyond that of non-venomous species) to researchers or others who may have need to handle them. Human health and safety should always be prioritized, and in order to ensure this with venomous snakes like the EMR, more specialized equipment, time, and concentration is usually required than that used to handle non-venomous species for data gathering. However, any such handling of EMR may also increase stress levels to individual snakes, potentially making EMR more susceptible to disease and infection. While mortality could occur from handling, the amount is expected to be discountable when practiced by trained individuals/experienced snake handlers, and using proper equipment. Appropriate precautions can be taken to reduce risk to both the snake and handlers.

The use of snake hooks should be restricted to lifting snakes by allowing body loops to loosely grip the hook. The popularly depicted method of "pinning" venomous snakes, where their heads and necks are pressed to the ground prior to handling could increase risk of injury or crushing the snake, and should not be used.

Use of modern animal handling tongs (e.g., Midwest Tongs, Inc., Gentle Giant ®; Stoney, Inc., Animal Equipment, Inc. or similar) that are engineered with pressure release springs, wide surface area jaws, and rubberized/padded coating should be used to grab snakes encountered in the wild. These designs ensure a secure grip while minimizing or even eliminating the risk of injury caused by crushing or cutting snakes when they are grabbed by experienced handlers.

Capturing or restraining snakes by using a length of clear plastic tubing to encircle the head and neck is a very popular method for restraining venomous snakes such as EMRs, especially in controlled or laboratory situations. This method is also used occasionally in the field though it can be much more difficult to use successfully when individual snakes are attempting to dart or escape into cover. This method involves encircling the head and neck of the snake with plastic tubing that is usually of a diameter slightly larger than the snake's head and neck, and then coaxing the snake to crawl into the tube. The handler can see when the snake has entered far enough (usually 25%-40% of the snake's body length) into the tube, and then can safely grab and handle the portion of the body remaining outside of the tube. Due to the small diameter of the tube, snakes are unable to turn around and bite the handler, thus allowing them to mark, draw blood from, anesthetize, swab, or otherwise examine the snake's body (see subsections below). When successfully practiced, use of clear plastic tubing is a very low risk form of handling for

both snake and handler. Researchers who use this technique often note that snakes appear to exhibit little indication of stress (for example they often stop rattling, biting, or resisting) while being handled, presumably because much of the snake's body is supported.

In most instances, EMRs removed from the wild for processing and data collection in ex-situ/laboratory situations shall be returned for release within 48 hours at the exact point of capture. Exceptions (for example if changes in weather conditions that would be hazardous to the researcher or the snake, finding of possible disease while the snake is being held) will be permitted on a case by case basis. When such situations arise, the permit holder holding the snake shall inform and work closely with the Service's Ecological Services Field Office(s) that cover the state(s) from which the snake was captured, and where it will be held (if different than where captured). When captured EMR are returned to the point of capture within 48 hours or less from the time at which they were captured, we anticipate the risk of lethal take to be minimal or discountable.

As stated elsewhere in this Biological Opinion, it may be beneficial and safer to both researchers and individual snakes if captured EMR are transported to a laboratory or other controlled environment for data collection and processing prior to release back to the wild. Once captured, snakes, including EMR are commonly placed into a cloth bag, the opening of which is then tied/knotted shut to secure the snake. Because snakes in cloth bags could be crushed, and because cloth bags are soft enough for EMR's to bite through the fabric, other precautions should be taken prior to transporting them to the laboratory. An example of a system that allows secure transport of live EMR a short distance from the field to laboratory devised by the Edward Lowe Foundation (Michael McCuistion, Edward Lowe Foundation, Vice President of Physical Resources, personal communication to Service Biologist M. Redmer, Feb 11, 2017) is described as follows. Following capture, and securely placing into and tying a cloth holding bag, the bag with snake is gently lowered into a five gallon bucket with securely closing lid, or other secure plastic container (e.g., "Rubbermaid®") with latching top. More than one snake can be placed into, and transported in such containers, but (1) small holes should be drilled to allow adequate ventilation, and (2) to avoid the possibility of agitated snakes biting/envenomating each other in the container, a piece of carpet remnant shall be placed between each bag, thus transporting the snakes in layers. No more than three snakes shall be transported in this manner. The outside of the container should be clearly labeled identifying the contents as venomous snakes/protected endangered species prior to transport on public roads. Care shall be taken that the hard containers are not exposed to direct sunlight or other conditions that may stress or kill EMRs contained within them. When captured EMR are transported in this manner we anticipate the risk of lethal take to be minimal or discountable.

Capture and handling of EMR in the field may be necessary to remove individual snakes from a hazardous situation. As stated in the Final Rule to list the species (U.S. Fish and Wildlife Service 2016), no permit is required for take when a listed species presents a hazard to human safety or property. Because the EMR is venomous, and individuals may enter human occupied areas (including a residence, yard, structure, sidewalk, road, trail, foot path, or campground), moving a snake from such a situation would not result in take and thus will not be prohibited. It is foreseeable that a field worker, trained nature center staff, or other individual familiar with EMR may be called upon to assist in removing the snake, and in turn may wish to collect data on

the individual as described herein. Brief handling required to remove a snake from a situation hazardous to humans or to the snake (e.g., to the nearest grassy habitat) would not require a permit, but any additional restraint or data collection taking longer than needed for release to a non-hazardous location would still require a permit. A study on translocated wild EMR indicated that individuals moved to adjacent habitat <200 meters form the point of capture did not return to capture locations, nor did they exhibit abnormal basking behavior (Harvey et al. 2014). When these conditions are met, we anticipate minimal or discountable risk of lethal take. In fact, if live EMR are successfully removed and translocated from a situation where they may otherwise present a hazard to human health (and this could be legally killed), such movements represent a net reduction in risk of lethal take.

We anticipate that studies rangewide will result in purposeful take of 400 or fewer EMR being captured and handled annually, or 2000 EMR captured and handled for data collection over a five-year period (WAU=200, EAU=200, CAU=1600). Based on rates of incidental take provided by two species experts involved in long-term studies involving capture and handling of wild EMR, mortality from incidental take will be nearly discountable. When EMR are captured and temporarily held and released using best practices described above, and following th Terms and Conditions provided below, we anticipate five or fewer individuals taken over a five-year period (WAU = 1, EAU = 1, CAU = 3).

Drift Fence Trapping

Because both funnel and pitfall trapping (and associated drift fencing) serve to capture animals that enter when they randomly encounter trap/fence arrays. An advantage to using these traps is that they are passive, and ensure encounters with animals that are captured within them. However, entrapment of EMR in either funnel or pitfall traps would represent take that would need to be permitted under section 10(a)(1)(A). In addition, animals that are unable to escape can also be subject to other sources of stress or mortality, for example due to exposure from heat or cold, or due to dehydration, or even flooding (if traps are set in flood prone areas during rain events). Other disadvantages of these types of traps are that, while not labor intensive to check, they are labor intensive to erect, they are typically not erected in ways that ensure complete coverage of a study site (thus many individual EMR that may be detected by other methods are missed when their movements are not intercepted by a fence). Drift fences and traps require deactivating when not in use. Drift fences may also contribute to mortality and incidental take because they are fairly non-selective, they do not prevent trapped animals from killing each other, or being killed by predators (e.g., black bears, raccoons, or coyotes) that may be able to remove captured animals from traps, or crush traps, exposure to weather can increase stress or mortality on captured individuals, and capture rate may be influenced by population sizes, activity levels, and size and nature of trap. All of these factors could increase the risk of lethal take to EMR that are captured in these traps. This may be reduced to a discountable, or low risk if researchers adhere to best practices, such as proving a source of shade or protection from rain and wind, providing a moisture source to help prevent desiccation of trapped animals, checking the traps no less than every 24 hours to remove captured EMRs, and removing or closing traps during periods when they cannot be checked at least once per 24 hours. While drift fences are widely utilized in surveys of snake communities we do not anticipate a high amount of purposeful take of EMR. Rangewide, we anticipate that drift fences and associated traps will result in the capture of 500 EMR rangewide (WAU=100, EAU=100, CAU=300) over a five-year

period of the permit. When best practices are used to minimize the risk of mortality to EMR captured in traps, we anticipate that no more than five EMR mortalities rangewide (WAU = 1, EAU = 1, CAU = 3) would occur over a five-year period as a result of drift fencing and trapping.

Wet pitfall traps (a dry pitfall trap containing a solution designed to trap, kill and preserve an animal or animals. Aqueous solutions used in these traps include; formalin (10% formaldehyde), alcohol, methylated spirits, trisodium phosphate and picric acid) are not considered in this biological opinion. Only dry pitfall traps are considered here. Due to the likelihood of lethal take at unpredictable numbers, wet pitfalls should not be used in areas known or suspected to have extant populations of EMR.

Individual Data Collecting

Because field studies using any of the above survey techniques often require handling to collect data from individual EMR for collecting data, a permit would be required whether the data were collected in the field or in a laboratory or other ex-situ environment. Measuring, weighing, photographing, and cloacal probing to determine the gender of individual snakes will be permitted in the field or in a laboratory or other *ex-situ* setting. Also parts (e.g., blood or other biopsied tissues used to study genetics, venom properties) may also be collected at the same time that individual data are gathered. Gathering these types of Veterinary/Health related data is addressed in the following sub-sections, below.

Regardless of the method used for capture, live individual EMR should be released and returned to their capture location within 48 hours from the day and time of actual capture. After capture, if EMR are returned to the point of capture within 48 hours or less, we anticipate the risk of lethal take from these forms of data collection to be minimal or discountable. As addressed above (see "Capture and Handling"), data collection, along with each of the methods with which EMRs may be captured and handled represent low risk of lethal take. Thus, when EMR are captured and temporarily held and released using best practices and Terms and Conditions provided below in this Biological Opinion, we anticipate a low or discountable risk of injury or mortality from incidental take related to collecting data on individual EMR. Release times exceeding 48 hours from time of capture would be extremely rare, and would be allowed only when conditions preclude release within this window (for example, if weather or field conditions are unsafe for personnel or snakes to be released, or if individual EMR are found to have symptoms of disease or pathology and veterinary care or additional exam is necessary). One other exception would be holding gravid female EMR in captivity in order to gather data on litter size or on individual neonate EMR then born in captivity to these females. Neonates born in captivity shall be released with the female at the point of her initial capture within 48 hours of birth. Circumstances that require holding wild-caught EMR in captivity longer than 48 hours should be documented in project proposals and also coordinated closely with the Service's Ecological Field Office(s) covering the area(s) where the project is taking place or where the snake will be held.

Veterinary Care

Instances may arise when EMRs held by permit holders may need veterinary medical care. In such instances, the permit holder would have discretion to seek veterinary care, and no additional permit would be needed by a licensed veterinarian to carry out medical procedures, up to and

including humane euthanasia, based on their professional medical judgement. In cases where a permit holder seeks veterinary care for an EMR they hold, they shall provide a copy of their permit to the veterinarian to maintain in their records. If the animal is left in the care of the licensed veterinarian, the permit holder shall document this by informing the Service's Ecological Services Field Office(s) that cover the site where the EMR was captured and/or where the animal will be housed to receive treatment. In most foreseeable circumstances where a licensed veterinarian is collaborating with or assisting a holder of a valid 10(a)(1)(A) permit, and where they would be attempting to treat the snake for a pre-existing condition, we do not anticipate lethal take as a direct result of their efforts. For example, if a wild-caught snake were presented in grave condition to a veterinarian, who then judged euthanasia as the best or most humane course of action, this would not represent take, because (1) purposeful take occurred when the animal was captured, and (2) the animal would have most likely died in due course. However, licensed veterinarians who actively conduct their own research, and use wild caught EMR, or captive EMR born following effective date of the final rule would still be required to obtain 10(a)(1)(A) permits for the snakes they may take and hold.

Prevention of Disease Transmission

Recent concern over the emergence of snake fungal disease in the EMR (Allender et al. 2011) has increased awareness for the potential to spread pathogens between populations. Taking precautions to reduce the risk of disease transmission may enhance the survival of the species. As a best practice, and precaution against disease transmission, researchers who work at multiple sites should disinfect their boots and field equipment (such as tongs, snake hooks, buckets, or snake bags) by soaking in or spraying with a 10% bleach solution, or laundering fabrics with bleach before traveling between field sites (Rzadkowska et al. 2016). We do not anticipate activities to disinfect equipment to prevent disease transmission between EMR populations to result in lethal take.

Prior to the Final Rule to list the EMR, there were circumstances (especially following initial information about snake fungal disease) in which wild EMR showing signs of pathology were taken and held under quarantine. Rationale for this was to research the pathogen, provide or experiment with potential approaches to veterinary support of the animal, treatment of the pathogen, as well as to reduce risk of spreading infection by returning them to the wild population. Approaches and decisions to take wild EMR into quarantine situations have also varied, with some states or land managers preferring to release exposed or infected snakes to track survival in the wild rather than hold them in quarantine. Snake fungal disease is still emerging as a pathogen of concern, and other novel diseases may be identified in the future. The relative importance of snake fungal disease to snake communities and prevalence in the wild are still poorly understood. For example, some individual EMR found in the wild that were swabbed and found to carry the causative fungus (*Ohidiomyces ophiodiicola*) had no signs of actual infection or pathology. Thus allowing states and researchers flexibility to decide when EMR should be placed into long-term quarantine (defined here as holding from 168 hours/7 days to an indefinite time) may have long-term value to informing the enhancement of survival of the species. There are several concerns with this. One concern is that when snakes infected with snake fungal disease are detected, they may be found in numbers. For example, in one season, nine individual EMR were found at a single Illinois population, and then held captive quarantine for up to several months or until they died (Dr. Matthew Allender, personal

communication to M. Redmer, February 15, 2017). In addition, in EMR that show symptoms of infection, snake fungal disease also has a high rate of mortality. Finally, over long periods of captivity, it is possible that individual snakes may lose recognition of their home range in the wild, or lose other behaviors needed to survive in the wild. Thus, placing wild snakes into quarantine may have the immediate effect of being considered lethal take. The amount of such take cannot be quantified at this time, however, so is not set in this Biological Opinion. To ensure that we achieve a greater understanding of this and other disease threats to the EMR, as well as to prevent additional transmission, the Service will permit long-term quarantine. However, if wild-caught EMR are to be held for periods much greater than 168 hours from the time of capture, permit holders and cooperating veterinarians or pathologists should contact the Ecological Services Field Office(s) that cover the state(s) involved. Upon learning of a need to hold EMR in long-term/indefinite quarantine, an activity-specific Biological Opinion should be prepared and appended to this Biological Opinion. Additional considerations in an appended Biological Opinion should include a rationale for why the snakes are to be held, a reporting schedule, establishing who will be responsible for the care of the individual EMR, and also a determination of the disposition of individual snakes to be authorized in the event they are cleared of the disease, or carcasses/parts thereof if they expire or are euthanized while in quarantine.

Nasal-Facial/Bodily Swabbing

Swabbing of the nasal cavities, heat-sensing pits, or bodies of EMR would require handling, and is expected to occur by personnel trained in appropriate sterile sampling and sample storage procedures. Since the first report of snake fungal disease in wild EMR (Allender et al. 2011) an increasing number of (though not all) EMR researchers have included swabbing as an additional portion of data collection from individuals. Thus, we do not anticipate that this action would result in purposeful take greater than that which would already occur from capture and handling as a result of other field research. Although EMR must be captured and handled, effects of this action are expected to be insignificant and discountable. Nevertheless, because individual EMR would be handled in order to acquire these samples, a 10(a)(1)(A) permit would be required for collecting samples. If compliant with methods described above, we anticipate minimal or discountable risk of lethal take from handling snakes to collect samples with swabs. Actual samples taken by swabbing these external surfaces of an EMR are not “parts” of a federally listed species. Thus, once collected no additional permit would be required for long-term storage, sharing, or shipping (including interstate) of samples (e.g., to study collaborators who may examine them in different laboratories).

Tissue collection

Collecting tissues, including blood, from EMRs can be useful for assessing health and/or exposure to diseases, studies of conservation genetics, paternity/maternity, isotopic studies of prey or surveys for environmental contaminants. In snakes, including EMR, tissues are usually sampled by biopsy through scale clipping, drawing blood from the subcaudal vein of the snake, or by salvaging whole carcasses or tissues from dead specimens found in the field or on roads. Taking blood from animals, if not done appropriately by a trained researcher could result in injury, hematoma, infection, or even death. Only licensed veterinarians or trained individuals, with experience drawing blood or collecting biopsies from snakes will be authorized to conduct these activities, therefore reducing the probability of injury (to live EMR). Sterile

procedures shall be followed. While in most cases lethal take from tissue collection is expected to be discountable if these conditions are met, we conservatively estimate lethal take totaling up to three EMR range wide (WAU=1, EAU=1, CAU = 1) could result from tissue collection over a five-year period. All forms of tissue collected from EMRs would constitute "parts" as defined by the act. Because of this, and because individual EMR would be handled in order to acquire these samples, a 10(a)(1)(A) permit would be required for collecting samples as well as for their long-term storage, sharing, or intrastate/interstate shipping to other individuals, including study collaborators.

Salvage

Whole or partial carcasses of EMR found dead in the field or on roads, or portions of shed skins from EMR can still be useful for inclusion in several types of studies (for example genetics) that can inform conservation of the species and should be collected. Because possession of "parts" of listed species is prohibited by the act, a 10(a)(1)(A) permit would be necessary for field workers to salvage these. However, shed skins from EMRs, or EMR carcasses found dead due to predation or natural causes, or found dead on roads due to being struck by motorists other than the permit holder would not represent lethal take resulting from their permitted actions, nor would these be included in monitoring of levels of take required of the Service's action (permit issuance). Researchers, land managers, or others permitted to salvage EMR parts found in the wild should clearly note the circumstances and disposition of the salvaged parts in their annual reports.

Individual Marking

PIT tags and microchips are usually injected (by syringe) subcutaneously into snakes, and thus could be detrimental by introducing external pathogens into the animal's body. However, studies to date have not found lasting detrimental effects of using PIT tags in snakes (Gibbons and Andrews 2004; Jemison et al. 1995; Keck 1994). The tags appear to be a safe and reliable method for individually marking snakes, including EMRs. Once inserted, a PIT tags can be scanned in a manner that allows identification without having to repeatedly disturb, capture and handle the animal. PIT tags circumvent identification problems sometimes encountered when trying to identify animals based on morphological characteristics. PIT tag frequencies last indefinitely, unlike other marking methods such as scale clips, which may fade over long periods of time. While some vertebrates are known to have the ability to expel PIT tags from the body, known incidences of this in snakes are very low (Jemison et al. 1995), with most known cases likely due to improper implantation (Gibbons and Andrews 2004). Such procedures can be stressful to snakes. Also, because injection by syringe can result in a small skin wound, sterile procedures and sealing the wound with veterinary bond glue should be used when injecting any type of transponder into a snake. If these precautions are taken, the risk of mortality from marking EMR with PIT tags should be minimal or discountable.

Painting rattle segments of EMR can be done with fast-curing fingernail polish, or permanent marking pen such as Sharpie® markers. Using different colors can allow a rattle to be coded and allow repeated visual recognition of individual EMR without having to repeatedly capture or handle the animals. Because both polish and marker ink wear off or fade within 4-6 months, this is considered a temporary marking method usually only useful within the active season in which the individual snake was initially captured. Also EMR may coil in a way that the rattle can be

concealed (thus necessitating some minor disturbance to see the rattle), and EMR also may break or otherwise lose rattle segments within the season, which can reduce the value of this technique. The incidental take resulting from injury or mortality caused by this method is expected to be minimal or discountable.

For snakes, scale clipping involves using a fine cuticle scissors to cut one or more notches in ventral scales, using an alphanumeric code dependent on the number and position (usually counting posterior to anterior starting at the first scale in front of the snake's cloaca) of the scale(s) clipped. However, ventral scales can wear due to continuous contact with the ground, and also because they continue to grow out with the clipped notches healing throughout the life of the snake. Thus, scale clipping is considered to be a temporary marking technique (usually lasting only 2-3 active seasons). In addition, for scale clips to be evaluated subsequently to the initial encounter and time the scales were clipped, EMR must be captured and handled. While effects of scale clipping are expected to be minimal to discountable, they are not without some risk. Increased winter mortality and weight loss have been observed in some scale-clipped snakes (Parker 1974). Use of topical disinfectants to sterilize the scales before clipping, or use of cautery pencils following the clip may reduce this risk. While in most cases incidental take resulting from injury or mortality due to scale clipping is expected to be discountable if these practices are used, we conservatively estimate up to three EMR injuries or deaths rangewide could result from this practice over a five-year period(WAU=1, EAU=1, CAU=1).

Dorsal saddle pattern recognition is similar to fingerprinting, and involves photographing EMR and noting unique shape of their individual saddles (the brown "spot" or color pattern on the backs of the snakes). Within populations, saddle patterns are variable enough between individual EMR that future recognition is possible by noting the sequential number (usually by counting anterior to posterior, starting at the first saddle behind the head) and describing the shape of saddles that are of atypical shape. The snake and atypical saddles are photographed, and maintained in a database along with standardized/unambiguous (and thus searchable) written descriptions of the atypical saddles. This allows photographic comparisons to determine if individual EMR within a study population were previously captured. Dorsal saddle pattern recognition is used by a number of EMR researchers, and has proven useful for identifying individuals over complete lifetimes (Dreslik, personal communication, January 11, 2017). While this method has the benefit of not requiring actual capture, it is usually used in conjunction with at least an initial capture and collection of data on the individual snake. Similarly, subsequent captures are not required (as long as a photograph showing saddle patterns can be acquired), though there may be reasons (e.g., updating measurements) to capture the snake during subsequent encounters. While individual EMR may experience stress due to handling and gentle posing to optimize the photograph, risk of incidental take caused by injury or mortality from photography is expected to be discountable.

Some EMR researchers prefer to use more than one marking technique to ensure redundancy and allow identification in the event that one method fails over time (e.g., rattle marking or scale clips becoming less visible with age). Because this provides the most robust approach to marking EMR used in studies of wild snakes, we encourage this practice in issuance of permits that allow marking.

Radiotelemetry

Radiotelemetry studies, especially using internal (surgically implanted) transmitters have been used extensively in studying the EMR, and other snakes, usually with the assumption that implanted transmitters have no effect on the study animal (Lentini *et al.* 2011). However, despite the efficiency of the internal method, the surgical intervention results in stress and the risk of injury, exposure to infection, decreased feeding and increased mortality (Lentini et al. 2011; Lutterschmidt and Rayburn, 1993; Plummer, 1990). It has also been documented that EMR receiving surgically implanted transmitters experience a significantly higher mortality rate than that for marked snakes without implanted transmitters encountered repeatedly (Baker et al. 2016) over the course of a study. It has also been documented that surgery can increase the risk of infection and implanted transmitters may affect snake behavior (Lentini et al. 2011). Furthermore, in some cases, the implantation of transmitters may have undesirable physiological consequences in the animals, thus negatively interfering in the reproductive process. In addition, these potential health affects also raise questions about the validity of the behaviors that are studied using this technique (Lentini et al., 2011). Thus, potential health risks associated with future use of this technique to research EMR should be carefully weighed with the benefits of such studies. We suggest minimizing research involving surgical implantation of radio transmitters to reduce risks of surgical injury and mortality. Research questions resulting in the need to conduct studies with radiotelemetry should be crucial to furthering understanding of recovery needs of the species, and not driven solely by site-based or locally based questions, and should also be weighed carefully against the risk of mortality (Baker et al. 2016). The number of surgically implanted transmitters will be determined by the analysis unit (western, central, eastern) where the research would occur. Surgical implantation of radio transmitters should be done by licensed veterinarians using sterile procedures. Only *ex-situ* surgical facilities should be used to conduct surgical implantation of radio transmitters in EMR. When surgically implanted radio transmitters are used, adequate recovery times (up to seven days) should be planned for close post-surgical evaluations and to ensure proper healing of the incision site and sutures before releasing the snake.

Externally attached transmitters (held on with glues or elastic bands) have been used with mixed success to study the EMR. One study used externally attached transmitters (using super glue, or one of several very similar veterinary suture glues) to successfully study the movements of neonate EMRs (Jellen and Kowalski 2007). However, other attempts to attach transmitters externally, especially to adult EMR, have resulted in frequent detachment of the transmitters (M. Redmer, FWS, personal observation; Dreslik, personal communication, January 11, 2016). Externally attached transmitters also fall off when snakes shed their skin, which may occur three or four times (depending on growth rate of the snake) during a typical six month active season. One adult male EMR with an externally attached transmitter shed its skin twice in less than four weeks, though it had not gained mass. When shown to and discussed with a cooperating veterinarian, it was suggested that veterinary bond glue (similar to super glue) may have triggered premature shedding due to irritation, and as a precaution the transmitter was not reattached (M. Redmer, U.S. Fish and Wildlife Service, personal observation, May 2008). In addition, whip antennae used on transmitters can become entangled in vegetation and can restrict movement and limit access to narrow burrows, again compromising the health and life of individual EMR.

Both approaches to affixing/implanting radio transmitters to EMR involve health risks to individual snakes. For example, 33% of a captive group (n=12) experimentally implanted with radio transmitters showed inflammatory response including bacterial infections at the surgical site (Lentini et al. 2011). We thus anticipate that mortality/lethal take may result from studies that use radiotelemetry. Any loss of individuals resulting from infection or other complications of surgery or carriage of transmitters would be counted against take limits for analysis units. We anticipate requests to use up to 50 EMR annually in radiotelemetry studies rangewide (EAU=10, CAU=40). We do not anticipate any requests for permits to conduct radiotelemetry studies in the WAU for two reasons. First, the only self-sustaining population (as defined by Szymanski et al. 2016) in the WAU has been the subject of extensive spatial ecology studies (Dreslik, 2005), and recently the research group who works there self-imposed a moratorium on further use of radiotelemetry at that population (Baker et al. 2016). We thus estimate that incidental take of up to 20%, or ten EMR rangewide (WAU=0, EAU=2, CAU=8) annually (or 50 total over a five-year period) could occur as a direct result of radiotelemetry or surgical procedures to implant transmitters. Radiotelemetry studies on EMR populations within the WAU would not be precluded, but potential permit applicants should coordinate early with their state conservation agency as well the Service's Ecological Services Field Office(s) that cover the state(s) in which they would conduct the study. If the study is to be approved, an activity specific Biological Opinion will need to be prepared and appended to this Biological Opinion.

While we will permit the use and surgical implantation of radio transmitters for such studies to help inform knowledge gaps on the species, a number of best practices, and checks will be required to ensure incidental take is adequately monitored and minimized. Thus, we will require that surgical implantation of radiotransmitters into EMRs be specifically mentioned in a 10(a)(1)(A) permit before it can take place. These include:

- Surgical implantation shall be conducted by licensed veterinarians who have experience with sterile surgical procedures for reptiles.
- Animals shall be monitored in a captive setting for not less than 120 hours following surgery before they may be released.
- Transmitters shall not exceed 5% of body mass of the snake.
- Regular monitoring (at least twice weekly) of radiotelemetered snakes will be required, and shall include examination for signs of infection or declining body condition, unusually high amounts of basking (to warm the body temperature possibly in response to infection).
- If any of the above are observed, the animal should be removed from the field for veterinary evaluation and care.
- In cases of mortality where all or portions of the carcass can be retrieved, a necropsy by a qualified veterinary pathologist will be required to determine if death was a result of internal infection, decline in body condition, or other symptoms that may indicate that the transmitter, or procedures used for attachment/implantation contributed to death.

Controlled Propagation and Management of Captives

EMR that were held prior to the date when listing became effective (October 31, 2016) are not unlawful to possess. An EMR currently in a display at a visitor center, or held in a zoo does not

need a permit as long as it wasn't collected post-listing. However, progeny born in captivity after October 31, 2016 are protected as if born in the wild, therefore captive propagation programs must be permitted by a 10(a)(1)(A) permit.

Captive Maintenance and Propagation
The Service and National Marine Fisheries Service have a stated policy regarding controlled propagation of federally listed endangered or threatened species (U.S. Fish and Wildlife Service and National Marine Fisheries Service 2000; "Propagation Policy"). The scope of the Propagation Policy includes:

- Establishing refugia or assurance populations.
- Producing individuals for research and technology development needs.
- Producing individuals for supplementing extant populations.
- Producing individuals for reintroduction into suitable habitat within the species' historic range.

Controlled propagation refers to any of the above actions when used to enhance the survival of the EMRs through propagation of individuals held in captivity.

The Propagation Policy (U.S. Fish and Wildlife Service and National Marine Fisheries Service 2000, p. 56917) also encourages the Service and others to follow as practical the protocols and standards of the Association of Zoos and Aquariums (AZA), and other appropriate organizations.

The Service works closely with the AZA's "Eastern Massasauga Rattlesnake Species Survival Plan (EMR SSP)". The EMR SSP is a collaborative management program of the AZA. Within AZA (the governing and accrediting body of most major zoos in North America), SSP species like the EMR are considered "flagship species." Functions of all SSPs, including the EMR SSP include:

- Development of oversight of a studbook and database of the species pedigree in the managed captive population, including data on animals held in non-AZA facilities but shared voluntarily by the holder.
- Establishing management, research, and conservation priorities.
- The development of an annual report and breeding transfer plans.
- Increasing public awareness of wildlife and conservation issues, including development of ex-situ and in-situ education strategies.
- Development of in-situ reintroduction programs if needed.
- Serving as an AZA expert group and providing a discussion forum for topics applicable to the species.
- Providing species-specific/taxon specific care for AZA animal care manuals.

In addition to producing its annual report and breeding and transfer plan (most recent version prior to this Biological Opinion is Jundt et al. 2016), the EMR SSP has produced a manual for standardized care of the species within AZA facilities (Lentini, 2010), and has also identified and developed crucial conservation related *in-situ* and *ex-situ* field research. This included studies into population characteristics, dietary needs, a study of stress hormones, assessment and

identification of healthy body conditions of captive and wild EMRs, and disease risk management for the EMR. Many of the activities of the EMR SSP will require it, and/or individual member institutions or their non-AZA collaborators to obtain 10(a)(1)(A) permits as described for topics already listed above. Also, because EMR SSP member institutions hold and actually prioritize captive breeding within the AZA population of EMRs, and because progeny are protected as if born in the wild, captive propagation programs must be permitted by a 10(a)(1)(A) permit. The EMR SSP also cooperates on a case by case basis with non AZA facilities (for example Nature Centers that are run by local units of government) that hold captive EMR, usually for outreach purposes.

Permits issued for the EMR SSP would include permission for intrastate, interstate, and international (e.g., to and from Canadian Zoos) breeding transfers of snakes born into the population following the Service's Final Rule to list the species. Transfers between facilities of adult animals held prior to the EMR listing would not require a 10(a)(1)(A) permit because lawfully taken and held endangered and threatened species may be shipped interstate as a bona fide gift or loan if there is no barter, credit, other form of compensation, or intent to profit or gain. A standard breeding loan, where no money or other consideration changes hands but some offspring are returned to the lender of a breeding animal, is not considered a commercial activity and, thus, is not prohibited by the Act, and does not require a permit. Documentation of such an activity, and a statement of origin of history of individual EMR (including gender, any externally identifiable markings, PIT tag/microchip frequencies, etc.) legally held prior to listing, but transferred for captive propagation should accompany shipment. Placement of wild caught animals into captive management must be authorized by a 10(a)(1)(A) permit (see below).

Mortality in the captive population is anticipated. This may be a result of circumstances beyond the control of facilities permitted to hold captive EMR, even when following standardized care measures recommended by Lentini 2010). Such cases could be the result of natural senescence or death of older individuals, from natural disease factors (e.g., tumors, complications from ectopic pregnancies), or novel pathogens. Mortality within the AZA population has fluctuated between 4% and 31% since the inception of the EMR SSP. Based on the SSP's goal of maintaining a population of at least 100 EMR, we anticipate up to 31 deaths per year. However, injuries or deaths of captive EMRs resulting from circumstances beyond the control of permit holders or which occurs due to natural conditions is not considered to be incidental take. Under rare circumstances permits may also be issued to non- AZA facilities to accept and house new captive EMRs (for example, if they are confiscated by law enforcement actions, see below), though in such cases the facilities should communicate with the EMR SSP so that the fate of the individual captive snakes can be tracked. Also, if captive snakes held outside of the SSP population prove to be of valuable pedigree or if they may have originated from a poorly represented genetic grouping (e.g., the Western or Eastern Analysis Units), their placement into the SSP population for potential captive breeding may be valuable to the enhancement and survival of the species. On occasion the SSP holds surplus animals of well-represented genetic lineages. Prior to the Final Rule, non-AZA facilities have expressed willingness to donate or place their animals on breeding loan into the SSP, in exchange for loan of a surplus animal that could be used as a replacement for use in local outreach. Such transfers would be permissible and would require no 10(a)(1)(A) permit if the exchanged snakes were held prior to the Final Rule to list the EMR. In the event that one or both individual animals were acquired following

the Final Rule to list the EMR, then both facilities should hold valid 10(a)(1)(A) permits prior to the exchange taking place.

Placement of Surrendered Captives into Captive Management
Legally maintained captive EMRs, as well as illegally maintained captive EMRs confiscated by law enforcement, but that cannot be released to the wild (due to unknown origins) could provide opportunities to diversify genetic material in captive breeding programs or could be useful for display in public outreach situations. Both uses have value to efforts to enhance the survival of the species. In cases where individual EMR are placed into captive populations or used for display, no 10(a)(1)(A) permit would be required for animals held legally prior to the Final Rule taking effect. However, animals that were held illegally, or the offspring of animals held legally prior to the listing, but born afterwards would require issuance of a 10(a)(1)(A) permit. The number of legally or illegally held EMR that may be placed in a permitted captive program is not possible to predict, though in one well-publicized case, 33 EMR collected from the wild in Canada were seized during a law enforcement action in New York. The offender cooperated with law enforcement agents who were able to return the snakes to the location of origin, but if he had not cooperated, these snakes would have likely been placed into the captive population. However, because placement of captive animals into a permitted captive population would not represent the actual take, this is not considered in our analysis of effects of the action.

Collection and Removal from the Wild for Captive Breeding
In rare circumstances, individual EMRs may be collected from the wild in order to enhance survival. For example, if best available data from monitoring of an individual population demonstrates that it has declined to such a small size that it is unlikely to recover on its own, remaining individuals may have value for enhancing survival of the species if placed into captive management. Such attempts to remove the few remaining individuals would require 10(a)(1)(A) permits. This would also be contingent on independent review of the data and additional permission by state and local land management agencies or landowners, as well on as availability of an AZA or other appropriate facility to receive individual EMR into a captive program for propagation or educational purposes. Because the number of animals that would be taken into captivity cannot be predicted, we have not set a limit on this specific type of action in this Biological Opinion. Instead, when situations arise where states, local stakeholders, landowners, and researchers demonstrate that a population has dropped to critically low numbers and there is a desire to salvage some of the final few individuals, they should contact the Ecological Services Field Office that covers the site in question. An action-specific Biological Opinion should be prepared and appended to this Biological Opinion prior to permit issuance.

Population Augmentation and Reintroduction
This includes use of EMR propagated and reared in captivity, or collected from the wild and translocated to augment wild populations, to reestablish populations at sites where the species has been extirpated, or to potentially attempt to establish new wild populations. Because the EMR is a venomous snake, we anticipate any such effort would be controversial. Similarly because collection and removal of wild individuals of listed species would also be controversial, both approaches would likely be attempted only under rare circumstances. This would also be contingent on agreement and additional permits issued by the state conservation agencies, as well as agreement from local land management agencies or land owners having authority over sites

where this would occur. Previous attempts to release EMR reared in captivity have met with mixed results (King et al 2004; Harvey et al. 2014), but did provide information on the viability of this method and suggestions for future direction, so any additional such attempts should continue to follow an experimental approach. If stakeholders agree that such approaches are worth attempting in an effort to enhance or inform better understanding of management needed to enhance survival of the EMR, a 10(a)(1)(A) permit would be required. In cases where individual EMR already held in captivity would be used for population augmentation or reintroduction, this would not represent lethal take from the wild. In cases where translocation is to be attempted, each instance where an individual EMR is captured from the wild and moved to new locations would represent lethal take to the population where the animals originate, and would need to be quantified at that time. When situations arise where states, local stakeholders, landowners, and researchers demonstrate a valid need to attempt to augment or establish a population by translocation, they should contact the Ecological Services Field Office that covers the site in question. An activity specific Biological Opinion should be prepared and appended to this Biological Opinion prior to permit issuance.

Research Involving Captives

Research using captive EMR could provide data that is useful for the enhancement of survival or to inform recovery of the species. Use of individuals held in captivity as surrogates may be useful in a number of potential types of studies of EMR biology that that may not require wild individuals, or could be used to augment data gathered from studies of wild individuals. Examples include studies of the physiology, metabolism, reproductive cycles, or reproductive behavior of captive EMR, or pathology and disease management, all of which could inform conservation of wild EMR populations, and thus contribute to enhancement of the survival of the species. Use of captive EMR held prior to when the final rule to list the species took effect would not require a 10(a)(1)(A) permit, but research using progeny born in captivity after the final rule to list the species would require a 10(a)(1)(A) permit. Unless mortality to EMR held under a 10(a)(1)(A) permit is expected as a result of the proposed research, we anticipate that lethal take resulting from research using captive EMR would be minimal or discountable.

Summary of Effects of the Proposed Action

The effects due to the proposed actions, discussed above, may result in harassment, reduced fitness, injury, or in rare cases, mortality of individual EMRs. Adverse impacts may result from conducting surveys for wild EMRs, capturing and handling them for collection of data (such as size, mass, body condition, gender, reproductive condition), collection of blood or tissue samples, marking for individual recognition, or implanting/attaching radio transmitters for studies of spatial ecology. Most take would be purposeful and temporary, with the intent to release individuals back in the wild. All of the reviewed actions (above) would be for scientific studies that could inform conservation and enhancement of recovery of the species. Thus, while issuance of 10(a)(1)(A) permits for the actions reviewed in this Biological Opinion may have some adverse effects to the EMR, there will also be a net benefit realized from the information provided by the actions themselves.

Cumulative Effects

Cumulative effects include the effects of future state, local, or private actions that will not be subject to section 7 consultation in the areas being considered in this Biological Opinion. Future Federal actions that are unrelated to the proposed action are not being considered in this Biological Opinion, since they would require a separate consultation pursuant to section 7(a)(2) of the ESA, as amended. Throughout its range, the EMR persists mostly in small isolated populations (Szymanski et al. 2016) which are vulnerable to environmental and demographic stochasticity, the identification and mitigation of threats is critical for their conservation. This should be considered when analyzing cumulative effects in other Biological Opinions.

Conclusion

Regulations define "jeopardize the continued existence of a species" as "to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of that species." After reviewing the current status of EMR, the environmental baseline for the action area, the effects of the proposed action, and the cumulative effects, we conclude that the proposed action is not likely to reduce reproduction, numbers, or distribution of EMR to such an extent as to reduce appreciably the likelihood of survival and recovery of the species. Certainly, some purposeful and incidental take of EMR is expected as a result of research and recovery related activities. The proposed actions are for the purpose of species recovery and will provide significant benefits for the species. It is the Service's biological opinion that the proposed action will not jeopardize the continued existence of EMRs.

Incidental Take Statement

Section 9 of the ESA prohibits the take of endangered species without special exemption. These prohibitions have been extended, by regulation, to threatened species. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to engage in any such conduct. Harm is further defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering. We define harass as intentional or negligent actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering. Incidental take is defined as take that is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity. Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking under the ESA, provided that such taking is in compliance with this Incidental Take Statement.

<u>Amount or extent of take anticipated</u>

The amount of purposeful and incidental take anticipated over a 5-year period due to activities authorized under section 10(a)(1)(A) permits is summarized in Tables 3 and 4.

In this Biological Opinion, most purposeful take of EMR would be a direct result of some method of capture, including handling and data collection, and may include a brief period of maintenance in captivity prior to release (usually within 48 hours of the time of initial capture).

Harassment (for example by posing a wild EMR for photos to be used for recognizing individuals) may also constitute purposeful take. We anticipate a maximum of 2650 individual EMR would be subject to some level of purposeful take from actions reviewed in this document, over a five year period.

Since before becoming a candidate for Federal listing in 1999, the EMR has been the subject of many field studies, and experts familiar with it have devised and used a number of methods to increase safety to both handlers and study subjects when the species is handled so that data on individual snakes could be gathered. When established best practices are used to capture and handle EMRs, most of the methods considered here would have discountable or low risk of incidental take resulting from injury or mortality to the animals involved. Data from additional population-based monitoring would be beneficial to better understanding trends in wild EMR populations. Thus, the anticipated numbers of purposeful take shown in Table 3 are considered to represent a "best case" scenario, or one in which robust, long term monitoring and studies of EMR are taking place across the species range in the United States. Due to lack of resources or other constraints, the numbers of studies that would result in the amounts of purposeful take, and provided in Table 3, may not be quickly realized in the first five years after listing.

In this Biological Opinion, most anticipated risk of incidental take would be a result of unintentional/accidental injuries or mortality occurring during or following purposeful take, for example, if a snake is accidentally injured during capture, or while being held briefly in captivity while awaiting collection of individual data or subsequent release.

This Biological Opinion also considers the possibility that incidental take may occur from injury or mortality of EMRs propagated in captivity after October 30, 2016, when the Final Rule to list the species took effect. At the time of this Biological Opinion (approximately 140 days following the Final Rule taking effect) no such "post-listing" captive progeny are known. Most incidental take of effected captive EMR would be a result of natural senescence, or other natural causes. Though injury or mortality due to accidental causes could occur, the risk of this is minimal or discountable, nor is it applicable to analysis of recovery of wild populations. We expect periodic proposals to use legally held captive EMR in research projects that could be informative to conservation and enhancement of the species survival. Unless there is reason to suspect such projects could result in incidental take from injury or mortality, the risk of this is considered to be minimal or discountable.

The anticipated purposeful and incidental take could not be estimated for several actions considered in this Biological Opinion, including long-term quarantine of wild-caught EMR for either prevention of disease transmission, placement of wild EMR into captive populations, or use of translocated EMR from one wild population to augment another. Because this Biological Opinion is programmatic in nature, and because scenarios under which each of these actions would occur are expected to vary, when those situations arise they should be addressed with action-specific Biological Opinions that can then be appended to this document.

Incidental take is expected in the form of harassment, injury, or mortality by actions incidental to the proposed permitted/funded actions. In order to determine the number of individual EMR that can be "taken" from a population before the "take" will negatively impact that population

(meaning when the take would pose a risk of extinction to the population) ideally we would need to know how many individuals are in the population. We have assumed in the SSA analysis that a self-sustaining population consists of 100 individuals, therefore we assume that those populations determined to be self-sustaining support 100 individuals.

Thresholds where EMR population dynamics become unstable leading to an extinction vortex by 100 years have been estimated at 20% (Seigel and Sheil 1999, Middleton and Chu 2004). Because disease and other stochastic events, as well as other causes of incidental take not quantifiable here may also affect population recovery following a known loss of 20%, we believe that no more than 20% of a self-sustaining population can be taken over a five-year period from permits issued under this Biological Opinion.

Our Species Status Assessment analysis estimated that there are 54 self-sustaining EMR populations rangewide: one self-sustaining population in the WAU, 47 self-sustaining populations in the CAU, and 6 self-sustaining populations in the EAU (Szymanski *et al.* 2016). Because the numbers of self-sustaining populations within each analysis unit differ, the amount or extent of take anticipated within each analysis unit will also differ.

The WAU supports one self-sustaining population of 100 individuals. We estimate that 20% of the population, or 20% of 100 individuals can be taken before an extinction risk to that population would become evident. Twenty percent (20%) of 100 (0.2 X 100) individuals is 20 individuals, therefore only 20 EMR individuals may be lost from incidental take in the WAU over a five-year period.

The CAU supports 47 self-sustaining populations with 100 individuals in each of these populations, therefore we believe this unit supports 4700 (47 X 100) individuals. We estimate that 20% of 4700 EMR individuals (4700 X 0.2 = 940) or 940 individual EMR may be lost from incidental take in the CAU over a five-year period.

The EAU supports 6 self-sustaining populations with 100 individuals in each population, therefore the EAU supports 600 (6 X 100) individual EMR. Twenty percent (20%) of 600 EMR individuals (0.2 X 600 = 120) is 120 individuals, therefore we estimate that 120 individual EMR may be taken from the EAU over a five-year period.

We anticipate that incidental take, resulting from injury or mortality of no more than 78 (6 + 16 + 56) EMR will occur rangewide over a five-year period as a result of the activities addressed by the proposed action. This take will not exceed 16 individuals in either the WAU, 940 individuals in the CAU, and 120 individuals in the EAU.

Table 3. The maximum anticipated amount of purposeful take of eastern massasauga rattlesnakes over a five-year period as a result of actions considered in this biological opinion, by analysis unit and rangewide. WAU = Western Analysis Unit, CAU = Central Analysis Unit, EAU = Eastern Analysis Unit (from Szymanski et al. 2016); N/A = purposeful take not applicable to action unless the actions "Capture, handling, data collection" or harassment takes place; "Append new BO" = a separate Biological Opinion would be required on an action by action basis, and appended to this programmatic Biological Opinion.

	ANALYSIS UNIT/PORTION OF RANGE			
ACTION	WAU	EAU	CAU	RW
SURVEYS				
Visual Encounter Surveys	N/A	N/A	N/A	N/A
Road Cruising Surveys	20	20	60	100
Cover Boards	N/A	N/A	N/A	N/A
Drift Fence trapping	100	100	300	500
Capture, handling, data collection[1]	200	200	1600	2000
VETERINARY CARE	N/A	N/A	N/A	N/A
Long-term Quarantine	Append new BO[2]	Append new BO[2]	Append new BO[2]	Append new BO[2]
Nasal-Facial/Bodily Swabbing[1]	200	200	1600	2000
Tissue/Blood Collection[1]	200	200	1600	2000
Salvage	N/A	N/A	N/A	N/A
Individual Marking[1]	200	200	1600	2000
PIT Tag/Transponder injection[1]	200	200	1600	2000
Marking rattle segments[1]	200	200	1600	2000
Scale clipping[1]	200	200	1600	2000
Photographic recognition[1]	200	200	1600	2000
Radiotelemetry	Append new BO[2]	10	40	50
CONTROLLED PROPAGATION/CAPTIVE MANAGEMENT				
Within AZA EMR SSP Population	N/A	N/A	N/A	N/A
Placement of captives into permitted captive program	N/A	N/A	N/A	N/A
Collection from wild for placement into captive program	Append new BO[2]	Append new BO[2]	Append new BO[2]	Append new BO[2]
Population Augmentation				
Using captives	N/A	N/A	N/A	N/A
Using translocated wild EMR	Append new BO[2]	Append new BO[2]	Append new BO[2]	Append new BO[2]
Research Using Captives	N/A	N/A	N/A	N/A

[1]For purposes of this Biological Opinion, the maximum number of EMR that we anticipate would be captured and handled for data collection over a five-year period is assumed to be the same number that would be used for tissue/blood collection, swabbing for external pathogen detection, and marked for individual recognition using any/all methods, thus maximum amount of purposeful take is not summed for all actions.

[2] Activities to be considered on a case by case basis and through separate consultation and Biological Opinion appended to this one.

Table 4. The anticipated amount of injury or mortality from incidental take of eastern massasauga rattlesnakes over a five-year period as a result of actions considered in this biological opinion, analysis unit and rangewide. WAU = Western Analysis Unit, CAU = Central Analysis Unit, EAU = Eastern Analysis Unit (from Szymanski et al. 2016); N/A = quantified lethal take lethal take not applicable to action; "Append new BO" = a separate Biological Opinion would need to be required on an action by action basis, and appended to this programmatic Biological Opinion.

	ANALYSIS UNIT/PORTION OF RANGE			
ACTION	WAU	EAU	CAU	RW
SURVEYS				
Visual Encounter Surveys	None/ Discountable	None/Discountable	None/Discountable	None/Discountable
Road Cruising Surveys	2	2	6	10
Cover Boards	None/Discountable	None/Discountable	None/Discountable	None/Discountable
Drift Fence trapping	1	1	3	5
Capture, handling, data collection	1	1	3	5
VETERINARY CARE	None/Discountable	None/Discountable	None/Discountable	None/Discountable
Long-term Quarantine	Append new BO[1]	Append new BO[1]	Append new BO[1]	Append new BO[1]
Nasal-Facial/Bodily Swabbing	None/Discountable	None/Discountable	None/Discountable	None/Discountable
Tissue Collection	1	1	3	5
Salvage	N/A	N/A	N/A	
Individual Marking	None/Discountable	None/Discountable	None/Discountable	None/Discountable
PIT Tag/Transponder injection	None/Discountable	None/Discountable	None/Discountable	None/Discountable
Marking rattle segments	None/Discountable	None/Discountable	None/Discountable	None/Discountable
Scale clipping	1	1	1	3
RADIOTELEMETRY	Append new BO[1]	10	40	50
CONTROLLED PROPAGATION/CAPTIVE MANAGEMENT				
Within AZA EMR SSP Population	N/A	N/A	N/A	N/A
Placement of captives into permitted captive program	N/A	N/A	N/A	N/A
Collection from wild for placement into captive program	Append new BO[1]	Append new BO[1]	Append new BO[1]	Append new BO[1]
Population Augmentation				
Using captives	N/A	N/A	N/A	N/A
Using translocated wild EMR	Append new BO[1]	Append new BO[1]	Append new BO[1]	Append new BO[1]
Research Using Captives	None/Discountable	None/Discountable	None/Discountable	None/Discountable
TOTAL	6	16	56	78

[2] Activities for which incidental take shall be considered on a case by case basis and through separate consultation and Biological Opinion appended to this one.

Reasonable and Prudent Measures.

We believe the following reasonable and prudent measures are necessary and appropriate to minimize effects to the EMR during the proposed actions considered in this programmatic biological opinion.

1. Ensure that proposed activities will result in the maximum benefit to the species and least possible levels of direct and incidental take necessary to accomplish beneficial management and recovery objectives.

2. Monitor the extent of take occurring under authority of section 10(a)(1)(A) permits issued by the Regional Offices, and modify permitted or funded activities as necessary or appropriate to minimize adverse effects (reasonable parties: permit holder/grantee and Service).

Terms and Conditions

The Chicago Field Office (or all Field offices) will provide review to ensure that any proposed research and population management activities use the most recent information available for the EMR.

The Service will ensure that permits issued under section 10(a)(1)(A) comply with the following terms and conditions, which carry out the reasonable and prudent measures. These terms and conditions are non-discretionary.

Terms and Conditions for Research, Handling, and Captive Management
Eastern Massasauga Rattlesnake (*Sistrurus catenatus*)

1) Permit applicants shall demonstrate education and experience in handling venomous snakes in a manner that is both safe to them and any EMR they may handle, as well as knowledge of the principles of general biology and ecology of EMR, including field identification of snakes and habitat. Applicants should submit a list of qualifications and references, including regional peers or persons under whom they have trained in EMR surveys.

2) Permit holders shall inform state and local conservation agencies, or other landowners of locations where they intend to work, and are responsible for obtaining any additional state or local permits, and private landowner permission that may be required.

3) Permit holders shall follow the protocol for surveys and data collection developed for the Service (Casper, 2001), unless otherwise indicated below or by the Chicago Field Office.

4) Only individuals named on the permit application are authorized to conduct activities pursuant to the issued permit. Each named individual must be responsible for compliance with the terms and conditions of this permit. For changes to names included on the permit, the permittee must submit a request to the Service. Persons (for example, graduate students,

new field assistants or other staff hired to work on permitted projects) working under the direct on-site supervision of a named permittee may assist with these activities.

5) Senior managers (including Directors, General Curators, or Reptile Curators) at accredited AZA Zoos or other public facilities that hold captive EMRs for display or captive management, and named on permits may use their authority to designate trained staff (for example animal keepers) who may handle or provide day to day care for EMR they hold without adding the names of each such individual. However, only named permit holders may oversee research using captive EMRs, or initiate breeding transfers of animals in their care to other institutions, and appropriate records of transfers must be kept. This term/condition applies only for EMR born in captivity after October 31, 2016.

6) The permittee shall not commence activities authorized by their permit in a new area, or at a previously authorized site at a new time, unless and until permission is received by the Service's Field Office that covers the new geographic area.

7) The use of snake hooks to capture EMR in the field should be restricted to gently lifting snakes. The practice of "pinning" the snake's head or neck to the ground prior to handling shall not be used.

8) The use of animal handling tongs to capture EMR in the field shall be restricted to gently lifting snakes with types (e.g., Midwest Tongs, Inc., Gentle Giant ®; Stoney, Inc., Animal Equipment, Inc. or similar) that are engineered with pressure release springs, wide surface area jaws, and rubberized/padded coating to prevent crushing the snake.

9) Permit holders may transport wild EMRs to a laboratory or other secure structure to provide a controlled environment to gather data such as size/weight, sex, reproductive condition, to collect skin swabs, blood/tissue samples for health or genetic studies, hold gravid females until they give birth (and collect data on neonates prior to release) or to perform individual marking as listed below.

10) Individual EMR captured in the wild may be placed in secure cloth bags. To be transported to a laboratory or other secure structure for data collection, bags containing EMRs shall be transported in hardened, secure containers. Containers with live EMR shall be kept out of direct sunlight, have adequate ventilation, and measures (such as "sandwiching" carpet remnants between bags containing live EMR) shall be taken to prevent snakes from biting through bags and envenomating each other.

11) Permit applicants who intend to collect blood, tissue biopsies, mark by scale clipping or swab facial pits or skin of live EMRs should either be licensed veterinarians or must demonstrate experience in performing these procedures in a manner that is both safe to them and any EMR they may handle. Applicants shall submit a list of qualifications and references, including peers or persons under whom they have trained to collect blood/tissues.

12) Holders of a valid permit may, at their discretion, present EMR legally taken form the wild and found with injuries, signs of disease, or other health concerns, to a licensed veterinarian for evaluation, treatment, supportive care, and if necessary (in the opinion of the veterinarian), euthanasia. The permit holder should provide a copy of their permit to the veterinarian performing such services, and the veterinarian or their staff will not need to be named on a separate permit to hold and provide treatment for such care.

13) Permit applicants who intend to collect blood, tissue biopsies, mark by scale clipping or swab facial pits or skin of live EMRs should submit a plan for conducting these procedures in a sterile fashion or describe their facilities to do so in the application.

14) When handling EMR in the lab, the technician's hands must be covered in latex, nitrile, or other gloves. Gloves shall be changed between processing individual EMRs.

15) When moving between sites, researchers should use rubber or other boots and disinfect the footwear and field equipment with a 10% bleach solution.

16) Animals captured in the field by permit holders shall be released within 48 hours unless they are receiving a surgical procedure, if they are gravid females being held so that data can be gathered on their offspring/neonates, or when unforeseen circumstances arise that would make release within 48 hours impossible, or unsafe (e.g., inclement weather), permit holders should document this by informing the Service's Ecological Services Field Office that covers the State(s) where the snakes originated and are being held, and provide an alternative release date.

17) All EMRs captured in the wild and handled, whether in the field, or while in the laboratory should be released at the exact location of the capture, unless the animals is found in harm's way (e.g., on a road or in/around a human dwelling) or at risk. At risk animals shall be moved to the nearest adjacent habitat, usually no greater than 200 meters from location of capture.

18) All proposed research that includes affixing internal or external transmitters to EMR shall include a veterinary protocol and statement of how research using radiotelemetry will advance further the recovery of EMR.

19) All surgical procedures involving EMR shall be performed by licensed veterinarians familiar with sterile and other standard procedures unique to reptiles.

20) Radiotransmitters implanted surgically or attached externally to wild EMR shall not exceed 5% of the animal's total body mass at the time surgery or attachment takes place.

21) EMR receiving a surgical procedure shall be monitored for at least 120 hours prior to release, to ensure that there are no obvious complications from surgery. If in the judgement of a licensed veterinarian individual EMRs require more than 168 hours for recovery from surgical procedures, the permit holder shall inform the Service's Ecological Services Field

Office that covers the state(s) where the snakes originated and are being held, and provide an alternative release date.

22) Radiotelemetered snakes shall be regular monitored (at least twice weekly, or every 86 hours), including examination for signs of infection or declining body condition, unusually high amounts of basking (to warm the body temperature possibly in response to infection). If any of the above are observed, the animal should be removed from the field for veterinary evaluation and care, and possible removal of the transmitter.

23) In cases of mortality of radiotelemetered EMR, where all or portions of the carcass can be retrieved, a necropsy by a qualified veterinary pathologist shall be performed to determine whether the transmitter, or procedures used for attachment/implantation contributed to death.

24) Radio transmitters will not be surgically implanted into snakes after August 15, unless there is a specific need or importance reason to do so, and after consultation with the Service's Ecological Field Office that covers the location where the project will occur.

25) Locations of cover boards placed at known or suspected EMR localities should be recorded with a GPS unit and marked to allow them to be found and removed in the future.

26) Name and contact information of the permit holder shall be attached to pitfall and funnel traps placed in known or suspected EMR localities. Applicants are responsible for following any state regulations covering standard labeling of wildlife traps that may also apply.

27) Pitfall, funnel, and other traps shall be secured in a way that would prevent them from easily being moved by wind or other weather conditions.

28) Pitfall, funnel, and other traps will be partly covered by shade cloth, weed guard fabric, carpet remnants, or other material fastened in such a way as to ensure that animals within the trap have a shaded retreat at all times during daylight hours.

29) Open pitfall, funnel, and other traps shall be checked no less than once every 24 hours. During periods when traps cannot be checked once within 24 hours, the traps should be disabled, closed, or removed from the field.

30) "Wet" pitfall traps (traps designed to kill fauna in an aqueous solution in the trap) shall not be used in habitat known to be occupied by EMR.

31) If capture, handling, data collection, blood collection, tissue biopsies, or other actions allowed by permit results injury or death to an EMR, within 48 hours the permit holder shall inform the Service's Ecological Services Field Office that covers the state where the trapping occurred so that incidental take can be documented.

32) Any dead specimens salvaged in the field should be preserved and labeled in accordance with standard museum practices for fluid preservation.

33) Tissues, blood or external swabs salvaged/collected for disease or genetic studies shall be either fluid preserved/cryopreserved using best practices for standard histological or laboratory exam. The permittee shall supply the depository with a copy of this permit to validate that the specimens were taken pursuant to the permit.

34) For dead individual EMR found in the field, record date it was found, GPS coordinates, age class (neonate, juvenile, or adult), sex (if/when possible), and document if the animal is a recapture.

35) Captive propagation of EMR held in facilities governed by the Association of Zoos and Aquariums (AZA) after October 31, 2016 shall be coordinated by the AZA's Eastern Massasauga Species Survival Plan.

36) Other institutions or facilities interested in captive propagation of EMR may apply for a 10(a)(1)(A) permit for enhancement of the species. Prior to doing so, applicants should be familiar with the Service's Policy regarding controlled propagation of species listed under The Endangered Species Act (USFWS and NMFS, 2000), and contact the Service's Chicago Field Office to discuss goals and justification.

37) Individual captive EMRs born into the AZA's Eastern Massasauga Rattlesnake after October 31, 2016 and deemed to be surplus by the Association of Zoos and Aquariums Eastern Massasauga Species Survival Plan may be transferred with documentation including age, gender, dorsal photograph(s) of the animal and date of transfer to other AZA facilities or non-AZA facilities, where they may be used for display, education, and outreach purposes.

Reinitiation – Closing

This concludes the Biological Opinion for Federally Listed EMR (*Sistrurus catenatus*) in Relation to the Issuance of Section 10(a)(1(A) Scientific Take Permits. We anticipate that up to 2850 EMR will be pursued and captured and that incidental take resulting from injury or mortality will result in up to 83 individuals over a five-year time period. The reasonable and prudent measures, with their implementing terms and conditions, are designed to minimize the risk and impact of incidental take that might otherwise result from the proposed action. If, during the course of the action, this level of incidental take is exceeded, such incidental take represents new information requiring reinitiation of consultation and review of the reasonable and prudent measures provided.

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