30 June 2017

Version 1.0

Ohio Conservation Plan:

Massasauga

Sistrurus catenatus

Prepared for the Ohio Division of Wildlife

by:

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Introduction and purpose

This plan outlines objectives, strategies, and methods for the conservation and recovery of the Massasauga (*Sistrurus catenatus*) in Ohio. In 1996, the Massasauga was listed as a state endangered species and is protected under Ohio Revised Code 1532.25 and Ohio Administrative Code 1501:31-23-01. In September 2016, the species was listed as federally threatened under the Endangered Species Act of 1973.

The plan was developed by Gregory Lipps and Nicholas Smeenk of the Ohio Biodiversity Conservation Partnership at Ohio State University, with input from the Ohio Division of Wildlife and partners. The plan will be updated as necessary to address new information and conservation concerns as they arise, with a thorough review scheduled for 2021.

Acknowledgments

This plan was greatly improved thanks to the data contributed and reviews of drafts provided by: Kate Parsons, Jeff Davis, Lisle Gibbs, and Angela Boyer.

Recommended citation

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List of changes to the plan

The following table identifies all changes that have occurred in the plan since v1.0 released on 30 June 2017. We appreciate feedback on any portion of the plan, including suspected typographical errors, omissions, or suggested changes. Send to: GregLipps@gmail.com.

Date	Version	Changes

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1. *Description of the Massasauga*

1.1. Appearance and coloration

Figure 1. Color morphs of the Massasauga. Most individuals have dark blotches on a lighter background (left), but up to one-third of some Ohio populations are melanistic (all black; right). Photo by Greg Lipps.

The typical base coloration of Massasaugas is gray, tan, yellow, or brown. The dorsum is covered with dark brown saddles or blotches extending down the length of the snake. The ventrum is black with occasional white or yellow markings. Often present on the head is a looping narrow white band surrounding a thicker dark-brown band. Juvenile Massasaugas have similar patterns to adults, but tend to be paler in color and have a yellow tail tip. Melanism is occasionally observed in some Ohio populations resulting in an atypical dark coloration with little or no pattern apparent, aside from white markings on the chin and neck (Figure 1).

1.2. Size

The Massasauga is a stout-bodied rattlesnake and the smallest pit viper species in Ohio. Range-wide, adult total length ranges from 45.7 - 70 cm (18 - 27.6 in) with individuals rarely exceeding 86 cm (34 in). Similarly, in Ohio, adult length varies from approximately 50 - 70 cm (19.7 – 27.6 in) for both males and females with no apparent length sexual dimorphism.

1.3. Similar and confusing species

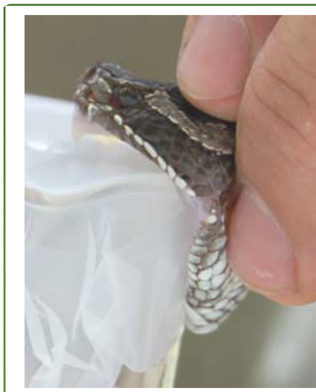

Figure 2. Loreal pit and fangs. The heat sensitive pit is visible just above the fang of this Massasauga whose venom is being collected for research. Photo by Greg Lipps.

In Ohio, the Massasauga is likely to be confused with other saddled or blotched snake species including the Eastern Milksnake (*Lampropeltis triangulum*), Northern Watersnake (*Nerodia sipedon*), Eastern Foxsnake (*Pantherophis gloydi*), and Eastern Hog-nosed Snake (*Heterodon platyrhinos*). Massasaugas can be differentiated from these species through the following characteristics:

1. Massasaugas, like the Northern Copperhead and Timber Rattlesnake, have elliptical pupils.
2. Massasaugas have heat-sensitive pits between the nostrils and eyes (Figure 2), as do the other two pit vipers found in Ohio.
3. Massasaugas have a distinct rattle at the end of their tail (although it is possible for this to be lost from an encounter with a predator). While many non-venomous species will readily vibrate their tail when disturbed, only venomous species possess rattles.
4. The belly of the Massasauga is dark with some irregular light markings. Foxsnakes have a yellowish

belly with a black checkerboard pattern. Milksnakes have a light belly, also with a black checkerboard pattern. The Northern Watersnake belly is cream-colored with irregular half-moon shaped crescents. The Eastern Hog-nosed Snake's belly is also light colored (except in melanistic individuals) sometimes having a light pattern.

5. The head of the Massasauga is more diamond shaped with white stripes along the jaw. The other (non-venomous) species have more oval-shaped heads. The exception to this is the Hog-nosed Snake, which can put on an impressive display that includes flattening its head into a triangular shape. The Hog-nosed Snake, though, has a distinctive upturned scale on the tip of its nose.

The majority of Massasauga sightings received by Ohio's herpetologists turn out to be misidentifications of more common species. Observations of Massasaugas should be accompanied by a photograph of the entire body, head, and tail. The public is encouraged to report possible Massasauga observations to wildinfo@dnr.state.oh.us.

2. *Taxonomy*

The Massasauga or "swamp rattler" is one of three species of the family Viperidae ("vipers") and subfamily Crotalinae ("pit vipers") and the only species of the genus *Sistrurus* in Ohio. Previously, the genus *Sistrurus* was divided into two species *S. catenatus* and *S. milarius,* each with three subspecies (Murphy et al. 2002). Prior classifications identified these subspecies based upon morphological variation in in scale characteristics, body size, coloration, and geographic distribution (Gloyd 1935; Gloyd 1940). The eastern subspecies, *S. c. catenatus*, ranges from New York to eastern Missouri, *S. c. tergiminus* and *S. c. edwardsii*, have ranges in the central and southwestern US, respectively.

Recent genetic analysis using 18 nuclear and 1 mitochondrial DNA loci, however, calls the validity of these of these subspecies designations into question (Kubatko et al. 2011). Inference from these multilocus data analyses provides strong and consistent evidence for the elevation of the eastern subspecies, *S. c. catenatus*, to full species status based upon the genealogical species concept and recognition as a distinct population segment under the ESA (Kabutko et al. 2011). Further genetic analysis suggests that this species has three genetically distinct subunits (Ray et al. 2013) (Figure 3).

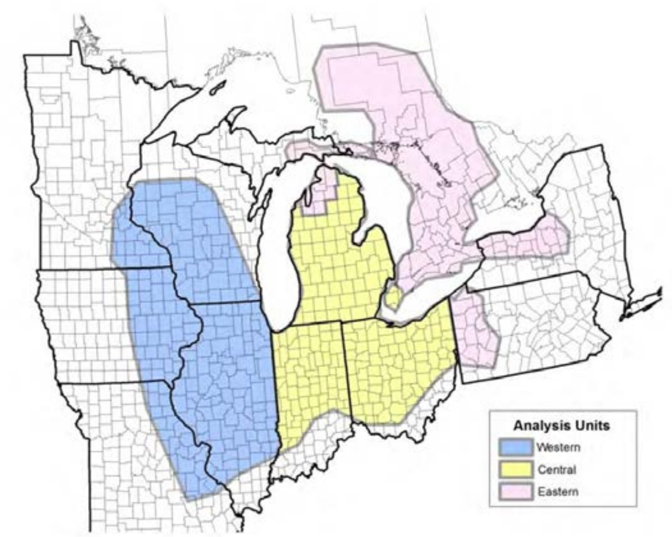

Figure 3. Distribution of the Massasauga. Shaded areas show three genetically distinct subunits (Western, Central, and Eastern) proposed by Ray et al. 2013. From Szymanksi et al. 2016.

The current recognized scientific and standard common name for the species was recently changed to *Sistrurus catenatus*, Massasauga (*SSAR North American Species*

Names Database. Society for the Study of Amphibians and Reptiles, n.d. Web. 28 Oct. 2014. <https://ssarherps.org/cndb/>). The USFWS continues to reference the species as the Eastern Massasauga Rattlesnake, and commonly uses EMR as an abbreviation.

3. *Geographic distribution*

The distribution of the Massasauga encompasses parts of ten states in the Great Lakes Region of the United States and one Canadian Province, including: western New York and Pennsylvania, Ohio, Indiana, Michigan, Illinois, Iowa, southeastern Minnesota, northern Missouri, southwestern Wisconsin, and southern Ontario (Figure 3) (Tennant et al. 2003). Although historically present in Minnesota, all populations are now believed to be extirpated. Extant but declining populations exist in all other areas of the Massasauga's range. The status of the species was recently reviewed by the US Fish and Wildlife Service in their Species Status Assessment (Szymanksi et al. 2016).

In Ohio, the Massasauga was historically documented in 28 counties located in the glaciated portion of the state; recent surveys, however, indicate that populations are likely extant in only 8 – 10 counties (Figure 4) (Wynn and Moody 2006). Remaining populations are found in four broad regions of the state: the lowlands associated with major river valleys in Ashtabula and Trumbull Counties; inland marshes and tall grass prairies in Erie and Huron Counties; remnants of the Prairie Peninsula in Wyandotte County; and, in scattered fens associated with esker-kame complexes near major end moraines in Champaign, Clark, Green, and Warren Counties.

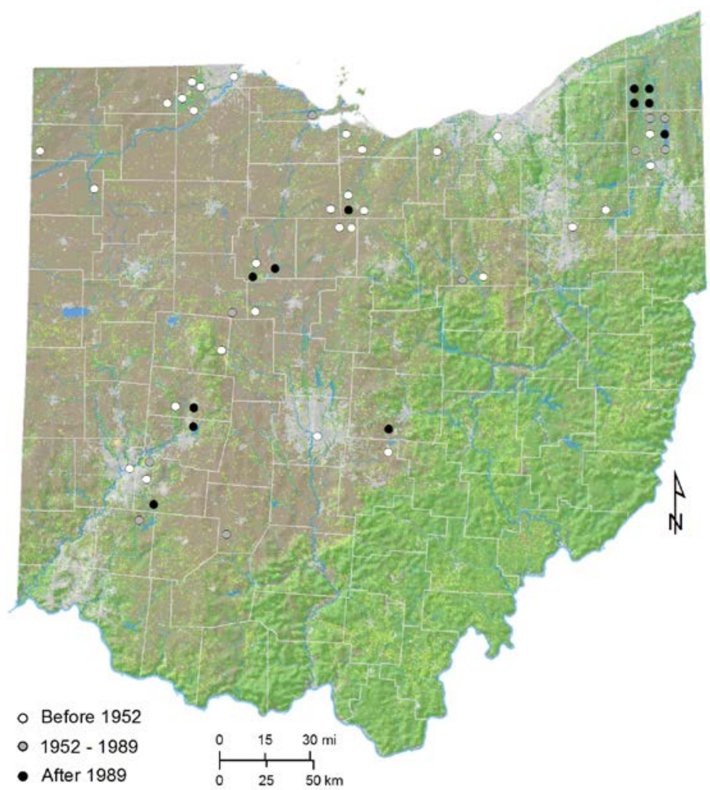

Figure 4. Occurrences of the Massasauga in Ohio by township. Only those occurrences that are backed up by a voucher specimen or a photovoucher in a museum collection are shown; anecdotal records are not included.

4. *State and federal status*

The Massasauga is legally protected as endangered or a species of special concern across its geographic distribution (Table 1). The degree of protection afforded by these designations varies in each state.

Table 1. Legal status and population status of the Massasauga within range states and Canada. Adapted from Szymanksi et al. (2016).

STATE	LEGAL STATUS	POPULATION STATUS
Illinois	Endangered	Extant
Indiana	Endangered	Extant
Iowa	Endangered	Extant
Michigan	Special Concern	Extant
Minnesota	Endangered	Likely Extirpated
Missouri	Endangered	Extant
New York	Endangered	Extant
Ohio	Endangered	Extant
Pennsylvania	Endangered	Extant
Wisconsin	Endangered	Extant
Ontario	Endangered	Extant

Species Status Assessments for the Massasauga have been completed by the USFWS in 1998 (Szymanksi 1998) and 2016 (Szymanksi et al. 2016). These are the most thorough assessments of our knowledge of the species and document declines in all parts of the Massasauga range.

The Massasauga was initially listed as a candidate species under the Endangered Species Act (ESA) in 1982 and again designated as a candidate species for federal listing by the U.S. Fish and Wildlife Service (USFWS) in October 1999, following the published status assessment completed in 1998 (Szymanksi 1998). As a candidate species, the Eastern Massasauga received no formal protection under the ESA, but the USFWS encouraged actions that may prevent the need for future listing. In 2005, the Center for Biological Diversity filed a petition to list the Massasauga as "endangered" under the ESA. This was followed in 2011 by a federal lawsuit (see *Center for Biological Diversity v. Salazar 10-cv-0230*) through which an agreement was made requiring the USFWS to make final decisions on the federal listing of 757 candidate species by 2018. On September 30, 2015, the USFWS proposed listing the Massasauga as threatened under the ESA, following the release of an updated status assessment (Szymanksi et al. 2016). The species was designated as federally threatened on September 30, 2016.

5. *Natural history and ecology*

5.1. Habitat

Massasaugas are associated with a wide variety of early-successional, vegetative communities across their range; in general, however, most habitats are composed of wet, herbaceous communities, such as wet meadows, prairies, sedge meadows, and old fields interspersed with shrubs and adjacent to mesic grasslands or lowland forests (Figure 5) (Szymanksi 1998; Tennant et al. 2003; Wynn and Moody 2006). Habitat use of these communities shifts seasonally and varies among males, gravid females, and nongravid females (Szymanksi 1998; Parent and Weatherhead 2000; Harvey and Weatherhead 2006a; Marshall et al. 2006). Potential suitable wetland habitats include fens, bogs, marshes, forested swamps, and wet meadows (Johnson 1995; Johnson and Leopold 1998; Kingsbury et al. 2003). Upland habitats include prairies, grasslands, savannas, and fallow fields (Smith 1961; Tennant et al. 2003; Wynn and Moody 2006).

Although Massasaugas are typically found in areas composed of both wetland and upland vegetative communities, it is the structural characteristics of sites, rather than vegetative composition that is the main determinant of habitat suitability (Harvey and Weatherhead 2006b; Moore and Gillingham 2006) Specifically, three habitat characteristics are consistent among all occupied sites: 1) open, sunlit areas intermixed with areas of shade for thermoregulatory and predator avoidance purposes; 2) the presence of the water table near the surface for overwintering; and 3) variable topography among wetland, lowland, and upland habitat areas (Szymanksi 1998).

Figure 5. Ohio Massasauga habitat. From top to bottom: Wyandotte Co., Huron Co., Trumbull Co., and Ashtabula Co. Photos by Greg Lipps.

Habitat use by Massasaugas is best understood through the lens of behavioral ecology, as snakes balance the need to thermoregulate, feed, find mates, and avoid predation. The preferred body temperature range is 30.0 – 33.6°C (86.0 – 92.5 °F), where most physiological functions are optimized (Harvey 2006). In addition, the various habitat requirements for these behaviors vary seasonally and among sexes and reproductive status.

5.1.1. Overwintering habitat

Massasaugas tend to hibernate individually or in small groups, usually in or near the same location from year to year (Johnson 1995; Harvey and Weatherhead 2006a; Smith 2009). Overwintering sites are most commonly crayfish burrows, but sphagnum hummocks, small mammal burrows, and tree roots have also been used (Figure 6) (Johnson 1995; Seigel et al. 1998; Szymanksi 1998; Harvey and Weatherhead 2006a; Smith 2009). A water table at or near the surface that does not freeze is the common element in all reported overwintering locations ((Maple 1968; Reinert 1978; Johnson 1995). Recent investigations have shown that it is common for snakes to be nearly entirely submerged underwater in these burrows (Smith 2009), and the presence of water may explain the body temperatures of snakes being higher than the surrounding soil (Kowalski 2007). In Pennsylvania, snakes typically spend 144 - 192 (mean = 165) days overwintering (Kowalski 2007).

Figure 6. Burrows. Crayfish burrows (top) and small mammal burrows (bottom) are used by many snakes including the Massasauga for overwintering. Photos by Greg Lipps.

Overwintering may occur in open-canopy sites such as grasslands, or in more forested areas (Johnson 1995; Harvey and Weatherhead 2006a; Kowalski 2007; Smith 2009). Apparently, the presence of water is a more immediate concern for overwintering than is solar exposure. Harvey and Weatherhead (2005) found more vegetative cover at overwintering sites than areas of activity and suggested that more canopy cover may correlate to warmer temperatures.

The specificity of characteristics at hibernacula locations in concert with the fact that most Massasaugas hibernate individually supports the conclusion that appropriate hibernacula are locally abundant, but regionally restricted (Harvey and Weatherhead 2006a). Within a landscape, areas having overwintering sites are often uncommon and snakes appear to show fidelity to these areas, returning year after year. Within such an area, however, there may be an abundance of burrows to choose from and the snake's fidelity does not extend to one specific burrow.

5.1.2. Post-emergence habitat

Massasaugas tend to stay very close to their overwintering locations after emergence and return to the same area to hibernate annually (Harvey and Weatherhead 2006a). Individuals probably continue to shuttle in and out of hibernacula during inclement spring weather, and do not move away until conditions are consistently warm. A trail camera monitoring a crayfish burrow used for overwintering in Ashtabula County documented emergence first occurring on April 6-7 (2014 and 2013, respectively) with use of the burrow continuing to as late as May 1. Kowalski (2007) found high stem density, canopy cover, and nearness of shrubs correlated to overwintering sites. Similarly, Harvey and Weatherhead (2006a) found that hibernacula were located near abundant vegetative cover, tall shrubs, and large rocks. The close proximity of hibernacula to cover suggests that the presence of these habitat characteristics may provide colder (and thus, more sluggish) snakes adequate cover from predators, especially prior to the growth of herbaceous vegetation.

5.1.3. Gestation habitat

The characteristics of habitat structure surrounding overwintering sites may be particularly important for gravid females who typically remain close to their overwintering area until parturition in the late summer. The need to maximize solar radiation during gestation means that gestation habitat is typically dominated by shorter vegetation than habitats used by non-gravid snakes (Reinert and Kodrich 1982; Marshall et al. 2006). In Indiana, this habitat was found along shorelines which were used by gestating females (Marshall et al. 2006).

Exposure does have risks, however, including predation and overheating. For this reason, gestating snakes commonly use edge habitats or the base of low-lying shrubs (e.g., *Vaccinium spp.*) where they can retreat from a predator or for shade (Harvey and Weatherhead 2006a; Kowalski 2007; Shoemaker and Gibbs 2010). Gestation habitats had the highest density of woody stems and the lowest canopy cover value of any utilized habitat in Pennsylvania (Kowalski 2007). However, within Killbuck Provincial Park (Canada), gravid females in high human disturbance areas remained in more hidden locations compared to those in low disturbance areas (Parent and Weatherhead 2000). This human-induced change in microhabitat use suggests the females perceived predation risk to be high, and chose to reduce the risk of direct confrontation with a potential predator, and thus the need to rely on flight for protection. Although researchers were unable to link this change to a decrease in litter size, avoidance of open areas for basking may result in delayed parturition and potentially decreased neonate survival.

Earlier research focused on the use of gestation sites by female snakes up to and including the time of parturition. It is important to note, however, that year-round radiotelemetry studies have found that these same individuals will make very large movements post-parturition, and use the same habitat types as non-gravid female and male snakes (Kowalski 2007). Therefore, focusing solely on maintaining gestation habitat is unwarranted, and habitat management and research efforts should instead be more holistic.

5.1.4. Active season habitat (spring - fall)

From the time that Massasaugas disperse from their overwintering areas, until they return in the fall, individuals may use a variety of habitat types, including uplands and wetlands (Johnson 1995; Harvey and Weatherhead 2005; Kowalski 2007). Movement into grassland areas is probably facilitated by warming temperatures, allowing individuals to leave the safety of overwintering burrows, in addition to increasing herbaceous cover, permitting greater cover from predators. During the spring-fall activity period, Massasaugas may use fallow fields, prairies, dikes, overgrown lawns, and the edges of woodland habitats, in addition to the bogs, fens, and wetland areas used during overwintering (Johnson 1995; Johnson and Leopold 1998; Kingsbury et al. 2003; Tennant et al. 2003; Wynn and Moody 2006).

Common herbaceous plant species associated with Massasaugas include: goldenrod (*Solidago* spp.), poverty grass (*Danthonia* spp.), blazing star (*Liatris* spp.), *Polytricum* spp. and *Sphagnum* spp. mosses, sensitive fern (*Onoclea sensibilis*), partridge pea (*Cassia fasciculata*), cinquefoil (*Potentilla* spp.), and strawberry (*Fragaria* spp.). Woody species include: dewberry (*Rubus* spp.), hawthorn (*Crataegus* spp.), dogwood (*Cornus* spp.), aspen (*Populus* spp.) and willow (*Salix* spp.). As indicated by the plant species listed above, Massasaugas can be found in a variety of habitat types. Furthermore, structure, rather than species composition, appears to be more important for Massasaugas. Specifically, snakes require a heterogeneous habitat, with areas of low/sparse vegetation to maximize their exposure to the sun (bask), close to more dense areas for foraging and refugia from predators and extreme weather. Weedy invasive species such as reed canary grass (*Phalaris arundinacea*), wild carrot (*Daucus carota*), and vetch (*Vicia* spp.) are commonly found in occupied fields, but as these species can form dense monocultures, they can greatly reduce this heterogeneity, and thus, reduce suitability of the habitat for Massasaugas.

5.2. Home range

The spatial ecology of the Massasauga is perhaps the most studied aspect of this species and may be the most well researched of any snake species. Home ranges of Eastern Massasauga have been widely reported (Table 2). Overall, male Massasaugas tend to have the largest home ranges and make more frequent and longer movements than non-gravid females. Gravid females have the smallest home ranges and move the least, but this is only true for the period prior to parturition in the late summer.

Home ranges may vary considerably among populations (see Table 1 in Durbian et al. (2008)). Szymanksi et al. (2016) reported homeranges of 1 – 136 ha (2.5 – 336.1 ac). Differences in home ranges may be the result of habitat heterogeneity, with snakes required to travel further in more homogeneous habitats where overwintering, gestation, and foraging habitats are widely separated (Reinert and Kodrich 1982; Marshall et al. 2006). However, when suitable habitat is in abundance, even in heterogeneous habitats, an increased density of snakes could increase competition and the need for larger home ranges to meet the individual's requirements (Kowalski 2007).

The maximum range length reported in the literature is 3,156 m (reviewed in Szymanksi (1998)). Mean range lengths between 89 and 1,331 m have been reported, and average daily movements observed at different sites have varied from 9.1 to 19.5 m. At Killbuck Provincial Park, snakes residing in areas of high human disturbance moved shorter distances and less frequently than snakes in areas with less human disturbance (Parent and Weatherhead 2000). During the mating season, males often make long distance, spatially directed movements to locate receptive females (Johnson et al. 2000).

Table 2. Statistics for minimum convex polygon (MCP) home range size (ha) for Massasaugas. Data comes from a review of reported home range sizes across the species range. See Appendix D. Massasauga Habitat Assessment for a more detailed table.

HOME RANGE	MALE	NON-GRAVID FEMALE	GRAVID FEMALE
MEAN	14.26	7.69	1.86
MINIMUM	1.64	1.13	0.70
MAXIMUM	38.3	41.4	5.10

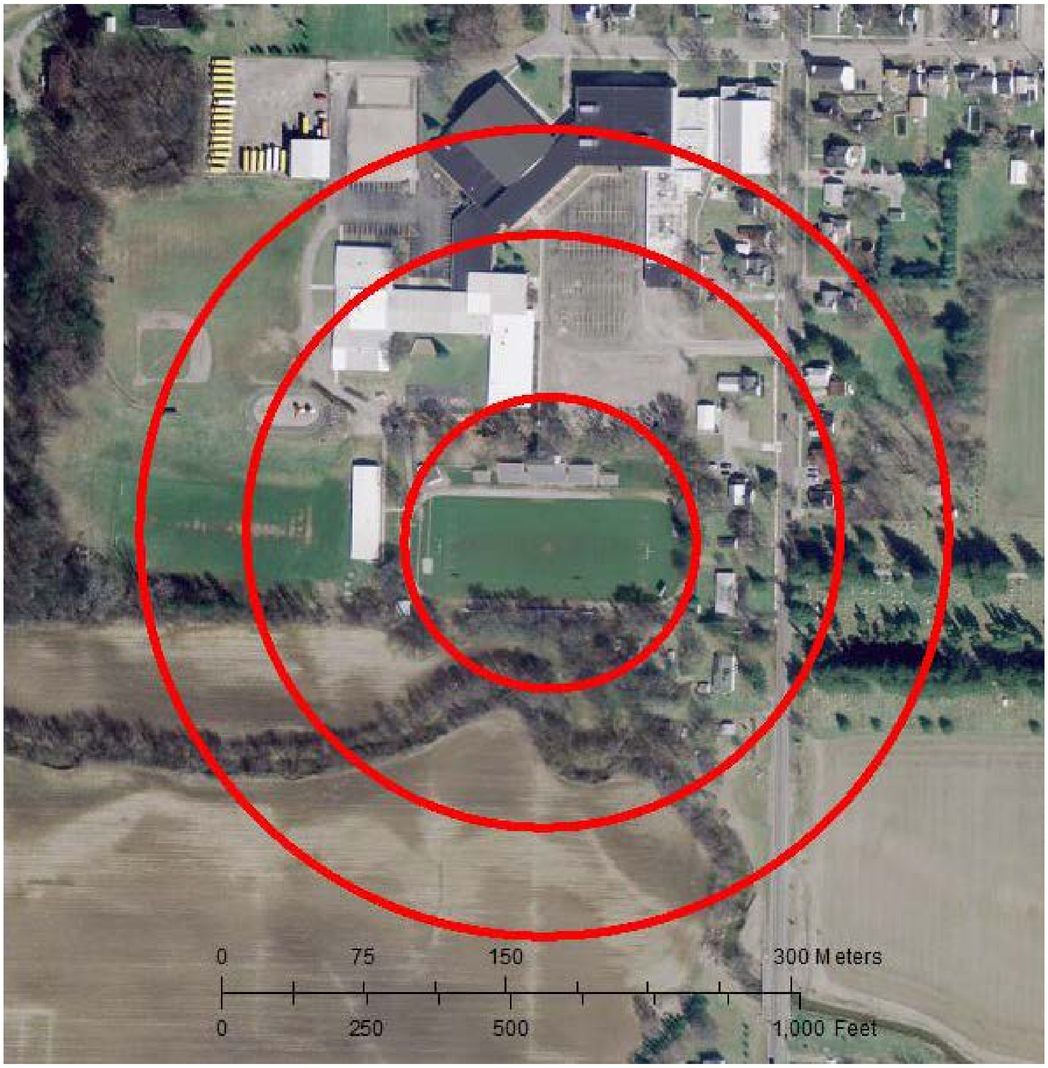

Figure 7. Homerange. Concentric rings around a football field (for scale) illustrate the mean home range sizes reported for Massasaugas (from smallest to largest): 1.86 ha (gravid females); 7.69 ha (non-gravid females); and, 14.26 ha (males).

5.3. Dispersal and gene flow

Our knowledge of the Massasauga's ability to disperse and colonize new areas comes from genetic analyses; there is little in the way of direct observations. Chiucchi and Gibbs (2010) found migration rates among Ohio populations are low and the rates currently and historically are similar in magnitude. This was true when comparisons were made between populations 50 km apart, but most surprising was that the magnitude of genetic differentiation was similar even for sites in close proximity (<7 km). This supports earlier findings of genetically distinct subpopulations <2 km apart (Gibbs et al. 1997). The available data does not provide any evidence for a more recent (<300 years) reduction in gene flow, although regional reductions due to barriers such as roads and water bodies may occur in some cases (Dileo et al. 2013). Overall, though, the low levels of migration and strong regional differences appear to be the historic norm for the species (Chiucchi and Gibbs 2010). This has two important implications. The first is that *"Massasauga populations should be managed as demographically independent units and that each has high conservation value in terms of containing unique genetic variation"* (Gibbs et al. 1997). Second, the data suggests that Massasaugas have very limited dispersal ability, and declining populations or extirpated sites are unlikely to be rescued or recolonized even if there are populations relatively nearby (2 km).

The available genetic data is difficult to rectify with the ecology of the Massasauga. As a species that is dependent on early successional habitats – those that would have historically been created through stochastic events (flooding, wildfire, etc.) – an ability to disperse and colonize these ephemeral habitats would be expected. Former agricultural fields located adjacent to fields occupied by Massasaugas have been restored at two sites in Ohio, and these were quickly colonized by snakes, as was a restored site in Missouri (Durbian et al. 2011). Further research is currently underway by Martin and Gibbs (Ohio State University) to examine genetic structuring at a much finer scale in Ashtabula County where several occupied sites are found in close proximity within the Grand River Lowlands.

5.4. Diet and feeding

Massasaugas are opportunistic predators, primarily feeding upon small mammals such as shrews, voles, and deer mice. They will also consume frogs, birds, eggs, and other snakes (NatureServe 2007). Hairs identified from fecal samples of Ohio Massasaugas identified the following food items (in decreasing order of prevalence): Northern Short-tailed Shrew (*Blarina brevicauda*), Masked Shrew (*Sorex cinereus*), Eastern Chipmunk (*Tamias striatus*), Meadow Vole (*Microtus pennsylvanicus*), Deer Mouse (*Peromyscus maniculatus*), Meadow Jumping Mouse (*Zapus hudsonicus*), Eastern Fox Squirrel (*Sciurus niger*), and Eastern Cottontail (*Sylvilagus floridanus*) (Weatherhead et al. 2009). In Michigan, Tetzlaff et al. (2015) reported adult Massasaugas feeding on an Eastern Gartersnake (*Thamnophis s. sirtalis*), two Red-bellied Snakes (*Storeria o. occipitimaculata*), and an American Red Squirrel (*Tamiasciurus hudsonicus*).

It has been commonly assumed that there is an ontogenetic shift in the diet of Massasaugas, with neonates including snakes in their diet in addition to small mammals, while adults feed primarily on small mammals. The available evidence does not support this notion, though, and a neonate snake feeding on a comparatively very large shrew (*Blarina* sp.) was documented in Illinois (Mike Dreslik, pers. comm.).

5.5. Reproduction

Like all rattlesnakes, Massasaugas bear live young. Although annual reproduction has been reported, biennial reproduction may be more typical. The frequency of reproduction is thought to be a result of habitat condition, prey availability, and latitudinal differences affecting the length of the active season. Although individuals may mate at any time they are active, late summer and early fall copulation is the dominant trend across the range (Reinert 1981; Kowalski 2007), and is associated with more frequent and longer movements by males seeking out mates. Sperm is apparently stored in the oviducts of the female until it is used to fertilize the oocytes the following spring (Ernst and Ernst 2003; Kowalski 2007)

Throughout their range, Eastern Massasaugas give birth from late July to early September (Ernst and Ernst 2003). In Missouri, Siegel (1986) reported birthing events between 17 August and 23 September. Pennsylvania females were observed giving birth from 4 August to 5 September (Kowalski 2007). In Ashtabula County, newly born young have been observed on August 12, 15, 18, and 20. In southwest Ohio, neonates have been found on August 4 (Champaign Co.) and August 13 (Clark Co.) (Jeff Davis, pers. comm.).

Gestation and birthing usually takes place very near the female's overwintering area (Kowalski 2007). Mother and young typically remain at the location of birth for several days after parturition, but neonates apparently receive no direct parental care. Three to five days after birth, the young snakes shed their skin for the first time and then gradually disperse (Johnson et al. 2000). Postpartum females may make very large movements, interpreted as a shift in focus from gestation to foraging (Kowalski 2007).

5.6. Growth

Massasaugas are born measuring 188 – 244 mm (total length) with a mass of 9.6 – 10.7 grams (reviewed by Jellen and Kowalski (2007)). In Pennsylvania, neonates grew an average of 29 mm and 2.4 g before their first winter (Kowalski 2007). By 12 months of age, they had a mean total length of 341 mm with a mean mass of 50 g. At 24 months, these had increased to 433 mm and 96 g; and, by 36 months, 543 mm and 185.5 g.

5.7. Predators

Predators of the Massasauga include birds (especially raptors), carnivorous mammals, and other snakes (especially *Lampropeltis* spp. – kingsnakes and milksnakes) (Szymanksi et al. 2016).

6. *Demographics*

6.1. Age at first reproduction

Sexual maturity in captive bred eastern Massasaugas has been documented as early as 27 months, but some studies suggest that females may not begin to reproduce until they are between five and seven years of age (Szymanksi 1998; Johnson et al. 2000). In Pennsylvania, sexual maturity was achieved during the individual's second full season of activity, resulting in an age of first reproduction at 36 months (Kowalski 2007).

Figure 8. Neonate Massasaugas. Photo by Greg Lipps.

6.2. Litter size

Brood size appears to vary greatly, between 2 and 20, and is positively related to female body size (Seigel

1986; Anton 2000; Ernst and Ernst 2003). Mean number of young was reported as 8 for 65 clutches (Ernst and Ernst 2003). Lipps (2006) found 14 live and 2 stillborn neonates together under a piece of tin on August 12, 2005 where two gravid females had been observed on several occasions. Only one, obviously spent, female was found with the neonates, and so it not certain whether these neonates came from one or two clutches. In 2015, three litters of neonates were found in Ashtabula County, numbering 6, 10, and 13. Similarly, Wynn (2003) reported a total of 14 neonates born to two females removed from Rome State Nature Preserve and temporarily held in captivity. Energy expended on reproduction is quite large, with females losing 44 - 55.7% of their mass (Anton 2000; Jellen 2005) or a mean of 106.8 g (Kowalski 2007) during parturition.

6.3. Survival

A range-wide analysis of Massasauga survivorship including data from 16 distinct locations found annual adult survival ranged from 0.35 to 0.95 (mean = 0.67) and increased from the southwest to northeast of the range (Jones et al. 2012). No consistent difference in survival between males and females was found. This data came from 499 telemetered snakes, an often used and very valuable tool for understanding the biology of snakes. Some caution is warranted, though, as Lentini et al. (2011) found adverse effects of transmitters placed into 8 of 12 snakes, including inflammation and infection, despite careful surgical procedures and advanced veterinary care being provided in a laboratory setting. If telemetry transmitters reduced survival in any of the studies referenced by Jones et al. (2012) this would result in a biased estimate.

6.4. Population density

Reported population densities for the Massasauga range from 0.59 to 9.2 per hectare, including an estimate of 1.97 per ha for a NE Ohio site (reviewed in Szymanksi (1998)). Recent work in NE OH has produced density estimates ranging from 0.6 to 10.8 snakes/ha (mean = 3.4) for 8 sites.

6.5. Population Viability Analysis

A range-wide extinction risk model for the Massasauga was produced by the population biologists at the Lincoln Park Zoo (Faust et al. 2011). A total of 64 sites were modeled, although many assumptions were made about most, as specific population parameters and demographic values used in the model are unknown at most sites. Current threats, as identified by researchers familiar with each site, were also included into the model. The PVA found the majority of the populations are likely imperiled, and that robust, viable populations generally require approximately 50 adult females.

Four of the six Ohio sites examined in the PVA were considered quasi-extinct, as their current populations were estimated to have less than 25 females. Probability of the other two sites reaching extirpation was 0 and 1, with a reduction to 0.82 for the latter if current threats are eliminated. These results should be cautiously interpreted, not only because of the uncertainty in the data used to populate the models, but also because the PVA was conducted by “site,” which may not correspond to populations (i.e., multiple sites may be part of a larger population or metapopulation). Furthermore, there is evidence that the Massasauga may be naturally adapted for persistence in small, isolated populations (see 5.3. Dispersal and gene flow), which would call into question the application of a generic minimum viable population (MVP) target of 50 adult females.

7. *Threats*

7.1. Habitat destruction/conversion

The outright destruction and conversion of habitat used by the Eastern Massasauga has been the greatest driver in population declines (Szymanksi 1998; Szymanksi et al. 2016). Over 90% of Ohio's original wetlands have been lost and a larger proportion of the state's original grassland and prairie habitats have been lost than any other habitat type.

7.2. Hydrologic alterations

Massasaugas are tied to open canopy, shallow water or wet soil, herbaceous wetlands that are often desirable for agriculture and development. Extensive networks of drainage ditches and field drain tiles have greatly reduced the water table throughout the state, impacting both Massasaugas and their habitat. Reduced water tables impact burrowing crayfish (the primary creators of Massasauga overwintering habitat) and increase overwintering mortality. Drainage also allows for greater encroachment of woody species, thus speeding succession. Flooding has also been implicated in mortality of Massasaugas (Seigel et al. 1998), although they appear to be able to withstand short-term (days) submersion during overwintering. Hydrologic alterations also can facilitate the establishment and spread of invasive plant species.

7.3. Vegetative succession

The succession of Massasauga habitat from an open canopy herbaceous-dominated system to a more woody-dominated forested system is currently the greatest threat to populations residing on protected properties in Ohio (Figure 9). These changes result in reduced solar insolation (required for the ectothermic Massasauga) and likely changes to their available prey base.

Figure 9. Succession. Visible at two scales, woody species move into unmanaged habitat, reducing its ability to sustain Massasaugas. Top: photo by Greg Lipps. Bottom: aerial images from Google Earth.

7.4. Invasive species

Invasive plant species degrade and even eliminate Massasauga habitat by converting areas to thick monocultures lacking the heterogeneity required for carrying out all aspects of the species' life history (Figure 10). Invasive species can also cause greater rates of transpiration, further reducing water tables, and populations of burrowing crayfish and Massasauga prey. In Ohio, the invasive plant species of greatest concern at Massasauga occupied sites are Reed Canary Grass, *Phragmites* sp., and Buckthorn.

Figure 10. Invasive species. Reed Canary Grass (seen here in winter) reduces habitat heterogeneity that is necessary for Massasaugas. Photo by Greg Lipps.

7.5. Land-management activities

Land management activities include prescribed burning and mowing, two techniques often used to improve and restore Massasauga habitat. The behavioral ecology of the Massasauga, however, makes them particularly vulnerable to increased mortality from these activities. First, Massasaugas may congregate at overwintering sites, making a large proportion of the population vulnerable to fire, mowing, plowing, disking, no-till drills, etc. Second, Massasaugas do not flee from approaching danger, but instead tend to remain motionless and rely on their camouflage (Parent and Weatherhead 2000; Lipps 2005). Mortality associated with mowing, burning, and tilling/disking of fields has been well documented (Lipps 2005; Durbian 2006; Cross et al. 2015).

7.6. Road mortality

Ohio's 198,258 km of roads equates to a density of 1.87 km/km^2, the seventh highest of all 50 states according to the U.S. Department of Transportation (2011). Over 9.8 million vehicles are registered in the state, and nearly 112 million miles are traveled on Ohio's roads each year. All of Ohio's Massasauga populations are located near roads, and road-killed Massasaugas are reported nearly every year in the state.

7.7. Persecution

Historically, persecution of the Massasauga was widespread and common. Old newspaper articles and landowners routinely recount stories of killing large numbers of snakes, especially during bailing hay or construction projects. These stories make it clear that the killing went far beyond simple take incidental to the activity; snakes were actively pursued to be killed. As most Ohio populations now reside on protected properties, the current role of persecution in population declines is likely greatly reduced, but is worthy of further study.

7.8. Poaching

As with any rare species, a market exists for Massasaugas among those who like to keep, display, and sell rare reptiles (Figure 11). Before the DOW adopted regulations concerning Ohio's native amphibians and reptiles, the collection and sale of Massasaugas was common, with many being sold to out of state wildlife wholesalers. Today, the extent of poaching is unknown, but landowners have reported to researchers that they have been approached by individuals looking to collect Massasaugas in recent years at two Ohio sites and one individual was convicted after admitting to collecting Massasaugas at a third site.

timber rattlesnakes. In May of 2008, and again in October of 2008, the target brought a total of 35 endangered snakes and one spotted turtle (also listed as endangered in Ontario) into the

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United States and traded them to me (see photo below). During the October transaction, the target was arrested on State, Federal, and Canadian charges when he delivered 33 massasauga rattlesnakes across the border. I am informed by Ontario Provincial officials that his collecting activities could have decimated an entire population of a rare animal, and rehabilitation work is under way currently to return the snakes to their habitat in the spring.

Rattlesnake secreted in a speaker box in a mini-van

Measuring a smuggled endangered massasauga rattlesnake

Figure 11. Poaching. Portion of a search warrant describing the sale of 33 illegally collected Massasaugas to an undercover wildlife agent as part of Operation Shellshock.

7.9. Increased predator populations

Despite being venomous, Massasaugas are vulnerable to a number of predators. Raptors are the most commonly cited predator of the species, with Red-tailed Hawks the most commonly observed raptor at Ohio Massasauga sites. The Red-tailed Hawk is a known snake predator and the North American Breeding Bird Survey annual index for Ohio populations of the species has steadily increased from 0.52 to 2.53 from 1966 to 2015 (https://www.mbr-pwrc.usgs.gov/bbs/bbs.html).

Other species that will consume Massasaugas include Raccoons, Striped Skunks, Coyotes, Mink, and Virginia Opossums. Populations of many mesopredators have greatly increased in Ohio. Opossums are immune to pit viper venom and a trail camera captured one feeding on a neonate Timber Rattlesnake (*Crotalus horridus*) in southern Ohio (Carl Brune, pers. comm.).

7.10. Disease

Snake Fungal Disease (SFD) is a recently described disease of free-ranging snake populations resulting from infection by *Ophidiomyces ophiodiicola* (Allender et al. 2015b). Typically presenting as skin lesions near the head and neck, SFD consistently results in morbidity or mortality and may cause severe localized population declines (*Sistrurus catenatus*, Allender et al. 2013; *Crotalus horridus*, Clark et al. 2011; *Thamnophis radix*, Dolinski et al. 2014). SFD has been reported in free-ranging snake populations in 15 Eastern and Midwestern US states (Allender et al. 2015a) and at least 11 species to date (Cheatwood et al. 2003; Rajeev et al. 2009; Allender et al. 2013; Dolinski et al. 2014; Guthrie et al. 2015; McBride et al. 2015). In Ohio, SFD was first reported from retroactive testing of five Lake Erie Watersnakes (*Nerodia sipedon insularum*), an Ohio state listed species, collected from Ottawa Co. in 2009 (Lorch et al. 2016).

Swabs collected from Massasaugas in NE Ohio failed to detect SFD (Smeenk et al. 2016), but it has since been detected on an Eastern Gartersnake and Eastern Milksnake living sympatrically with Massasaugas in the region. Some swabs of other symptomatic snakes collected around the state were also positive, so it would appear that this disease is widespread in Ohio, although it has not yet been detected on any Ohio Massasaugas. The potential role of this and other diseases in Ohio population declines is unknown. A USGS fact sheet about SFD is included as Appendix A. Snake Fungal Disease fact sheet.

8. *Environmental review procedures*

The Ohio Division of Wildlife (DOW) conducts environmental reviews on projects that require a federal permit or utilize federal funding. These reviews are generally requested in order to comply with the National Environmental Policy Act (NEPA). NEPA establishes the requirement that all federal agencies' funding or permitting decisions be made with full consideration of the impact to the natural and human environment.

When a project involves work within the range of the Eastern Massasauga, this is noted during the environmental review. If the work is within the range and is to occur in, or adjacent to, an area of shallow water wetlands where land use is not entirely composed of forest, row crops, and/or urban land-use, then the DOW recommends that an assessment of the habitat be conducted by a herpetologist approved by the DOW. Should the assessment find suitable active season and/or overwintering habitat, the DOW recommends that a survey be conducted by a herpetologist approved by the DOW to determine the presence or absence of the species using the currently accepted methods (see 1.12.3.1. Current survey technique). The DOW's position is that findings from these surveys are valid for two years; if proposed activities are delayed beyond two years of when the survey was conducted, then additional surveys may be necessary for staff to appropriately assess the potential impacts of the action.

As not all of Ohio has been adequately surveyed for the Massasauga, a range map showing where the species is known to occur and where it may potentially occur has been created using the same 3-mile hexagon units described below (1.8.1. Sensitive data). This map is used for environmental review procedures to determine if a project is within an area where the species is known to occur or where it may occur, but the area has not been adequately surveyed. In addition, a flowchart has been developed to aid reviewers in making recommendations during environmental reviews. Finally, should impacts to the Massasauga be unavoidable even after appropriate minimization and avoidance measures are taken, or if a project timeline does not permit for a survey to be conducted and instead the presence of the species is assumed, a snake exclusion procedure has been developed to ensure no Massasaugas are harmed during project construction. The range map, flowchart, and snake exclusion procedure are provided as Appendix B. Environmental review tools.

8.1. Sensitive data

In order to strike a balance between providing data to clients that may help avoid, minimize or mitigate impacts to listed species populations while at the same time preventing the unauthorized release of data, especially for certain sensitive species like the Massasauga, the DOW enacted Policy 52 concerning the sharing of species location data. For requests related to a single project site, the client will be told that their project is within a 1-mile radius of an Massasauga occurrence. To hide specific locations for larger projects or for the file sharing service, the state has been divided into 3-mile hexagon units within the Ohio Natural Heritage Database and if a sensitive species is located within the project boundary, the client will receive the shaded hexagon without information on its specific location.

Ohio Revised Code 1531.04 permits sensitive site locations for wildlife and unique natural features to be excluded from public records if the Chief determines that the release of the information could be detrimental to the conservation of a species or unique natural feature.

9. *Human-Massasauga conflicts resolution*

Occasionally, Massasaugas will turn up in areas where they come into conflict with people usually resulting in calls to local wildlife officials. The person receiving such a call should remember that greater than 80% of reported Massasauga sightings that are investigated turn out to be more common, non-venomous species (see 1.3. Similar and confusing species). Ask the caller if they are able to safely take and send a digital photograph via email or text message so that the animal can be properly identified.

The public should always be reminded that the Massasauga is a venomous – and therefore potentially dangerous – wild animal. Children and pets should be kept away from the snake, and no body part should be allowed to come within striking distance of the snake (one-half of the body length of the snake). More people are bitten by venomous snakes when they attempt to capture or kill them, than are people who unintentionally come into contact with a snake.

At the same time, there is no need to cause undue panic or fear. Many landowners coexist with populations of Massasaugas on their property without incident. Bites from the Massasauga are extremely uncommon. Having a rare and endangered animal living on your property is to some a source of pride, evidence of being an excellent steward of the land and water.

To reduce potential conflicts with Massasaugas, residents are encouraged to keep their lawn mowed short, and to keep the area where they live and work free from trash and debris. Anything that provides cover, such as construction materials or brush piles, will be attractive to Massasaugas. Likewise, anything that attracts prey items (small mammals), such as seed and trash, may also attract snakes. Of course, if a landowner has a large enough parcel, leaving some areas away from the residence wild and attractive to snakes is encouraged to aid in the conservation of the Massasauga.

Figure 12. Bites to pets. Dogs are occasionally envenomated by Massasaugas, often in the nose. Most recover with only supportive therapy. Photo by Greg Lipps.

Most encounters with a Massasauga do not require any action to be taken, as the encounters are not a “conflict.” As a shy and secretive animal, Massasaugas that are seen around homes usually do not stay long, much preferring to move to more suitable habitats. Adult male Massasaugas will sometimes make long distance movements during the late summer in search of mates, causing them to occasionally be found in areas far from anything that resembles suitable habitat. Again, left alone, these individuals will usually quickly move on.

In the rare case that requires moving a Massasauga due to concerns about public safety or the wellbeing of the animal, a professional herpetologist or other individual trained in dealing with venomous snakes should be called upon. Ideally, the captured snake will be processed, collecting all of the data that is normally collected during surveys (see 1.12.3.2. Data collection) and checking the animal for a passive integrated transponder (PIT tag). Moving Massasaugas up to 200 m (656 ft.) from their capture location appears to result in only mild behavioral changes (Harvey et al. 2014), a finding that has been noted for other snake species. Moving individuals further than 200 m, however, may result in abnormal behavior (i.e., long, straight-line movements interpreted as seeking familiar territory) associated with increased mortality, and should therefore be avoided. If releasing the individual within 200 m is not feasible, then the appropriate individuals within the DOW and USFWS should be consulted, and implanting a radio transmitter to track the snake should be considered. In emergency situations, all of Ohio’s AZA accredited zoos (Cleveland, Akron, Toledo, Columbus, and Cincinnati) are capable and willing to aid the DOW and USFWS with temporarily housing Massasaugas.

OHIO RECOVERY EFFORTS

The goal of the Massasauga Conservation Plan for Ohio is to have multiple self-sustaining (viable) populations located throughout the state. Following the model of the USFWS Recovery Plan (in preparation), our goal is to ensure resiliency, representation, and redundancy in our populations. When successfully implemented, populations in Ohio will be: (1) large enough in numbers and available habitat of sufficient quality and quantity to ensure population viability; (2) all populations will have some level of protection (i.e., fee-simple ownership or easements held by an agency or conservation organization); and, (3) occupied sites will be geographically spaced so as to prevent loss due to disease outbreaks or stochastic events (i.e., floods, droughts, fire, etc.). Furthermore, as suitable habitat requires management to set back succession and prevent the establishment of invasive plant species, plans outlining the frequency, timing, and type of management as well as the responsible party for conducting this management and the monitoring of the population's response will be developed for each occupied locality.

10. *Objective 1: Protect habitat*

The majority of sites where Massasaugas are known to occur in Ohio are publicly owned, most by the Ohio Division of Wildlife. Other owners include the Ohio Division of Natural Areas and Preserves, the US Army Corps of Engineers (managed by other agencies), Ohio History Connection, Western Reserve Land Conservancy, and private owners. It is possible that snakes occur on other properties owned by other conservation partners, including The Nature Conservancy and The Cleveland Museum of Natural History. As the outright loss of habitat due to conversion to other uses (i.e., agriculture or development) or through succession is the greatest cause of the Massasauga's decline, efforts to protect additional properties where the snake occurs are of the utmost importance to the species' conservation. Tasks necessary for accomplishing this objective include:

10.1. Identify locations where the Massasauga occurs in Ohio

Explore the efficacy of the Ohio habitat model (McCluskey 2016) in predicting suitable habitat. Engage with partners to reach private landowners who may have Massasaugas on their property. Explore opportunities for reaching landowners using direct mailers and/or articles in newsletters, newspapers, magazines, and social media.

10.2. Acquire properties having Massasaugas

Identify and pursue opportunities to acquire properties with Massasaugas and/or protect these lands through conservation easements. Engage partners to fulfill this task, including county SWCDs, park districts, NGOs, and land trusts, including the Western Reserve Land Conservancy and The Nature Conservancy. Successes in protecting and managing newly documented populations have been the result of building relationships of trust with private property owners, and understanding their interests and motivations.

As vegetative succession will naturally render areas unsuitable over time, it is important that agencies or organizations that acquire occupied sites either have the resources to devote to management, or that plans are in place for management by partners. Unlike species living in climax communities, even permanently protected sites will be temporary for the Massasauga, if not managed. When opportunities to protect sites become available, agencies and NGOs should work cooperatively to determine the entity best able to provide the management necessary to ensure continued suitable habitat.

10.3. Acquire and restore adjacent properties

Identify and pursue opportunities to expand currently occupied sites through fee-simple acquisition or conservation easements of adjacent, restorable property. Massasaugas have been seen to quickly colonize adjacent former agricultural fields at two sites in Ohio. Where acquisition is not feasible, explore opportunities to use farm bill programs to provide additional habitat, corridors, and buffers on adjacent properties.

10.4. Identify and pursue funding for property and easement purchases

In the past, funds to protect Massasauga sites have come from State and Tribal Wildlife Grants, Section 6, the Clean Ohio Fund, and compensatory mitigation.

11. *Objective 2: Manage habitat*

The goal of habitat management for the Massasauga is to maintain a mosaic of wetland and upland vegetative communities dominated by native herbaceous plants with scattered small trees and shrubs together in areas of sufficient size and quality to sustain viable populations (see 5.1. Habitat). While natural forces (e.g., beaver, storms, wildfire, etc.) can create such areas, all of Ohio's extant populations rely on management for the maintenance of suitable Massasauga habitat. Typically, areas that are left unmanaged will become unsuitable through natural succession of woody species or colonization of invasive species in 3-10 years, depending on local conditions. All management activities run the risk of having direct negative impacts on individual snakes, and managers should work to minimize these threats while promoting conditions necessary for viable populations. Management actions commonly used to accomplish this objective include mowing, prescribed fire, and herbicide applications.

Effective and timely management of Massasauga habitat is currently the greatest challenge to the recovery of the species in Ohio, with increasingly limited resources available for maintaining early-successional habitat and controlling invasive species.

There may be opportunities for managers to conduct activities targeted for other species (or suites of species) to benefit the Massasauga. For example, the National Bobwhite Quail Initiative encourages the use of light strip disking in the fall or winter on no more than 1/3rd of an area, preferably next to areas of shrubby cover, to reduce residue, create bare ground, and promote desirable broadleaf plants that produce seed (NBCI 2016). Should this be done outside of the Massasauga's activity season, while avoiding areas where the snakes overwinter (wetlands and areas with burrowing crayfish, see 1.11.1.2. Overwintering habitat), this management could be beneficial for the Massasauga. Other management, such as that targeting Woodcock, Snipe, and grassland birds can also greatly benefit the Massasauga, if conducted in a time and manner that takes into account the ecology and behavior of the snake.

11.1. Habitat components

Areas where the Massasauga occur should be managed to ensure adequate quality, quantity, and distribution of habitat for all life stages and seasons, as described below.

11.1.1. Patch size

Massasaugas in Ohio have been documented using habitat patches as small as 0.3 hectares (0.8 acres), although being located in close proximity to other suitable habitat. While the species has never been documented in forested areas in the state, they are apparently able to move through small patches of trees, as one individual marked in Ashtabula County was recaptured in an adjoining field separated by 50 m of forest. A "self-sustaining, robust population" is thought to require 50 adult female snakes (Szymanksi et al. 2016), which in turn would likely require **suitable habitat of at least 50 contiguous hectares (125 acres)**, assuming a population density of 2 adults per hectare and a sex ratio of 1:1. Realization of this 50-ha goal within many of

Ohio's occupied sites may not be realistic, and general rules of thumb for minimum viable population size (MVP) such as the 50 females cited above have been called into question (e.g., Shoemaker et al. 2013). Where this goal is not feasible, populations will likely be more susceptible to stochastic events (e.g., fire, flooding, disease) and inbreeding depression, requiring more active and vigilant management.

11.1.2. Overwintering habitat

Massasaugas survive the winter by avoiding freezing, usually in burrows that allow contact with groundwater. As suitable overwintering sites may be concentrated into only one or a few areas, research should be conducted to **determine the location of overwintering sites** (i.e., radiotelemetry). Lowering of the water table, either from drought or drainage can cause a significant increase in mortality. Managers should seek opportunities to **restore natural hydrology** at sites, including disabling drain tiles and drainage ditches, which will ensure adequate groundwater levels for overwintering, encourage colonization by burrowing crayfish, and - in some cases - reduce invasive plant species and succession. **Management activities at overwintering sites should be avoided during the time snakes are emerging in the spring**, as they are particularly vulnerable (sluggish) and populations are often concentrated. As the earthen burrows are susceptible to collapse (Smith 2009), **the use of vehicles and machinery in overwintering sites should be avoided** when snakes may be in the burrows and the ground is not sufficiently frozen.

11.1.3. Foraging habitat

It is unknown if food availability is a limiting factor in Ohio's Massasauga populations. Analysis of hair found in feces and stable isotopes from scale clips indicates that Ohio's populations are feeding mostly on shrews, voles, and mice. Management should **encourage at least some seed-producing grasses** within Massasauga habitat. One study found that meadow vole density increased rapidly with the proportion of grasses, but reached an asymptote at 40% grass cover (Adler and Wilson 1989).

11.1.4. Basking habitat

There is increasing evidence that basking habitat - areas where solar radiation can reach the ground allowing snakes to warm to their preferred temperature - may be a limiting factor in Massasauga habitat suitability (Johnson et al. 2016). As basking is a necessary but dangerous activity that puts snakes at risk of predation (especially by raptors), sites should **provide areas of short vegetation or bare ground in close proximity to areas of refuge** (see 11.1.5. Refuge habitat). This can be accomplished by encouraging more "patchy" prescribed burns, mowing of only a portion of large sites at a time, and/or leaving the occasional woody species (e.g., Dogwood, Blueberry, etc.) during management. Gravid females have especially demanding thermal requirements, and because they usually do not travel far from their overwintering sites until after parturition, **ensuring adequate basking sites near overwintering sites** is also important. Preliminary results of Digital Image Vegetation Analysis (DIVA) at occupied Ohio sites in June found that ideally when a 1 m^2 whiteboard is photographed from 5 m away and at a 1 m height, 60% of the board will be visible, on average. DIVA is a visual obstruction method for measuring both the height and thickness of vegetation, similar to (but more robust than) the Robel pole technique. For more information, see Appendix D. Massasauga habitat assessment.

11.1.5. Refuge habitat

Raptors appear to be a primary predator of Massasaugas, and to escape these predators as well as extreme temperatures, snakes will use underground burrows, thatch, and shrubs as refugia. Management should **ensure that small shrubs remain scattered throughout fields**, by encouraging more "patchy" prescribed burns and mowing only a portion of large sites at a time.

11.2. BMPs for Prescribed Burning

Generally, prescribed fires in areas inhabited by the Massasauga are only scheduled when snakes are underground during their overwintering period to reduce incidental take. Based on data collected by Doug Wynn at Killdeer Plains Wildlife Area, the Ohio Division of Wildlife previously produced Best Management Practices that directed managers to not conduct prescribed burns in areas with known Massasauga populations once the ground surface temperature is 60°F or higher for 4 consecutive days (Appendix C. Prescribed burning BMPs).

Recent research led by Northern Illinois University (Hileman 2016) indicates that a more predictive model of when snakes become active above ground comes from taking temperatures at 30 cm and 60 cm below the surface. During the winter, temperatures are warmer at the lower depth. In the spring, when this relationship inverts (30 cm temp > 60 cm temp) snakes usually leave their burrow. To avoid mortality from burning, Hileman (2016) recommends ending the burn season once the soil temperature at 30 cm has exceeded that at 60 cm for 10 days. It is anticipated that the findings of this USFWS-sponsored research will be incorporated into future guidance on burning Massasauga occupied sites (i.e., the Service's Biological Opinion). Temperature logging units used in Hileman's research were in place at Killdeer Plains Wildlife Area and on private property in Ashtabula County. The addition of cellular devices to transmit this data and solar panels for power should be further investigated, as well as adding loggers to cover sites in SW and north-central Ohio.

11.3. BMPs for Mowing

The Division of Wildlife's current BMPs for mowing are based largely on the findings of research conducted on the Plains Gartersnake (*Thamnophis radix*), another state endangered snake that is sympatric with the Massasauga at Killdeer Plains Wildlife Area. The BMPs include the following recommendations:

- *Spot mow rather than full field mowing.*
- *Mower decks should be set at 6 inches (preferably 12 inches) or higher.*
- *In the summer, mow from 11 a.m. to 3 p.m. when reptiles are least active.*
- *Consider mowing in rows (e.g., back and forth) as opposed to circular mowing (where you finish in the middle of the area). This mowing method allows ample opportunity for snakes to seek refuge away from the area being mowed.*

- *Mowing conducted from June - August should be done during the heat of the day (11am – 3pm) to coincide with the snake's period of inactivity and mowing done before June and after August should be done in the mornings before 11am again to coincide with the snake's period of inactivity.*

The findings of researchers and their observations call into question the effectiveness of these BMPs for minimizing impacts to the species. Although Johnson et al. (2000) suggested that a mower deck height of 10 – 15 cm (4 – 6 in) will *"miss most Massasaugas and other snakes,"* Durbian (2006) killed 3 of 7 Massasaugas with radiotransmitters when he mowed a field to a height of 20 cm (nearly 8 inches). One of these snakes was crushed by the tractor tire, while the other two were killed by the mower blades. A fourth snake was depredated the day after mowing, apparently by a raptor.

Unlike the Plains Gartersnake, Massasaugas do not flee from approaching danger, but instead tend to rely on their camouflage and remain motionless. This behavioral characteristic makes them especially vulnerable to land management activities occurring while they are on the surface. In addition, while it is true that snakes are generally less active during the heat of the day in the summer, those that are on the surface are generally gravid females due to their increased thermoregulatory needs. These individuals have a greater contribution to the viability of populations, and, therefore, every effort should be made to reduce their incidental take.

The Division of Wildlife will update mowing recommendations based on the forthcoming Biological Opinion from the USFWS (expected by September 2017).

11.4. Herbicides

The application of herbicides is a commonly used management technique in wetlands and grasslands, especially for controlling or eliminating invasive plant species. The most commonly used herbicides contain glyphosate (e.g., Roundup®, although this formulation is not approved for wetland use). Herbicides that target woody species might be useful in temporarily setting back succession when other techniques (i.e., burning or mowing) are not feasible. Additional research on the potential impacts of herbicides to snakes (including Massasaugas) is needed.

11.5. Invasive species management

Invasive plant species have greatly reduced the suitability of many wetland and herbaceous habitats and are one of the greatest threats to the Massasauga on protected properties. For each site where Massasaugas occur, an invasive species management plan should be developed to include surveillance methods and frequency for detecting invasives and species-appropriate techniques for their control. The initial focus should be on **maintaining invasive-free areas known to be important to Massasaugas** (an early detection, rapid response focus, i.e., National Invasive Species Council (2003)) prior to moving on to addressing large established stands of invasives. At one occupied site in NE Ohio, hydrologic restoration (by disabling field drain tiles) resulted in the near elimination of an extensive monoculture of teasel.

11.6. Hydrologic management

Massasaugas are tied to wetland habitats, but too much or too little water can be detrimental to populations. Changes in hydrology can also lead to the establishment of invasive plant species, reducing the suitability of habitat. Hydrologic management should focus on **restoring natural hydrology, by disabling drain tiles and drainage ditches.** Further reductions in ground water levels, for example through deepening of drainage ditches, should be avoided. While Massasaugas can sustain short-term submersion during overwintering, longer periods of flooding have been documented to increase mortality. Where beaver occur, managers should **monitor beaver activity and resulting flooding** and take actions if these threaten Massasauga sites.

11.7. Additional Habitat Management Needs

11.7.1. Delineate population boundaries

It is currently unknown if snakes located within one management area (e.g., Killdeer Plains Wildlife Area) are a single population, multiple populations, or a metapopulation. Similarly, occupied sites in NE Ohio are often in close proximity, but gene flow between sites has not been established. By **delineating the physical boundaries of populations**, management can be directed at ensuring each population's habitat needs are met. Also, delineating population boundaries will permit estimation of demographic parameters, which are necessary for predicting population viability. The recent Population Viability Analysis (Faust et al. 2011) treated each site as a population, an assumption that may be invalid and could result in erroneous conclusions. For example, snakes residing on a small patch of private property may be deemed as a not viable population, when they may actually be part of a much larger (and viable) population extending over multiple parcels.

11.7.2. Update BMPs

In partnership with USFWS, **update DOW's prescribed burning and mowing recommendations** based on the Service's Biological Opinion (expected in September 2017).

11.7.3. Develop partnerships for management

Reach out to partners, especially other organizations having Massasauga-occupied sites, to encourage species-appropriate management and monitoring. **Explore methods for cooperative management agreements to ensure all sites are being managed** for succession and invasive species, including those on private land. Identify additional sources of funding to pay for routine maintenance of habitat to control succession and invasive species.

11.7.4. Investigate effectiveness of management practices

Do burning, mowing, and herbicides all provide the same quality of habitat for Massasaugas?

11.7.5. Research link between management, habitat quality, and population health

Continue research to link habitat management and resulting changes in habitat components and characteristics to Massasauga population parameters (see Objective 3).

12. *Objective 3: Develop an adaptive management framework*

> *Adaptive management is "learning by doing" (Lee 1999). It is a way of thinking about and implementing natural resource management that recognizes our understanding of ecosystems (even simple ones at small scales) is very incomplete and that any management we impose on the system is essentially an experiment (Gunderson 1999; Walters and Green 1997). There are three goals of adaptive management: 1) manage currently to the best of our knowledge, 2) learn from management, and 3) improve management in the future. In adaptive management, learning is as important as doing – monitoring is as important as management.*
>
> --- Elzinga et al. 2001. Monitoring plant and animal populations: a handbook for field biologists. Blackwell Science, Inc. Malden, MA, USA.

Adaptive management is identified as the cornerstone of Ohio's State Wildlife Action Plan (Ohio Division of Wildlife 2015). Understanding the link between management actions and resulting changes in habitat conditions to the viability of Massasauga populations is critically important for the recovery of the species. While the general habitat requirements of the Massasauga are well known - perhaps better than any other snake species (see 5.1. Habitat) - the actual linking of management and habitat to the viability of populations has proven difficult to quantify (Johnson et al. 2016). Central to implementing an effective adaptive management strategy is the necessity of repeated collection of data using standardized techniques to allow for comparisons over time and between sites. This includes data on: (1) Habitat characteristics; (2) Management actions; and, (3) Population responses.

12.1. Habitat assessment techniques

Smeenk and Lipps (2015; Appendix D. Massasauga habitat assessment) developed an initial methodology for collecting habitat data in relation to the Massasauga, called the Ohio Massasauga Habitat Assessment Method (OMHAM). The method borrows heavily from the BBIRD protocol (Martin et al. 1997), using random 5 meter circular plots to characterize vegetation and structure within fields. The data collected is meant to measure and assess characteristics which are thought to influence thermoregulation, crypsis, foraging, and refugia as they relate to Massasaugas. The suitability of habitat for the Massasauga is hypothesized to be highest at sites that provide: (1) high quality basking areas (high solar insolation); (2) in close proximity to adequate refugia from predators; (3) suitable overwintering sites; and, (4) habitat for prey species (dense vegetation, thatch, and burrows).

12.2. Data on management actions

To understand how management impacts snake populations directly and through changes in the habitat, it is necessary that all management actions be faithfully recorded. At a minimum, this should include:

1. Location of the field(s), preferably a polygon shapefile or kml (Google Earth) outline of the area where the management was carried out. If this is not provided, then a detailed description of the site location from a fixed point (i.e,

the nearest intersection), the area (hectares) of the field, and a paper map outlining the approximate boundaries.

2. The date, start/end time, and general weather conditions (temperature, precipitation) when the activity occurred.
3. The objective of the management (i.e., controlling woody species, invasive species management, establishing/increasing grasses, etc.).
4. The management technique employed (prescribed burn; mowing; herbicide type, concentration, and application; hand cutting; manipulation of hydrology, etc.).
5. A measure of the scale and effectiveness of the activity, preferably quantified (e.g., 90% of area burned; 75% of RCG - located in three large patches - sprayed; beaver dam removed resulting in drainage of standing water which covered 10% of the NE corner of the field for past 30 days).
6. Any searches conducted afterwards to look for snake mortality, and the results of those searches.
7. Before and after photographs of the field taken in the same location and facing the same direction.

12.3. Monitoring Massasauga populations

While population monitoring can be carried out for a variety of reasons, here it is presented in the context of the adaptive management framework. Specifically, Massasauga populations are monitored in order to understand how they respond to management activities (or lack thereof) and the resulting habitat changes, so that these activities can be further refined ("adapted") to achieve the overall goal of population viability.

12.3.1. Current survey technique

The currently accepted survey technique for the Massasauga in Ohio uses artificial cover objects consisting of metal barn roofing material ("tin" 2' x 6-8') placed in transects within the field being surveyed. Tin density usually ranges from 5-7 tins/ha (2-3/ac). Tins are generally placed prior to snake emergence (mid-April). These tins are then checked by carefully lifting one side with a snake hook or tongs and capturing any Massasaugas that have taken refuge under the tin (Figure 13). Surveys (checks of tin) take place during the activity period of the Massasauga (mid-April through mid-September, depending on latitude and local conditions). Weekly checks (~25 surveys) without detecting Massasaugas has been interpreted as evidence of its absence.

In addition to artificial cover, the Massasauga can also be located through visual encounter surveys (VES) that are carried out in addition to, or concurrent with, checks of artificial cover. VES is especially effective when snakes are first emerging from their overwintering sites, as vegetation is low and snakes spend a great deal of time basking. Methods for conducting VES vary, but generally involve slowly walking through fields searching for exposed snakes. As exposure is linked to basking, VES is generally more effective earlier in the season and earlier in the day, when ambient temperatures are lower than the preferred body temperature for the species.

Figure 13. Artificial cover. Sheets of old barn roofing (tin) are attractive to snakes for their warmth and refuge. Photo by Greg Lipps.

Based on 2-years of data from multiple sites in NE Ohio, we estimated that the probability of detecting a Massasauga where they occur exceeds 90% when surveying for at least 1.5 person-hours/hectare and using artificial cover (tin). In Illinois, detection of Massasaugas using VES increased when surveys: (1) began earlier (c. 8 AM); (2) had a warmer start time; (3) had a lower UV index; (4) occurred on days when the maximum temperature for the previous three days was cooler - a mean of 11ºC (51.8ºF) was ideal; and, (5) the humidity at ground height was low (Mike Dreslik, pers. comm.). Additionally, Dreslik found having fewer people work for a longer time was more effective than multiple people surveying for a shorter duration, but effectiveness of surveyors dropped after 3 hours of searching.

12.3.2. Data collection

The following information should be collected once at each site surveyed for the Massasauga:

1. Location of the field(s), preferably a polygon shapefile or kml (Google Earth) of the site being surveyed. If this is not provided, then a detailed description of the site location from a fixed point (i.e, the nearest intersection), the area (hectares) of the field, and an outline on a paper map.
2. Location and number of artificial cover objects, preferably a point shapefile or kml (Google Earth) file made from GPS coordinates taken at each tin location.
3. Representative photograph(s) of the site and vegetation.

With each survey event, the following information should be collected:

1. Date, begin time, end time, and number of participants involved in the survey.
2. At the start of the survey: the ambient temperature at waist height in the shade, the humidity at ground level, and the UV index.
3. Method(s) employed (checking tin and/or VES).
4. The number of Massasaugas encountered (indicate captured, escaped, and total).
5. All reptile species encountered at the site.

When a Massasauga is encountered, the following information is recorded:

1. Location, consisting of the latitude and longitude from a GPS unit.
2. Behavior, including whether the animal was using artificial cover.
3. Snout-vent length.
4. Total length.
5. Mass.
6. Sex (include a count of the subcaudal scales).
7. Number of rattle segments and if the rattle is complete (still having the original button) or incomplete (broken).
8. Color pattern: blotched/patterned or melanistic.
9. Gravid or not (adult females only).
10. The presence and description of any abnormalities, deformities, or scars.
11. Samples collected, including snake fungal disease swab, blood, scale clips, feces, etc. and the corresponding vial/container ID. Every effort should be made to collect a blood sample from each captured snake for genetic analysis. This is most easily accomplished by collecting c. 0.1 mL of blood using a hypodermic needle inserted into the caudal vein posterior to the cloacae (Figure 14). Blood should be placed into a vial of 95% ETOH and stored in a freezer while awaiting transport to the Gibbs' laboratory at OSU.

Figure 14. Blood collection. A hypodermic needle is used to collect blood from the tail of a Massasauga while its head is safely restrained in a tube. Photo by Greg Lipps.

12. First capture or recapture of a previously marked snake.
13. Passive Integrated Transponder (PIT) ID. All Massasaugas having a SVL >20 cm should be marked using a PIT placed under the skin, anterior to the cloaca near the midline of the snake. To ensure the PIT is retained, a drop of surgical adhesive is placed over the injection site. In accordance with DOW regulations, PITs should be 125kHz, 134.2kHz, or 400kHz.

12.3.3. Considerations for future surveying and monitoring

12.3.3.1. What are we trying to measure?

This is the critical first question for each and every survey, and the answer will certainly be different across sites and time. Similarly, this will alter the timing, frequency, intensity, and methods used to survey and monitor. While the current surveying techniques (12.3.1) have proven successful in detecting Massasaugas and the use of tin has increased capture rates, future surveying and monitoring should be more explicit in detailing: (1) the question being asked; (2) the data required to answer that question; and, (3) the best technique(s) and resources required for acquiring the data.

Within the adaptive management framework, *What are we trying to measure?*, is not yet an answered question. Ideally, for each site we would be able to generate a population estimate of sufficient precision in order to detect changes and correlate this to management and habitat conditions. But detection probability is notoriously low for reptiles, particularly snakes, meaning that inferring population size (or even relative abundance) from traditional capture-mark-recapture (CMR) surveys may not be feasible (see Steen (2010) and Durso and Seigel (2015) for excellent treatments of this topic). Specifically, catch per unit effort (CPUE) has been demonstrated to be an extremely poor predictor of actual population size and increasing the duration of CMR surveys may only increase the amount of low precision data collected (Steen 2010).

12.3.3.2. Alternative measures of snake response

If CMR surveys using tin and VES are not providing the data to measure population sizes with sufficient precision, then perhaps alternative measures of snake response should be considered for monitoring Massasauga populations in Ohio. Some of these possibilities are explored below.

12.3.3.2.1. Proportion of areas occupied

The ability to detect at least one Massasauga where they occur appears to be high using tin and VES. Estimates of detection probability can be used to develop occupancy models to monitor the proportion of habitat (herbaceous fields) being utilized as another method of assessing populations (Figure 15). By accounting for the covariates found to be significant in the detection probability model, non-detection of Massasaugas can be confidently interpreted as the species being absent from the field. There may also be opportunities to explore less labor-intensive methods for determining presence-absence, such as the use of camera traps in conjunction with drift fences. This technique was successfully used to document the closely related Pygmy Rattlesnakes (*Sistrurus miliarius*) and several other snake species in Florida (Martin et al., in press).

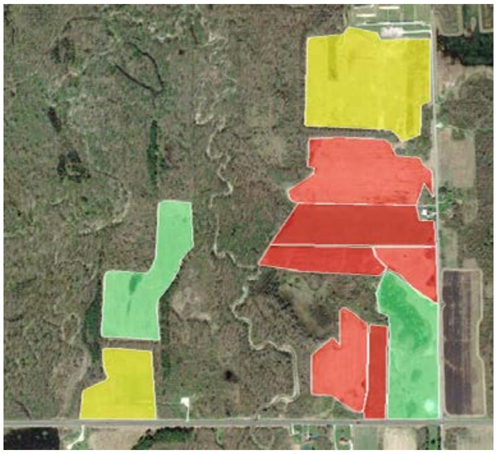

Figure 15. Proportion occupied. Fields are color-coded by snake presence. Green=occupied. Yellow = not occupied. Red = unavailable/row crops.

12.3.3.2.2. *Body condition index*

Body condition Index (BCI) is assumed to influence an animal's health and fitness and is measured by comparing the mass of an animal to its length (or values derived from these measures, i.e., modified BCI). The assumption (untested for Massasaugas) is that higher BCI relates to higher fitness, which in turn can be related to the quality of the habitat and effectiveness of management actions.

12.3.3.2.3. *Demographic parameters*

Healthy Massasauga populations are assumed to have a 1:1 sex ratio and include adults, juveniles, and neonates. Adult females are thought to give birth biannually in most populations. Yearly adult survivorship averages 0.67 (Jones et al. 2012). In many declining wildlife populations, changes in these demographic parameters are more profound and thus easier to detect than changes in population size.

12.3.3.2.4. *Disease, health, and wellness*

While the increasing role of veterinary medicine in wildlife conservation is an often discussed topic, the reality is that attempts to measure parameters of reptile health - whether they be parasite loads, blood values, or disease prevalence - has not yet been translated into data useful for monitoring the overall health of populations. Should this be attempted in the future, engaging with researchers willing to develop new species-appropriate tests and methods will probably be necessary, instead of relying on available methods which are usually developed for other taxa or domestic animals.

12.3.3.2.5. *Habitat use and home range size*

Tracking snakes with implanted radiotelemetry transmitters can provide insight into the habitat being used and the amount of area snakes are using to fulfill all of their life history requirements. For example, knowing that snakes are overwintering within the managed area versus moving off site to an unprotected area is evidence of successfully providing protected winter habitat. Providing protected habitat for *all* aspects of the life history of the Massasauga (see 11.1. Habitat components) is an important component of ensuring population viability. Similarly, the success of management intended to provide basking sites could be demonstrated through a reduction in previous long distance movements to reach basking sites (an energy-expensive and dangerous activity that likely reduces annual survivorship).

12.3.3.2.6. *Genetic tools*

Modern molecular techniques can provide information on populations including: effective population size, signs of inbreeding depression, and low genetic diversity, including the loss of rare alleles (Chiucchi and Gibbs 2010).

12.3.3.2.7. Combination of measures

On their own, each of the above measures is unlikely to provide the sort of rigorous monitoring required in an adaptive management framework. Together, however, and especially with estimates of population size (however imprecise), the combined data may provide sufficient evidence of the status of a population necessary to inform management decisions. An example of a worksheet combining all of these metrics for assessing a site is provided as Appendix E. Combined measures worksheet.

12.3.3.3. Alternative techniques for population monitoring

If the answer to the question of *"What are we trying to measure?,"* is population size (and changes to that value), then increasing sampling effort and expanding techniques beyond artificial cover and VES will be necessary to provide estimates of reasonable precision. These techniques, discussed below, are necessarily more time and labor intensive, and thus will require greater resources to implement and are likely not an efficient use of the current limited resources for monitoring all occupied sites in the state.

12.3.3.4. Drift fences and funnel traps

Bartman et al. (2016) found that using drift fences paired with funnel traps was 6 times more efficient for capturing Massasaugas and 28 times more efficient for capturing male Massasaugas than VES. Cover objects (wooden boards in this study) were more effective than VES, but were female-biased, similar to our findings of using tin. Drift fences require construction of

Figure 16. Drift fence and funnel trap. A setup in Florida for capturing snakes. Photo by Rex Rowan. http://fieldguide.blogs.gainesville.com/701/snake-trapping/

funnel traps, ground disturbance to install the fences and traps, and daily or twice-daily checks and repairs of the traps and fence.

12.3.3.5. Intensive VES surveys at emergence

Some researchers in other states rely on intensive visual encounter surveys during the early spring emergence period to capture Massasaugas. These are usually conducted with larger groups of individuals spending several days during optimal conditions, but often result in a similar number of captures and recaptures compared to Ohio's current methods (12.3.1. Current survey technique). Paired with the current season-long surveying, conducting early season Massasauga survey "blitzes" could increase capture rates to provide for more precise population estimates.

Figure 17. Spring intensive survey. Biologists search for Massasaugas emerging from their overwintering burrows on April 5 at a site in Missouri. Photo by Greg Lipps.

12.4. Analyze and adapt

The final step in the adaptive management framework is to analyze the data collected (habitat conditions, management actions, and population responses) to determine changes to be made to the recovery program and prioritize future activities. To fully implement this action, the following recommendations are made:

12.4.1. Refine the current OMHAM

Include the minimum data necessary for adequately measuring habitat quality. (Additional data collection may be required for this to be accomplished.) Explore opportunities to collect this data at all occupied sites, by employing seasonal technicians, training DOW staff, or enlisting volunteers. Assign one individual the responsibility for compiling and analyzing the data each year.

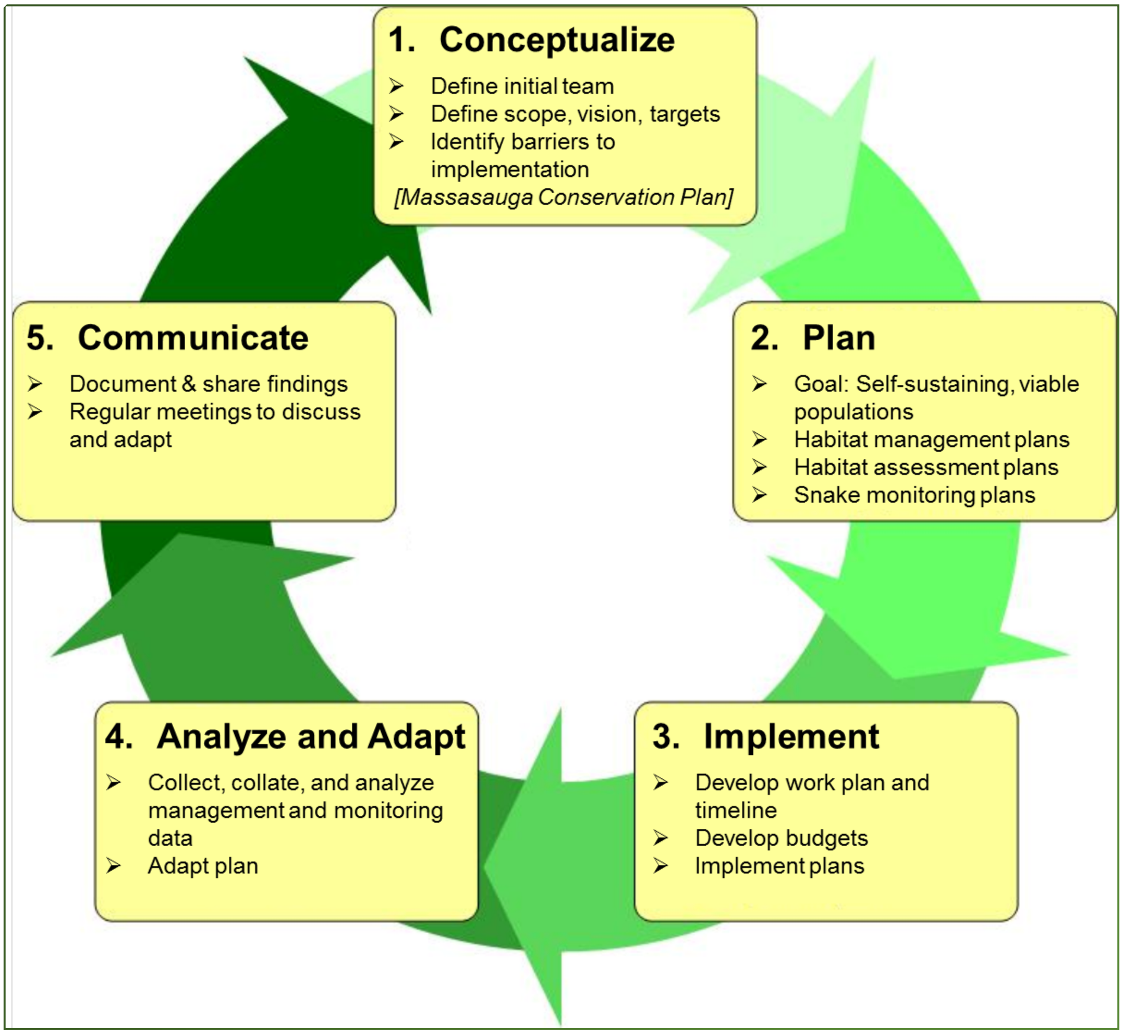

Figure 18. The cycle of adaptive management. Modified from the Conservation Measures Partnership Open Standards (**http://www.conservationmeasures.org**).

12.4.2. Collect and analyze management data

At the end of each field season, have land managers provide data on all management activities to one individual for compilation and analysis.

12.4.3. Collect and analyze Massasauga data

At the end of each field season, have researchers provide Massasauga surveying and monitoring data to one individual for compilation and analysis.

12.4.4. Schedule regular meetings of stakeholders

Hold twice yearly meetings (pre- and post-season) of all the individuals involved in management, research, data collection, and analysis to review findings, propose activities, and finalize yearly plans.

13. *Objective 4: Monitor and mitigate disease impacts*

There is no evidence that disease is currently playing a role in the decline of Massasauga populations in Ohio. However, given the reports elsewhere of catastrophic mortality associated with Snake Fungal Disease (see 7.10. Disease), researchers and managers should be on guard for this or other emerging pathogens.

13.1. Disease surveillance

Snake Fungal Disease has now been documented on multiple species and in nearly every portion of the state, including at two sites where Massasaugas occur. Researchers should – as part of their normal surveying – record any scarring, abnormalities or deformities observed on captured Massasaugas (see 12.3.2. Data collection). Familiarity with how the disease presents is important and is described further in Appendix A. Snake Fungal Disease fact sheet. When a snake is captured with symptoms associated with SFD, a swab of the area should be collected and the swab stored in a cool place (on ice in the field, then in a freezer) prior to submission for analysis (Figure 19). Swabs are analyzed using qPCR by Dr. Matt Allender in the Wildlife Epidemiology Lab at the University of Illinois.

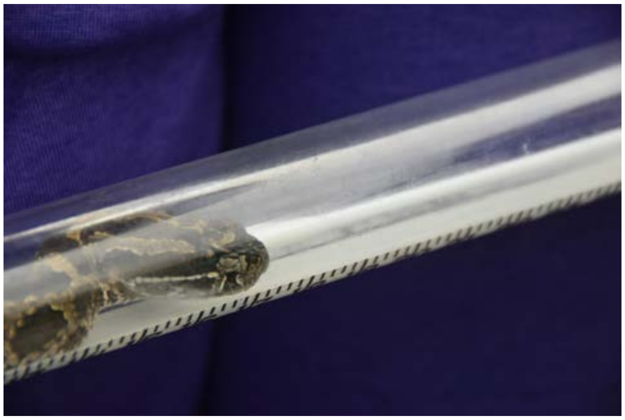

Figure 19. Snake Fungal Disease surveillance. A sterile swab is used to collect a sample from the loreal pit of a Massasauga. Photo by Greg Lipps.

Reports of snake die-offs or multiple individuals presenting with unusual symptoms should be further investigated. In such a case, engaging with experts such as Dr. Allender or the staff of the USGS National Wildlife Health Center is recommended.

13.2. Biosecurity requirements

The Ohio Division of Wildlife requires the implementation of basic disinfecting procedures designed to prevent the unintended spread of pathogens between sites. At a minimum, persons conducting herpetofauna field activities must adhere to the following protocol: Once sampling is complete and before moving to a new site, all field equipment (i.e., boots, rubber gloves, nets, traps, tripods, water quality instruments, etc) that comes into contact with animals, surface waters, or soils shall be washed and disinfected. All debris and mud must be scrubbed off prior to disinfectant application, because organic matter and soil can reduce its effectiveness. Disinfection is accomplished by putting 4 ounces of bleach in 1 gallon of clean water and using the solution to rinse off all field equipment prior to going to another site. The bleach solution should be allowed to evaporate from the equipment, or rinsed off after a minimum of 15-minutes of contact.

When field work is completed for the day/night, equipment and personal gear should be thoroughly washed and disinfected again. Equipment and gear should be hung and allowed to completely dry. In many cases, drying serves as a means of inactivating pathogens. Bleach breaks down with exposure to air, sunlight and organic material, thus bleach solutions should not be stored or used after 5 days. An illustrated guide to disinfecting procedures is provided as Appendix F. Illustrated disinfection protocol.

13.3. Other disease concerns

While Snake Fungal Disease is currently receiving much of the attention, it should not be forgotten that there are other reptile diseases of concern, some of which are particularly deadly to pit vipers (e.g, ophidian peromyxovirus). Emerging pathogens that have not yet been identified should be as much – if not more – of a concern than those we are aware of. Researchers and anyone who comes in contact with snakes or their habitats should carefully follow biosecurity requirements, even when there is no evidence of disease.

Of particular concern to biosecurity are the unauthorized visits by herp enthusiasts to Massasauga sites. One individual photographed making an unauthorized visit to an Ohio Massasauga site carried a very distinctive field hook; on social media, a photo of him was discovered using the same hook to capture a Central American pit viper. We are aware of unauthorized visits by herp enthusiasts to at least four Massasauga sites in Ohio. Many of these individuals have captive collections of exotic snakes, raising further the possibility of their transmitting novel pathogens into Ohio's Massasauga populations. Law enforcement should be made aware of occupied Massasauga sites and their cooperation sought to enforce endangered species and trespassing laws and regulations.

14. *Objective 5: Reduce poaching*

Like disease, the extent to which poaching is impacting Ohio's Massasauga populations is unknown. Nevertheless, it is a concern, especially when artificial over objects (tin) are located, which can aid a poacher in the same way that it aids a researcher. DOW-sponsored researchers have been provided with stickers to be placed on their artificial cover objects identifying these as part of ongoing wildlife research projects and warning that it is a crime to disturb them.

We are aware of individuals approaching landowners near two occupied sites asking to either collect Massasaugas or offering to pay for snakes. On several other occasions, herp enthusiasts (not necessarily poachers) have been found to be visiting sites to search for Massasaugas for fun and photographs. Aside from biosecurity concerns, these individuals often share their exploits on herp-centric social media platforms, like fieldherpforum.com. Photos of Massasaugas under Ohio researchers' artificial cover objects have appeared on this forum multiple times.

While supporters of these sites often point out that posting of exact locations is prohibited, connections made among members often lead individuals to meet and visit sites together. One Ohio fieldherpforum.com user has twice brought people from out of state to a researcher's Ohio site without permission of the researcher or the DOW. The danger of this activity was brought to light during the USFWS' Operation Shellshock. An individual arrested for selling 33 Massasaugas to an undercover agent first learned of the location where he poached the animals from a fieldherpforum.com user who wouldn't reveal the location on the forum but agreed to take him to the site for what was supposed to be an opportunity to photograph the snakes. The poacher later returned to collect the snakes to sell and trade for other endangered reptiles (Figure 11).

As locations where Massasaugas can be found are greatly sought after by poachers and enthusiasts, exact localities should be treated as sensitive data that is not disclosed (see 8.1. Sensitive data). Individuals allowed to accompany researchers to sites should agree to strict confidentiality of locations, not to return without permission, and to ensure that photographs shared or posted to social media are stripped of location information. Finally, researchers and managers should strive to improve communication with law enforcement, especially the DOW's wildlife officers and investigators, making them aware of sites and potential poaching and trespass issues.

15. *Objective 6: Reduce intentional persecution*

As with disease and poaching, the outright persecution of Massasaugas is an unknown contributor to the decline of Ohio's Massasauga populations. In the past, persecution appears to have been widespread and no doubt caused declines and local extirpations. With the majority of sites now located on protected property, however, persecution may no longer be as much of an issue. This subject is worthy of additional research by those who study the human dimensions of wildlife conservation.

15.1. Education and outreach

In order to reduce persecution, increase respect and tolerance, and encourage species-appropriate habitat management on private property, education and outreach should be directed towards landowners in areas where the Massasauga occurs. The DOW has produced a Massasauga poster as well as a large trifold display that is routinely used at events to provide information about the Massasauga (Figure 20).

Figure 20. Massasauga poster. This large, tri-fold, portable display, developed by the Ohio Division of Wildlife, supports conservation education and outreach efforts.

Appropriate messaging and methods of contact for education and outreach would be best addressed by experts in human dimensions research. Christoffel (2007) conducted a series of investigations concerning people's perceptions of the Massasauga in Michigan. Her findings suggest that outreach should include efforts to minimize the perceived threats of Massasaugas. This can include providing information on the infrequency of human envenomations, the lack of any deaths attributed to the species in Ohio, and providing people with the opportunity to view Massasaugas in a non-threatening, controlled atmosphere. Graphics of the Massasauga used in outreach materials should show them in their normal (relaxed) position, not in the stereotyped coiled-rattling-and-ready-to-strike pose of an agitated snake.

Finally, studies in zoos have found that visitor's interactions with people (i.e., a zoo docent) can often be more effective at increasing positive attitudes towards reptiles than media or interactive displays alone. Identifying individuals that work within areas occupied by the Massasauga (e.g., SWCD staff, park naturalists, etc.) to deliver a very simple message about Massasaugas to landowners may be a more effective and practical method of education and outreach than producing and distributing more brochures, websites, and other media.

16. *Objective 7: Repatriations and augmentations*

Repatriation (releasing animals to an environment where they once occurred) and augmentation (adding animals to an extant population) have both been considered as possible strategies for the conservation of the Massasauga. There are several obstacles – both biological and political – to these strategies, however, that would first need to be overcome to consider this as a tool for conserving the Massasauga in Ohio.

The DOW has long fought an urban legend (common throughout the country) that they are already actively stocking rattlesnakes in the state. Every snake researcher has encountered individuals who have some knowledge about rattlesnakes being "dropped in balloons from helicopters" to control the coyote/turkey/deer populations. Ohio has never engaged in a stocking program for any snake other than the Plains Gartersnake, a message that has been clear and easy to communicate.

As a venomous – and therefore, potentially dangerous – animal, a program to repatriate or augment Massasaugas could potentially face opposition from local residents and politicians. Public backlash to Massachusetts' plan to release Timber Rattlesnakes (*Crotalus horridus*) onto a remote island in the Quabbin Reservoir made national news in 2016 and led the state senate to delay the plan while naming a 13-member Rattlesnake Review Group to review the plan and seek additional questions, comments and suggestions from the public. As with all education and outreach, it may be wise to engage with specialists in human dimensions to determine a course of action for public outreach prior to any such program being undertaken.

Aside from the human obstacles facing such a program, there is also the issue of the unproven nature of repatriations and augmentations as a conservation strategy for the Massasauga. King et al. (2004) reported on the first attempted repatriation of Massasaugas at a site in Wisconsin. They released a total of 31 captive-born and reared snakes (1 – 3 yr. old) to two areas in the summer and fall. Fall released snakes had much higher mortality, with 5 of 15 being killed prior to winter. There was no mortality prior to winter in the group released in the summer. Eight of the 10 fall released snakes that made it to winter died before spring, and one was killed within a week of spring emergence.

Likewise, 7 of the 15 summer released snakes died during the winter. The higher mortality associated with the fall release may have been at least partially due to late season surgeries to implant telemetry transmitters. One of the released snakes was observed mating and gave birth in March while in captivity.

In a second repatriation study in Ontario, Canada, 27 captive-born and reared snakes (3 yr. old) were released in June (Harvey et al. 2014). Nineteen survived until winter and the snakes appeared to make movements and court mates similar to wild snakes. None of the remaining snakes survived winter, though, with some dying in their burrows, others coming out during the winter and dying of exposure, and some being predated upon by mink.

The experiences provided above illustrate the potential difficulties of attempting a repatriation project. To quote King et al (2004): *"We emphasize that repatriation* may *be an appropriate conservation tool but* cannot *be fully endorsed without further study."* Should Ohio choose to attempt a repatriation project, it should be designed as a research project to determine feasibility and effectiveness, before relying upon moving snakes as a recovery action. It is also important that the resources required to carry out a repatriation program be weighed against other conservation options, such as improving habitat through management, or restoring adjacent habitat to expand the carrying capacity of populations.

There are good reasons why repatriations and augmentations should be considered. The Massasauga's apparent limited dispersal ability (Chiucchi and Gibbs 2010) and the increasing fragmentation of habitat making travel across the landscape difficult or impossible for the Massasauga, means it is likely that suitable sites will remain unoccupied without human-assisted migration (King et al. 2004). This is especially problematic at formerly degraded sites where habitat has been restored or where persecution has been curtailed, but there are no nearby source populations to supply immigrants to naturally colonize the area. Furthermore, genetic analysis has already found evidence of a bottleneck in at least one Ohio population (Chiucchi and Gibbs 2010), indicating that augmentation may be required to improve genetic diversity.

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Appendix A. Snake Fungal Disease fact sheet

https://www.nwhc.usgs.gov/disease_information/other_diseases/snake_fungal_disease.jsp

USGS
science for a changing world

National Wildlife Health Center
Wildlife Health Bulletin 2013-02

Snake Fungal Disease in the United States

To: **Natural Resource/Conservation Managers**
From: Dr. Jonathan Sleeman, Center Director, USGS National Wildlife Health Center
Date: **April 22, 2013** (revised genus name, May 2, 2013)

Snake Fungal Disease (SFD) is an emerging disease in certain populations of wild snakes in the eastern and midwestern United States. While fungal infections were occasionally reported in wild snakes prior to 2006, recently the number of free-ranging snakes with fungal dermatitis submitted to the USGS National Wildlife Health Center (NWHC) and other diagnostic laboratories has been increasing. Laboratory analyses have demonstrated that the fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola* is consistently associated with SFD, but often, additional fungi are isolated from affected snakes. At this time, definitive evidence that *O. ophiodiicola* causes SFD is inconclusive. As its name implies, SFD is only known to afflict snakes.

To date, the NWHC has confirmed fungal dermatitis (or the suspected fungal pathogen in association with skin lesions) in wild snakes from nine states, including Illinois, Florida, Massachusetts, Minnesota, New Jersey, New York, Ohio, Tennessee, and Wisconsin. However, it is suspected that SFD is more widespread in the United States than is currently documented. Multiple species of snakes have been diagnosed with SFD at the NWHC (see attached figures; view additional photographs at http://www.nwhc.usgs.gov/disease_information/other_diseases/snake_fungal_disease.jsp), including northern water snake (*Nerodia sipedon*), eastern racer (*Coluber constrictor*), rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and milk snake (*Lampropeltis triangulum*).

The most consistent clinical signs of SFD include scabs or crusty scales, subcutaneous nodules, premature separation of the outermost layer of the skin (*stratum corneum)* from the underlying skin (or abnormal molting), white opaque cloudiness of the eyes (not associated with molting), or localized thickening or crusting of the skin (hyperkeratosis). Skin ulcers, swelling of the face, and nodules in the deeper tissues of the head have also been documented. Clinical signs of SFD and disease severity may vary by snake species. Aside from the presence of fungi with disease-associated lesions, specific pathological criteria for the disease have not yet been established.

While mortality has been associated with some cases of SFD, population-level impacts of the disease are not yet widely known and are difficult to assess due to the cryptic and solitary nature of snakes, and a general lack of long-term monitoring data. In New Hampshire, clinical signs consistent with SFD were associated with a 50 percent decline of an imperiled population of timber rattlesnakes from 2006 to 2007. In areas where susceptible snake species occur in small, isolated populations, the added threat of SFD may threaten viability of these populations. In other regions, SFD has been observed without suspected or, as yet, documented population declines.

Several agencies, organizations, researchers, and other key stakeholders, including the NWHC, are working together to investigate this potentially emerging disease and to learn more about the impacts that SFD is having on snake populations in the eastern and midwestern United States. We encourage conservation agencies and natural resource managers to contact the NWHC if snakes with clinical signs consistent with SFD are encountered.

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To report or request assistance for wildlife mortality events or health issues, please visit the NWHC Web site at http://www.nwhc.usgs.gov/mortality_events/reporting.jsp or contact Dr. Anne Ballmann, 608-270-2445, aballmann@usgs.gov; Dr. LeAnn White, 608-270-2491, clwhite@usgs.gov; Barb Bodenstein, 608-270-2447, bbodenstein@usgs.gov; Dr. Thierry Work, 808-792-9520, thierry_work@usgs.gov (Hawaii and Pacific Islands); or Jennifer Buckner, 608-270-2443, jbuckner@usgs.gov (single animal mortalties, nationwide).

To see past Wildlife Health Bulletins, click here.

WILDLIFE HEALTH BULLETINS are distributed to natural resource/conservation agencies to provide and promote information exchange about significant wildlife health threats. If you would like to be added to or removed from the mailing list for these bulletins, please contact Gail Moede Rogall at 608-270-2438 or e-mail: nwhc-outreach@usgs.gov.

Figures

Figure 1. Eastern racer (*Coluber constrictor*) showing signs of fungal skin infection. Obvious external abnormalities are an opaque infected eye (spectacle), roughened crusty scales on the chin, and several discolored roughened scales on the side of neck. Snake captured in Volusia County, Florida, in January 2013 (case 24266). Photograph by D.E. Green, USGS National Wildlife Health Center.

Figure 2. Eastern rat snake (*Pantherophis alleghaniensis*) showing signs of fungal infection. Obvious external abnormalities are an opaque infected eye (spectacle) and roughened, crusty scales on the snout. Snake captured in New Jersey in March 2012 (case 23906). Photograph by D.E. Green, USGS National Wildlife Health Center.

Figure 3. Northern water snake (*Nerodia sipedon*) with crusty and thickened scales overlaying raised blisters as a result of a fungal skin infection, captured from island in western Lake Erie, Ohio, in August 2009 (case 22747). Photograph by D.E. Green, USGS National Wildlife Health Center.

Appendix B. Environmental review tools

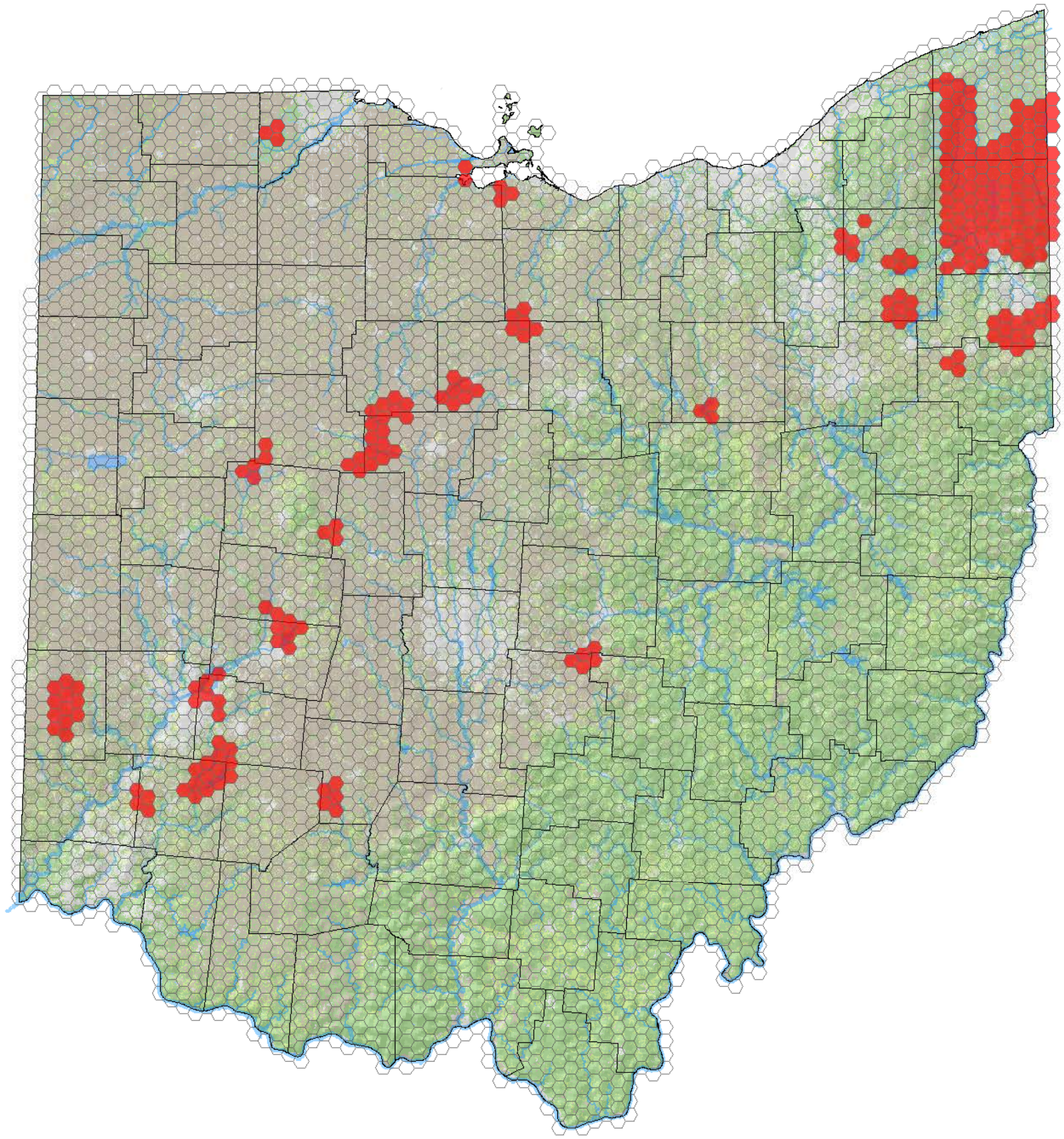

Figure B-1. Potential occurrence map for environmental review. Map of Ohio showing areas where the Massasauga may occur (red shaded hexagons) for use in environmental reviews. Shaded hexagons include areas: (1) having a recent documented occurrence of the species; (2) having an historic (>25 years) documented occurrence of the species, and the possibility that the species may still occur there; (3) having anecdotal (undocumented) reports of the species occurring, with suitable habitat still likely to exist; or (4) having what is thought to be suitable habitat, but the area has not been adequately surveyed

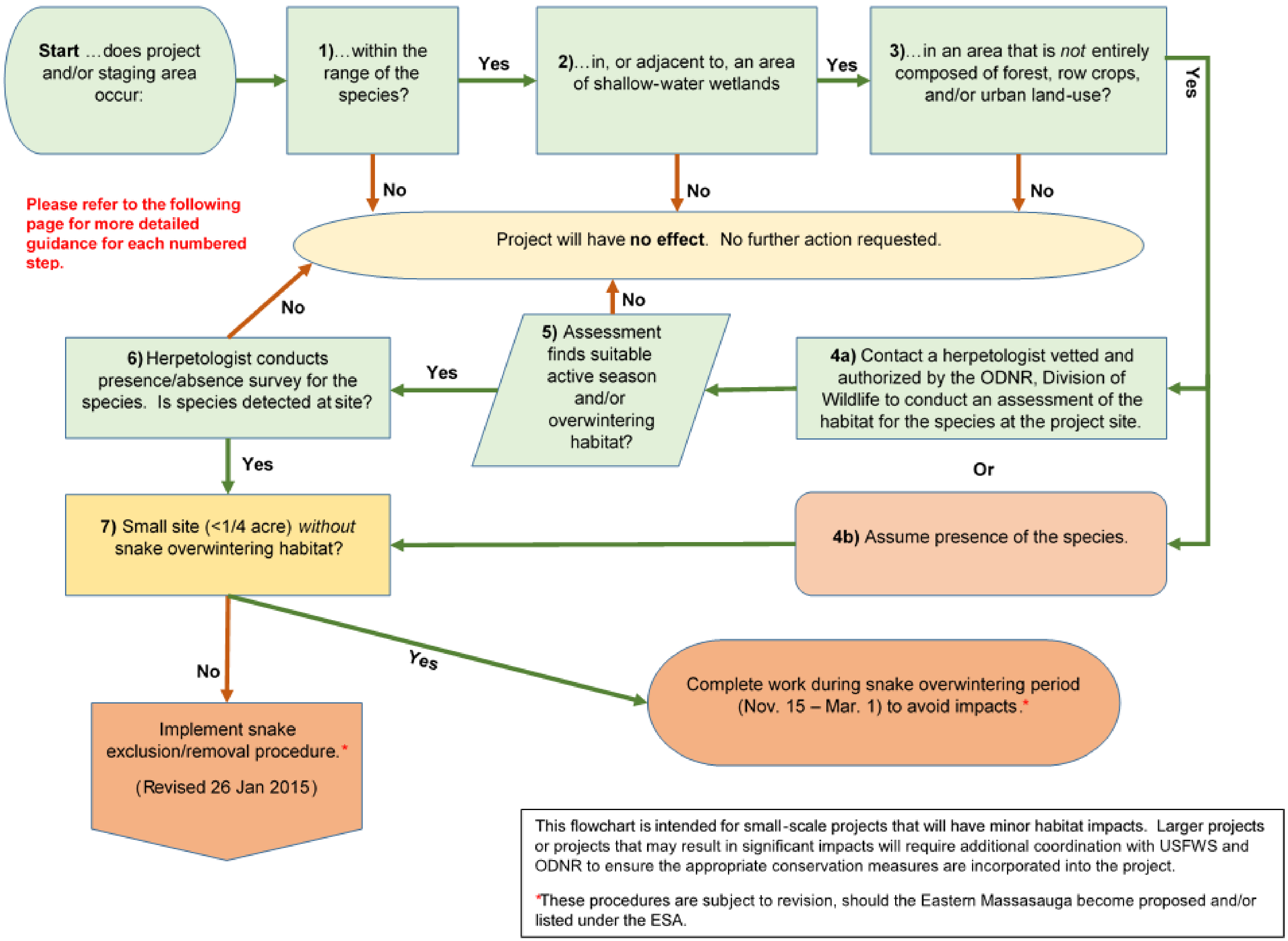

Figure B-2. Flowchart for environmental reviews. This tool, along with the potential range map (Figure B-1), is for use by reviewers to determine if proposed projects are likely to impact the Massasauga, and, if so, recommended actions to take.

Snake exclusion/removal procedure

If a survey to determine the presence or absence of Eastern massasauga rattlesnakes within a proposed project area is not conducted, the contractor/consultant or other land manager shall assume the snake is present within the project area and the following shall be implemented. These steps also apply if massasauga presence has been confirmed in the project area.

Please note that if rattlesnakes are encountered during construction, operations should cease until the snake has moved out of the area and our office should be notified immediately. *Due to the potential for the snakes to occur near this area, all workers should be instructed not to harm or kill the snakes and to use caution, as the eastern massasauga is a venomous species.*

1. Prior to initiating any activity within the project limits, mow all standing vegetation within the work limits to a height not to exceed one inch. Unless otherwise approved in writing by the Chief of the Division of Wildlife, mowing for this purpose must be done only during the snakes' hibernation period which is typically November 15th-March 1st and only on a cloudy, cool day when snakes are not likely to be above ground basking. Mowing before the snakes emerge from hibernation will make the area less attractive because there isn't any hiding cover. Mowing before emergence further ensures no snakes will be killed by the mower.

2. Before initiating any activity including but not limited to earthmoving and/or construction within the project limits, all potential Massasauga habitat must be encircled with a snake-proof barrier (silt fencing or metal flashing, at least 30 inches high above ground) that prevents snakes from crossing over or under the barrier. “Curlex” (or similar material) shall not be used in construction of the snake-proof barrier. The barrier should be buried at least 6 inches below the surface and the trench backfilled to support the barrier and prevent animals from burrowing under the barrier. The integrity of this barrier must be ensured throughout the period of activity, and breaches of the barrier must be repaired promptly. The snake-proof barrier must be in place at least 15 days prior to any activities occurring on the site, and be completed between April 15 – September 15.

3. For small linear projects that include an active roadway through the site (e.g., a culvert replacement), the preferred method is that all areas of suitable habitat be completely enclosed in a snake-proof barrier, which could potentially result in up to 4 enclosed areas. Where this is not feasible, the barrier may be extended an additional 300 ft. from the work area (or to the limit of suitable habitat) before joining the road. This alternative method assumes that the road will discourage snakes from entering into the area, and if they should, the additional length may provide some buffer from the work area.

4. *Snakes within the area enclosed by the snake-proof barrier are to be captured using cover boards (sheet metal) placed within the area and/or funnel traps placed along the fencing. Captured snakes are to be moved to the outside of the project limits, but no further than 1,000 feet from their point of capture. The capture-removal of snakes should be conducted daily for a minimum of 14 days prior to initiating any activity within the project limits. The 14 day snake capture-removal may be completed in 14 consecutive days or over a period not to exceed 28 days. If funnel traps are used, these must be checked no less than once every 24 hours.

5. After 14 days of snake capture-removal, activities may begin in the area enclosed by the snake-proof barrier, so long as the integrity of the barrier is maintained. The barrier should only be breached for a few minutes at a time to move equipment into and out of the area; the barrier must then be put back in place. Should the integrity of the barrier be compromised for more than 24 hours, it will be necessary to repeat the 14 days of snake capture-removal.

6. Furthermore, on the ground outside of the snake-proof barrier cover boards (sheet metal) must be placed around the perimeter as protection for snakes trying to access the project area. Cover sheets should be placed parallel to the fence with no more than 25 feet between each sheet.

7. *Construction activities must be conducted in accordance with guidelines outlined in the USFWS "The Eastern Massasauga Rattlesnake: A Handbook for Land Managers 2000." This handbook can be found on the U.S. Fish and Wildlife website at http://midwest.fws.gov/Endangered/reptiles/eama-mgmt-guide.pdf.

8. *If Eastern massasaugas are found in the work area, their locations must be marked on a topographic map and GPS coordinates recorded. *The snake must then be moved, unharmed outside the work limits. Contact Nathan Reardon, Compliance Coordinator, by telephone at (614) 265-6741 to report all massasaugas. Mail topographic maps and GPS coordinates to Mr. Reardon at the Division of Wildlife, 2045 Morse Rd., Building G, Columbus, Ohio 43229-6605

*The work indicated in items 4, 7 & 8 above must be performed by one of the following professionals approved by the U.S. Fish and Wildlife Service and the ODNR, Division of Wildlife:

Jeff Davis 625 Crescent Road Hamilton, OH 45013 OH 43035 ohiofrogs@gmail.com	Greg Lipps 1473 County Road 5-2 Delta, OH 43515 GregLipps@gmail.com	Doug Wynn 2375 Cross Creek Court Lewis Center, Sistrurus@aol.com

23 July 2008. Revised 26 January 2015.

Appendix C. Prescribed burning BMPs

Best Management Practices (BMPs) for Prescribed Burning on Ohio Division of Wildlife (DOW) Managed Lands

Historically fire has played an important role in creating, restoring, and maintaining certain natural ecosystems in the Midwest. Fire-adapted ecosystems, such as grasslands, oak savannas, and oak-hickory forests, depend on regular fires to control woody succession and invasive plants, as well as stimulate herbaceous species. The use of prescribed burning to manipulate habitats for the benefit of wildlife and plant species is a widely recognized and necessary land management practice. Prescribed burns are most often used by the Division of Wildlife (DOW) to establish and maintain native grasslands. It is by far, the most efficient and cost-effective habitat management tool utilized by the Division in these habitats. Other habitat management tools, such as mowing and herbicide application, do not provide the same benefits as prescribed fire. While prescribed burning is essential to the maintenance of quality habitat, it must be used responsibly to ensure that it does not have direct negative impacts on flora and fauna. There are five groups of species which deserve special consideration regarding the timing and use of prescribed burning on wildlife areas:

1) use of the area by state-listed reptiles and amphibians;
2) use of the area by ground-nesting birds;
3) use of the area by state-listed Lepidoptera;
4) use of the area by bats, particularly Indiana bats;
5) occurrence of state-listed plants.

It is likely that some level of disturbance will occur to one or more groups of these species with the application of prescribed burning. However, responsible management dictates that reasonable measures are taken to minimize any direct negative impacts that may be caused by prescribed fires. It is also recognized that in the absence of prescribed burning in these fire-dependent ecosystems, these habitats and the species they support may cease to occur on wildlife areas. Therefore, the DOW will use the following best management practices (BMPs) in the planning and execution of prescribed burns to minimize negative impacts on wildlife and plants, particularly the above groups of species. These BMPs will be incorporated into specific wildlife area burn plans to ensure they are implemented on the most appropriate wildlife areas, where occurrences of the above groups of species have been documented.

As a part of the Ohio certified prescribed burn manager program, wildlife area managers must prepare annual burn plans for each wildlife area where they intend to conduct burns. These burn plans are reviewed both by the district and central offices, and must be followed once they are approved. Each burn plan has a standard format which includes categories such as objectives, personnel and equipment, fuel descriptions, burn unit descriptions, acceptable weather conditions, smoke management, firing techniques, post-burn evaluation, and detailed maps of the burn units. In general, prescribed burns may be conducted in the spring (March-April) or fall (late October-November). Winter burns (December-February) are not effective for controlling woody vegetation, so they will typically not be conducted in this timeframe. Each burn manager must carefully consider the current weather conditions (specifically temperature, relative humidity, wind speed and direction, and mixing height), smoke management, and the predicted weather forecast before deciding to burn on a particular day.

The Division's prescribed burn managers will promote patchy burns and utilize a mosaic of burn units within a wildlife area on a burn regime of 3-5 years, depending on the condition of the habitat. If prescribed burning is not a feasible tool, or if the timing of the burn will have a negative effect on the groups discussed above, other land management methods will be utilized (i.e., herbicide application, mowing, discing, etc.). DOW prescribed burn managers will implement these BMPs and address any associated issues when developing burn plans, conducting prescribed burns, and evaluating the results of each burn.

Reptiles and Amphibians

If there is documented or suspected activity by state-listed reptiles and amphibians in a given wildlife area unit, all efforts will be implemented to conduct prescribed burning based on recommendations and current data from herpetologists submitted to the Division of Wildlife. Current data from Ohio researchers support the recommendation that prescribed burns should not be conducted in areas with known state-listed snake populations **when ground/soil surface temperatures are 60 degrees F or greater for 4 consecutive days prior to burning.** In general, burning after **April 15th** is discouraged in units where state-listed reptiles and amphibians are known to occur, however weather conditions vary greatly from year to year. Harm may be minimized for many species if unusually cool conditions (overcast, < 50 degrees F) have persisted for many days. Burns should not be conducted in close proximity to known state-listed snake hibernacula.

For wildlife areas with known populations of Eastern Massasauga or Copperbelly Watersnake *(i.e., Killbuck Marsh, Killdeer Plains, Mosquito Creek, Resthaven, Spring Valley, Willard, and Lake LaSuAn):*

On wildlife areas where there are known populations of Eastern massasauga and copperbelly watersnake, all efforts will be implemented to conduct prescribed burning based on current Ohio data and guidelines developed in partnership with herpetologists, recognized as species experts by the Division, and the USFWS-Ohio Field Office.

In 1993, the Division initiated intensive Eastern massasauga surveys and monitoring efforts at several locations. For 18 years, herpetologist Doug Wynn has collected data from more than 800 Eastern massasaugas captured across the species' Ohio range. Of these, a dataset of 121 massasaugas was assembled containing air, ground/soil surface, and soil substrate (4-6" depth) temperatures. These data represent snakes captured before June 1st, on the ground/soil surface, but not found under coversheets. Based on Doug Wynn's work, snakes may be above ground during warm sunny days in the winter. The data show that snakes move closer to the ground/soil surface from their hibernacula as springtime approaches and move in and out of their dens when conditions are optimal (also referred to as "shuttling behavior"). However, Wynn's work also shows that Ohio massasaugas are not fully emerged—spending the majority of their time on the ground surface - until ground/soil temperatures reach 60 degrees F for a minimum of four consecutive days. Until this threshold is met, the snakes are staying in close proximity to their burrow entrances coming to the soil surface and retreating to their burrows as evening temperatures drop.

Based on discussions with the USFWS-Ohio Field Office and other data from Midwestern states collected from Eastern massasauga populations, **the Division will not conduct prescribed burns in areas with known massasauga or copperbelly populations**

once the ground surface temperature is <u>60 F° or higher for 4 consecutive days</u>. Preparation of firebreaks (e.g., mowing, discing, plowing) should follow the same guidelines to minimize negative impacts to the snakes. In addition, burns should be conducted at least 50 meters (164 feet) from known hibernacula. Fall burns should be conducted after **October 30th.** Any snake mortality should be reported on the day of the burn (see DOW burn report). In addition, the attached Prescribed Burning Monitoring Plan for these snakes will be followed, with the use of the Prescribed Burn & Snake Monitoring Form for all data collection which is specified in this plan.

These conditions will apply to locations within the above referenced wildlife areas with known populations of Eastern massasauga and copperbelly watersnake. Maps of the current distributions of these two species on the 7 wildlife areas have been developed (2012-2013) and will be used in the preparation of annual prescribed burn plans. As our knowledge of known snake distribution changes, these maps will be revised and updated annually.

<u>Nesting Birds and Other Wildlife Species</u>

Native and restored grasslands, oak savannas, and oak-hickory woodlands provide important habitat for ground-nesting birds and Lepidoptera. In burn units where there is documented or suspected nesting activity or documented occurrence, care will be taken to ensure that prescribed burning will be conducted before the peak nesting time and/or early enough in the season to allow for additional re-nesting attempts for the specie(s) utilizing the wildlife area. **To minimize impacts to ground-nesting birds, burning after April 30th is discouraged.** In the case of known state-listed Lepidoptera, prescribed burning will be conducted to minimize impacts to specific life stages vulnerable to fire. Burn units should be burned on a rotational basis so that there is always similar unburned habitat left standing nearby and available to wildlife.

To minimize impacts to bats, particularly Indiana bats, the Division will follow the guidelines in the ODNR Indiana Bat Management Strategy. If prescribed burns will be conducted in forested units after **April 15th**, net surveys will be conducted to determine whether Indiana bats occur on the site.

<u>Plants</u>

If state-listed plants are known to occur in a given wildlife area unit, care will be taken to ensure that prescribed burning does not have a negative impact on the plant or its life cycle. In the case of early flowering species, a date may be incorporated into the burn plan to minimize negative impacts on emergence and flowering (e.g., small white-lady's-slipper). Some state-listed plants respond favorably to properly-timed prescribed burns (e.g., Eastern prairie fringed orchid, wild lupine), while others are intolerant of fire (e.g., Lakeside daisy, running buffalo clover). In particular, there are known populations of Eastern prairie fringed orchid (Federal threatened) and small white lady's-slipper (state endangered) on several wildlife areas; burning will not be conducted on these sites after **April 15th** unless the orchids have not emerged from the ground yet.

February 2013

Appendix D. Massasauga habitat assessment

Title: Ohio Massasauga (*Sistrurus catenatus*) Habitat Assessment Method v1.0
Date: 02/05/2015. Last revised: 03/22/2015
Authors: Nicholas Smeenk and Gregory Lipps

INTRODUCTION AND BACKGROUND

The Massasauga (*Sistrurus catenatus*) is a small-bodied rattlesnake with an adult length of 45.7 cm to 70 cm. Typical coloration of Massasaugas is gray-tan-yellow with darker spots along the dorsum with white stripes on the sides of the head. Melanistic (all black) individuals are common in some populations. The distribution of the Massasauga encompasses the Great Lakes Region of the United States and one Canadian Province, including New York, Pennsylvania, Ohio, Michigan, Indiana, Illinois, Iowa, Wisconsin, and Ontario (Tennant 2003). In Ohio, the Massasauga was historically documented from 30 counties located in the glaciated portion of the state; recent surveys, however, only documented individuals in eight counties (Wynn and Moody 2006). This range retraction is consistent with range-wide trends in population declines and localized extirpations (Symanski 1998). Reasons for historical and continued declines of Massasauga include: habitat destruction and fragmentation, overutilization and poaching, and emerging infectious diseases (Prior 1991, Symanski 1998, Allender et al. 2013). The Massasauga is a protected species throughout its range and is listed as a state endangered species in Ohio (Ohio Revised Code 1531.25) and is currently being considered for federal listing under the Endangered Species Act of 1973.

Massasaugas are associated with a wide variety of early-successional, vegetative communities across their range; in general, however, most habitats are composed of wet, grassy communities, such as wet meadows, interspersed with shrubs and adjacent to mesic grasslands or lowland forests (Fig. 1) (Symanski 1998, Tennant 2003, Wynn and Moody 2006). Historically, early successional communities, such as wet meadows, grasslands, scrublands, and forests were maintained through natural disturbances such as fires, beavers, floods, and windstorms (Askins 2001). Anthropogenic control, such as fire suppression, over-exploitation, and dams have changed or eliminated these natural regimes, leading to loss of additional habitat through succession. Continued loss of critical habitat through both anthropogenic and natural means remains the single most prevalent threat to the survival of extant Massasauga populations across their range (Symanski 1998). Understanding the macrohabitat (landscape) and microhabitat (local) structure and the distribution of remaining habitats is imperative for the continued persistence of Massasaugas and associated habitats. Through combined understanding of ecology, behavior, and habitat associations, more informed decisions can be made in regards to how, when, and where to manage Massasauga habitats.

Previous studies utilizing radiotelemetry techniques have provided insight into macro- and microhabitat selection, homerange size estimations, and daily movements of Massasaugas. Harvey and Weatherhead (2006) found that Massasaugas in Ontario select open, wetland and grassland habitats in relative proximity to retreat sites and shrubs; however, snakes were not selective at a landscape-scale, instead selecting habitat patches with suitable microhabitats. Similarly, snakes in Michigan selected open early-mid successional wetland and grasslands with low shrub and tree density (Bailey et al. 2012). The spatial ecology of the Massasauga is the most studied of any snake species, and results from across the species' range have come to comparable if not identical conclusions in regards to habitat use by these snakes (Illinois: Wright 1941; New York: Johnson and Leopold 1998; Pennsylvania: Kowalski 2007; Michigan: Moore and Gillingham 2006).

Although many studies have quantified habitat use by Massasaugas, few have quantified the importance of habitat use and availability relative to measures of individual or population health. The maintenance of appropriate body temperatures affects both the fitness of individual snakes and the viability of populations (Weatherhead and Madsen 2009). Reptiles rely on the external environment to maintain body temperature through basking; therefore, it is reasonable to assume that the availability of basking habitat may be a limiting factor for the maintenance of essential functions and reproduction. In one New York population, Massasaugas select the warmest basking sites within habitat patches and in open sites will select basking sites near retreat sites and cryptic cover (Shoemaker and Gibbs 2010). Similar results come from Massasaugas in Ontario, Canada, where gravid snakes select basking sites for optimal thermoregulatory opportunities relative to non-gravid females and males, indicating the potential importance of optimal basking habitat relative to fitness, recruitment, and survival (Harvey and Weatherhead 2010).

Based on habitat measurements from radiotelemetered snakes in Michigan, Bissell (2006) developed a Habitat Suitability Index (HSI) model for Massasaugas, which was later tested and refined by Bailey (2010). This model, however, did not examine fitness variation among individuals or the relationship between habitat quality and population health (Bailey 2010). Additionally, other work has focused on habitat use relative to availability at both landscape and local scales, but we are unaware of research that links measures of habitat quality to those of the population and individual fitness. Therefore, ***the primary purpose for the development of the Ohio Massasauga Habitat Assessment Method (OMHAM) is to provide a method to evaluate and describe the structure of Massasauga habitat in Ohio and its relationship to measures of both population and individual health.*** We expect the final method to provide a scientifically defensible standardized method for state, federal, and non-profit organizations to assess known and potential Massasauga habitat and be useful for determining, how, when, and where to conduct habitat management activities, such as burning and mowing.

Massasaugas have life-history characteristics similar to those of other rattlesnake species in that they are ectothermic sit-and-wait predators that rely upon crypsis for both prey capture and predator avoidance (Parent and Weatherhead 2000, Harvey and Weatherhead 2006, Bailey et al. 2012). Furthermore, Massasaugas will frequently use crayfish burrows, logs, and other similar refugia for both thermoregulation (including freeze-avoidance during the winter) and escape. Throughout their range, wetlands and adjacent grasslands with low canopy cover provide the habitat characteristics required by Massasaugas (Harvey and Weatherhead 2006, Wynn and Moody 2006, Shoemaker and Gibbs 2010, Bailey et al. 2012). Because of their reliance on habitat for both thermoregulation and crypsis, Massasaugas are an obligate early-successional wetland and grassland species.

Based upon these life-history traits, we hypothesize that measures of individual snake fitness and/or population health will be correlated to habitats that provide: (1) high quality basking areas (high solar insolation); (2) in close proximity to adequate refugia from predators; (3) suitable overwintering sites; and, (4) habitat for prey species (dense vegetation, thatch, and burrows). Therefore, the vegetative and structural habitat characteristics included in OMHAM are intended to measure and assess field-level microhabitat characteristics that are assumed to be important for thermoregulation, crypsis, and refugia as they relate to Massasaugas. Additionally, vegetative characteristics represent attributes that can be addressed through habitat management such as burning, cutting, and herbicide application.

Figure 21. Fields where Massasaugas are found generally consist of a mixture of grasses, forbs, and sedges with scattered shrubs and small trees in a mosaic of wetland and upland habitats. G. Lipps photo.

METHOD DEVELOPMENT

Development of the OMHAM included a literature review to assess factors of Massasauga ecology, home range size, movement, and both macrohabitat and microhabitat associations. From available information and local knowledge, we determined the appropriate scale at which to apply the OMHAM and important habitat characteristics to include in the method. Wherever possible, we have attempted to use procedures similar to those being utilized to assess grassland habitats in Ohio primarily for bird species (based on the BBIRD protocol, Martin et al. 1997) so that results may be comparable.

Federal, state, and regional experts in Massasauga ecology will review the initial draft of the OMHAM. We will conduct field testing of the method during June of 2015 concurrently with annual Massasauga surveys conducted in Ohio. During the fall of 2015, we will analyze the findings of OMHAM in comparison to measures of individual (i.e., body condition, leptin and/or cytokine levels, etc.) and population health (i.e., catch per unit effort, population size, population density, etc.) after which we will incorporate any appropriate changes to the methodology.

PURPOSE

The OMHAM is designed to primarily assess the structure and, to a lesser extent, the composition of Massasauga habitat at a field level. Because there is no need for plant identification, any individual can be quickly trained in the application of the method. This methodology is designed to rapidly assess Massasauga habitat and should take a trained observer only a few minutes per plot. The primary outcome of OMHAM will provide managers with the ability to rapidly assess known and potential Massasauga habitat and provide primary support for determining how, when, and where to conduct habitat management activities related to the conservation of Massasaugas.

ASSESSMENT AREA

Although Massasauga homerange size varies with reproductive condition, overall, the size of homeranges tends to be relatively large (Table 1). Variation within this homerange size likely relates to two main factors: the quality of habitat within the homeranges of a population and the size of tracts of land containing suitable habitat. Within a given area, Massasaugas do not appear to primarily select areas based upon macrohabitat characteristics, such as percent area of forest, wetland, or grassland; instead, snakes seem to select habitat based upon the relative availability of microhabitat within an available tract of habitat (Harvey and Weatherhead 2006). In addition, grassland and wetland management activities will typically be conducted at a field or site level, so any habitat assessment should provide insight at the same scale in order to be applicable to management of habitat used by Massasaugas.

Table 2. Mean (± SD) minimum convex polygon (MCP) homerange size (ha) for Massasaugas. When SD was not reported, it was calculated as SD=SE × √N, where N is the sample size.

State	Male	Non-gravid female	Gravid female	Source
IN	7.3 ± 1.4	3.4 ± 0.7	1.4 ± 0.5	Kingsbury et al. 2003
IN	7.3 ± 4.3	3.4 ± 2.0	1.4 ± 1.4	Marshall et al. 2006
PA	10.0 ± 12.3	4.2 ± 0.6	1.3 ± 0.7	Kowalski 2007
WI	16.2	6.7	2.8	King 1999
MI	1.6 ± 1.0	1.2 ± 1.3*		Moore and Gillingham 2006
MI	29.8 ± 24.6	14.4 ± 17.4	1.6 ± 2.0	DeGregario et al. 2011
MI	7.1 ± 4.9	3.1 ± 2.7	0.7 ± 0.6	Bailey et al. 2012
MI	5.3 ± 13.8	1.1 ± 2.7	1.1 ± 2.2	Bissell 2006
IL	5.0 ± 6.7	2.1 ± 1.5	2.5 ± 2.6	Dreslik 2005
NY	27.8 ± 16.0	41.4 ± 4.8	2.0 ± 1.2	Johnson 2000
Mean	11.8	8.1	1.6	
Minimum	1.6	1.1	0.7	
Maximum	29.8	41.4	2.8	

*Non-gravid and gravid females were not separated when calculating homerange size.

Because Massasaugas will select fields with available microhabitat and select particular microhabitats within a field, we will assess habitat at both the large-scale (field) and small-scale (individual snake and artificial cover, i.e. "tin") levels. In both instances, we will conduct habitat assessments within 5 m-radius habitat assessment plots (hereafter “plot”). To assess the habitat condition for a field, we will measure microhabitat characteristics at a density of 1 plot per 2 hectares, which encompasses all or part of the average homerange for all massasauga reproductive states (Table 1; Figure 2; Bailey 2010). For fields smaller than 2 ha in size, we will measure habitat characteristics in 3 plots. In all instances, plot locations will be randomly selected prior to field sampling.

Additionally, Massasaugas select for specific microhabitat characteristics within a field and as a potential validation for field level assessments, we will also assess habitat characteristics within plots at all locations where individual Massasaugas are encountered during visual surveys. In instances where a visual encounter occurs, the location of the snake will become the center of the plot (Figure 3A). In addition, we will randomly select a subset of tins prior to sampling, adjacent to which we will place a plot. To avoid assessing direct impacts of tins and human trails created by walking to check tins, we will place the center of plots 6 m perpendicular to trails (Figure 3B).

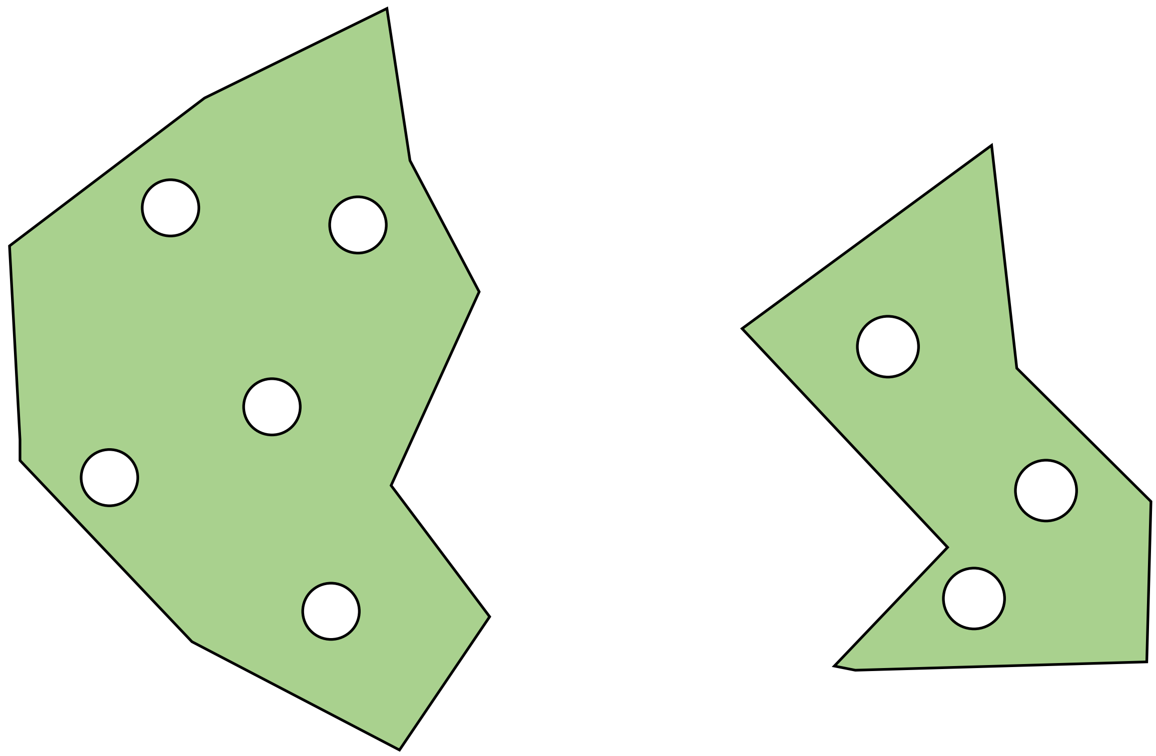

Figure 22. For fields larger than 2 ha, assess habitat characteristics in randomly placed plots at a density of 1 plot/2 ha. For fields ≤ 2 ha in size, we will assess habitat characteristics in 3 randomly placed plots. In all instances, the locations are random.

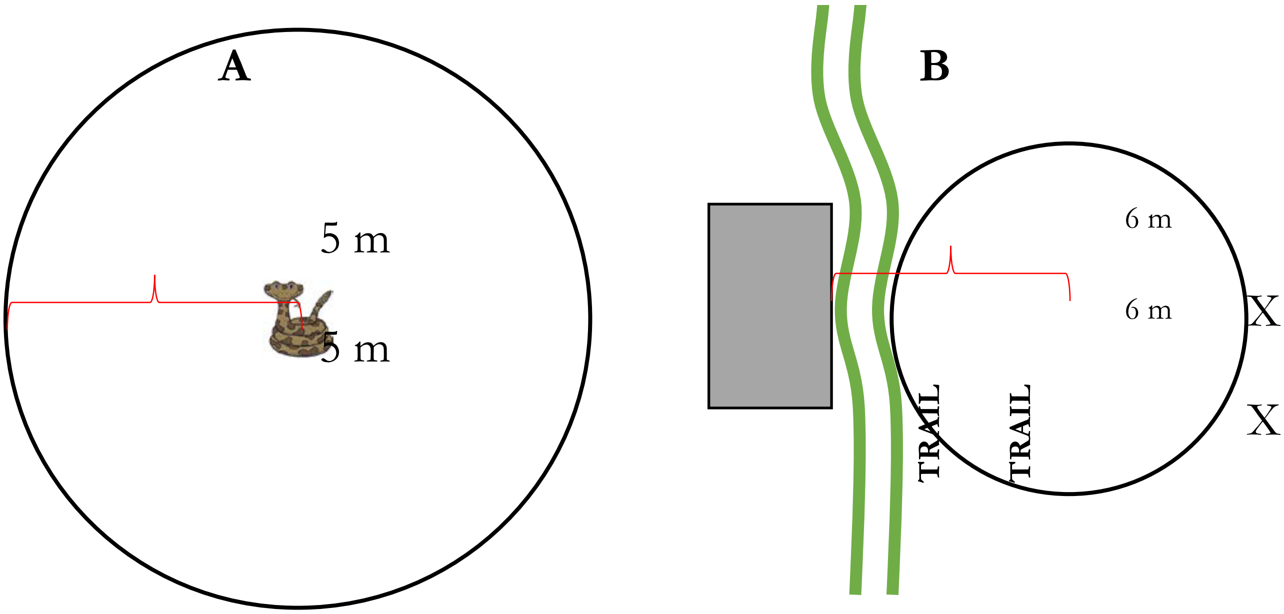

Figure 23. In addition to randomly selected plots, measure habitat characteristics in a 5m-radius plot surrounding all visually encountered Massasaugas (A). Also randomly select survey tins adjacent to which plots will be placed, with the center of the plot falling 6 m distant and perpendicular to the trail (B).

PROCEDURES FOR MEASURING HABITAT CHARACTERISTICS

This section comprises the main component of the OMHAM protocol. The procedures are broken down into two main sections: (1) tools required to conduct the habitat assessment and (2) descriptions, locations, and procedures for measuring habitat characteristics. The procedures should be read and fully understood prior to their application in the field.

Tools

The following tools are needed for the application of OMHAM:

1. ***Density Board***

   For the last 30 years, the "Wiens Pole", "Robel Pole", and "Nudds Board" have regularly been used to measure, compare, and report vegetative height and density in grassland communities (Wiens and Rotenberry 1981, Nudds 1977, Robel et al. 1970). These methods, however, rely on the subjective estimates of observers, which can lead to high variability in measurement (Limb et al. 2007). In addition, measurements from such methodologies have little predictive power (see Moynahan et al. 2006, Renfrew et al. 2005, Warren and Anderson 2005). Therefore, we propose the use of a density board in concert with digital photography and Digital Imagery Vegetative Analysis (DIVA; Jorgensen et al. 2013). A density board is a 1 m x 1 m solid white board as described by Jorgensen et al. (2013). The density board is used to measure vegetative density via digital photography and DIVA (Jorgensen et al. 2013).

2. ***Digital camera and 1 m tall monopod***

   Use of the board described above requires a digital camera. In general, a higher resolution camera is better, as methodologies used to analyze the photographs do so based upon pixels and thus higher accuracy will be attained in measurements. All photographs should be taken from a height of 1 m above the ground. Use of a set monopod or tripod allows for a consistent photo height. For 2015, we will be using tablet computers (Pantech Element) to collect data and also to acquire photographs for the DIVA. The tablet is placed upon a section of PVC pipe perpendicular to the ground so that the camera lens is 1 m above the ground.

3. ***Compass***

4. ***Tent peg or stake with four 5 m ropes attached***

   The tent peg is placed at the center of vegetation assessment plots. The 5 m long ropes allow for quick demarcation of the cardinal directions and plot edges. In addition to being 5 m long, each rope should be marked 1 m and 3 m distances from the stake. The ends of each rope will also provide a consistent point at which photographs should be taken relative to the plot center and vegetation density board.

5. ***Metric tape measure and meter stick***

Procedures for Assessing Habitat

Ground cover

Ground cover is estimated in 5% increments within the plots assuming a nadir view of the plot. Categories of ground cover are explained below and include: live standing herbaceous plants; live standing woody plants; and other ground cover. If a cover class has < 5% estimated cumulative cover within the plot, it is not included in cover estimates for that plot. This is a measure of ground cover; canopy cover from trees (if present) is measured separately, and should not be included here. Vegetation that originates from outside the plot may be included if it contributes to the ground cover within the plot. In all cases, total estimated cover for all ground cover classes must sum to 100%.

Vegetative cover

Estimate the % cover of the following cover classes within each plot:

<u>Live standing herbaceous cover</u>

1. Bunch-forming grasses

   Bunch-forming grasses are warm-season graminoid species that grow in tight clumps. In Ohio, these include species such as switchgrass (*Panicum virgatum*), big bluestem (*Andropogon geradii*), little bluestem (*Schizachyrium scoparium*), and Indian grass (*Sorhastrum nutans*).

2. Sod-forming grasses

   Sod-forming grasses are cool-season graminoids with tight, shallow root structures. Species in Ohio include Kentucky bluegrass (*Poa pratensis*), fescue (*Festuca spp.*), redtop (*Agrostis gigantean*), reed canary grass (*Phalaris arundinacea*) and, orchard grass (*Dactylis spp.*).

3. Sedges

   Many sedges (*Carex spp.*) form hummocks and tussocks in wet meadow and grassland habitats. They are readily identified by their triangular stems as "sedges have edges".

4. Forbs

   Forbs are non-woody, herbaceous, flowering plants and include what would commonly be called wild flowers. Examples of common forbs include goldenrod (*Solidago spp.*), wild carrot (*Daucus carota*), black-eyed susan (*Rudbeckia hirta*), purple coneflower (*Echinacea purpurea*), dogbane (*Apocynum cannabinum*), fleabane (*Erigeron philadelphicus*), and cup plant (*Silphium perfoliatum*).

<u>Live standing woody cover</u>

5. < 1 m in height

Plants with above ground woody stems standing less than 1 m in height. This may include saplings, shrubs, bushes, woody vines, and brambles.

6. > 1 m in height

   Plants with above ground woody stems standing greater than 1 m in height. This may include shrubs, bushes, woody vines, brambles, and trees.

Other cover classes

7. Moss

   Mosses growing on the ground within the plot.

8. Dead vegetation (standing)

   All dead vegetation that is standing $>45^{\circ}$.

9. Vegetative litter

   Litter includes all fallen and standing $\leq 45^{\circ}$ dead herbaceous vegetation such as leaf litter (forbs) and thatch (graminoids). As you are measuring ground cover, do not include litter that is overlain with live vegetation.

10. Bare ground

    Bare ground is any barren soil or mud that is not overlain with any live standing herbaceous vegetation, live standing woody vegetation, or vegetative litter.

11. Other ground cover

    Other items within the plot that do not fit into any of the above categories belongs in this category. This may include logs, rocks, or anthropogenic cover (i.e., trash). If there are standing puddles of water within the plot, record these areas according to the substrate on the bottom of the water (e.g., bare ground, live herbaceous, etc.)

Canopy cover

Canopy cover is defined as large woody vegetation (usually trees) and their leaves that overhang the plot causing shading of the herbaceous vegetation. In most instances, there will be no canopy cover within fields being assessed as Massasauga habitat. If there is canopy cover, estimate the percentage of the entire plot over which it occurs to the nearest 5%.

Vegetative litter depth and herbaceous height

The presence and depth of litter within a plot provides cryptic cover for Massasaugas and is used to avoid detection and to thermoregulate. Litter also provides habitat for common prey species. Using a meter stick, measure the depth of lying vegetative litter within the plot to the nearest cm at distances of 1 m, 3 m, and 5 m from the center (Figure 4). These depths should be measured along transects in all four cardinal directions (N, E, S, and W). In total, 12 measurements will be taken per plot.

At the same locations (1 m, 3 m, and 5 m) use a meter stick to measure the maximum height of the herbaceous vegetation for a total of 12 measurements. For both the vegetative litter and herbaceous height measurements, if one of your points lands on a rock or log, move your measurement location so that you are at a location that does have mineral soil.

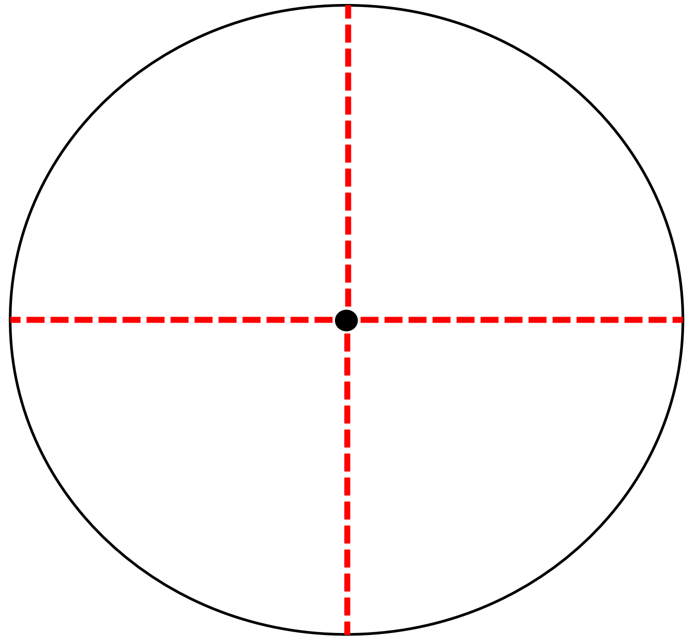

Figure 24. Vegetative litter depth measurements should be taken at 1 m, 3 m, and 5 m distances from the center of the plot in all four cardinal directions.

Number and distribution of above ground refugia

Above ground refugia are important for two reasons, they allow for thermoregulation (shade) and provide cover for predator avoidance. For each plot, measure both the number and distribution of refugia. First, count the total number of above ground refugia available to snakes including logs, rocks, and shrubs. Only include cover that a snake could use for shade or to escape from predation (e.g., a buried log or rock without access underneath would not be considered "available" and should not be counted). Second, describe the distribution of the above ground cover as clustered, moderate, or dispersed (Fig. 5). When assessing plots surrounding the location of visually encountered snakes, follow these same procedures; however, if no such refugia are present, use a tape measure and record the distance to the nearest above ground refugia.

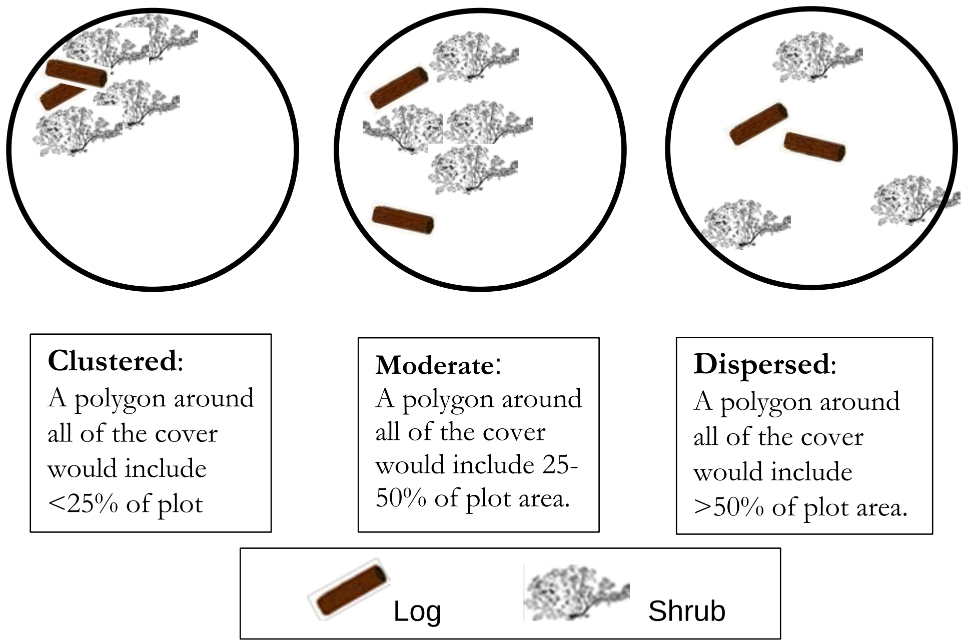

Figure 25. Categories for describing the distribution of above ground refugia available to snakes.

Figure 26. Examples of above ground refugia used by snakes include downed logs (left) and woody shrubs (right). G. Lipps photos.

Underground refugia

As with above ground refugia, access to subterranean retreats is important to snakes for thermoregulation and to escape predators. Crayfish burrows are the most commonly used overwintering sites for the Massasauga, and their presence is thought to be a critical component of their habitat. For each plot, search the ground for the presence of crayfish or small mammal burrows. Burrowing crayfish often create "chimneys" of soil around the entrance to their burrows which can aid in their location. Record burrows as either present or absent within the plot.

Figure 27. Examples of underground refugia used by snakes include crayfish burrows (left and center) and small mammal burrows, like those made by the Star-nosed Mole (right). G. Lipps photos.

Vegetation density

Dense vegetation obscures light, providing fewer opportunities for basking and thermoregulation, but provides cryptic cover for quick escapes from predators. Massasaugas are thought to benefit from a heterogeneous distribution of patches of high density and low density vegetation. Vegetation density is measured using a 1 m^2 solid white density board located at the center of the plot (Limb et al. 2007, Jorgensen et al. 2013). A series of photographs are taken, one from each cardinal direction (N, S, E, W), parallel to the ground at a height of 1 m and distance of 5 m from the center of the plot (Figure 5). Each photo is then cropped to the shape of the density board and converted to binary black and white image. Vegetation density is estimated using software to determine the percentage of cells in the photograph composed of vegetation (Jorgensen et al. 2013).

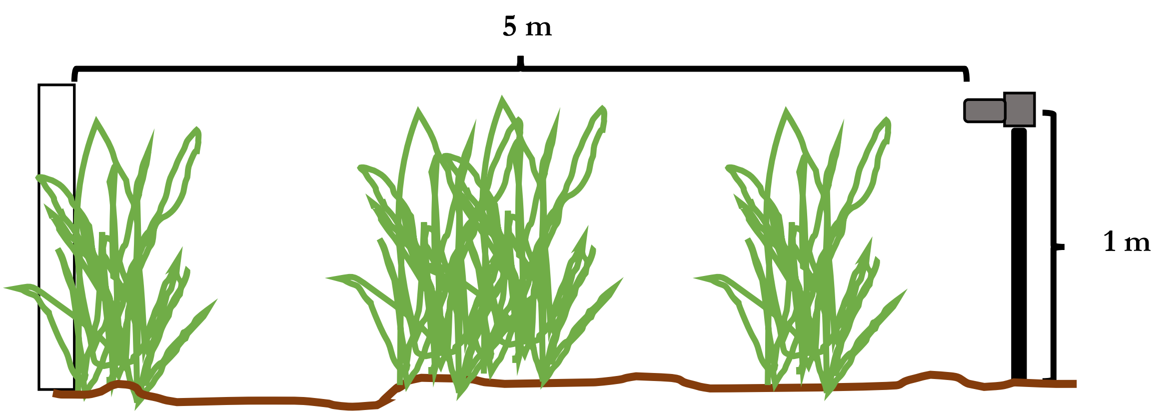

Figure 28. Vegetation density is estimated from four digital photographs taken from the cardinal directions at a height of 1 m from the ground and distance of 5 m from the base of a 1 m x 1 m white board.

ANALYSIS OF DATA AND METHOD VALIDATION

Data for OMHAM is primarily collected within 5-m radius plots located in randomly selected locations. Additionally, we will collect data in vegetation plots centered on all visually encountered snakes within a field. If no snakes are visually encountered, we will collect data in plots located adjacent to artificial cover locations where Massasaugas are encountered. As a validation of the OMHAM, we will compare results of data collected for randomly selected locations and assessment plots locations from visually encountered snakes and artificial cover using a multivariate Hotelling's t-square test and principal components analysis (PCA).

Any habitat assessment method is built upon the assumption that higher quality habitats support more robust and healthier populations and individuals. We will test the efficacy of the OMHAM against measures of both population health, such as population size and density in a given habitat, as well as measures of individual health and fitness. We are currently working to determine the best measures of individual health, but some options include body condition indices, neutrophil to lymphocyte (N:L) ratio (a measure of long-term stress), and parasite load. We will use multivariate analyses and multi-model inference to determine the relationship among variables and measures of population and individual health.

Habitat variables are described above.

Measures of community and population "health"
1. Population density
2. Population size
3. Population demographics
4. Fecundity
5. Snake species diversity

Measures of individual fitness and health:
1. Body condition indices
2. Blood values, i.e., N:L ratio; leptin; cytokines, etc.
3. Parasite load

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Appendix E. Combined measures worksheet

Table E-1. Example combined measures worksheet. This draft worksheet illustrates potential metrics that could be measured and scored to describe and monitor the overall status and health of Massasauga populations occurring within a management unit (e.g., a wildlife management area) in an adaptive management framework. Generating population estimates of sufficient precision to monitor populations is not feasible for all sites; this worksheet facilitates a more inclusive approach for qualifying the status of populations. All values are hypothetical and included for illustrative purposes only. See **12.3.3. Considerations for future surveying and monitoring** for further explanation.

Completed by: ______________________________ Date: ______________

Metric	Measure			Values and Comments
	Negative	Neutral	Positive	
Population Size				
Estimated adult female population size	<50	50	>50	
Change in estimated population size between two most recent surveys	Declining	Stable	Increasing	
Estimated population density in occupied field(s)	<2/ha	2/ha	>2/ha	
Change in estimated population density between two most recent surveys	Decreasing	Stable	Increasing	
Snake condition				
Modified body condition index compared to mean (118.3) +/- 1 SE (11.11) for OH	<107.17	107.19 – 129.41	>129.41	
Proportion of adults with scars/abnormalities	>10%	10%	<10%	

Metric	Measure			Values and Comments
	Negative	**Neutral**	**Positive**	
Snake Fungal Disease	Detected on Massasaugas	Detected at site, not on Massasaugas	Not detected at site	
Demographics				
Sex ratio	Significantly different than 1:1		Not significantly different than 1:1	
Adult survivorship	Significantly less than 0.67	Not significantly different from 0.67	Significantly greater than 0.67	
Proportion of adult females gravid (2-yr mean)	<67%	67%	>67%	
Percent juveniles (≤40 cm SVL)	<24 or >42%		24 – 42%	
Habitat				
Amount of available Massasauga habitat (not forested, inundated, row-crops, recreational fields, developed)	<125 ac	125 – 250 ac	>250 ac	
Change in the amount of available habitat between two most recent surveys	Decreasing	Stable	Increasing	
Proportion of available habitat dominated by invasive plant monocultures	>20%	10 – 20%	<10%	
Change in the amount of available habitat dominated by invasive plants between two most recent surveys	Increasing	Stable	Decreasing	

Metric	Measure			Values and Comments
	Negative	**Neutral**	**Positive**	
Proportion of available habitat occupied	<25%	25 – 50%	>50%	
Change in the proportion of available habitat occupied between two most recent surveys	Decreasing	Stable	Increasing	
Management				
Occupied fields: proportion dominated by invasive plant monocultures	≥20%	1 - 19%	0%	
Occupied fields: change in proportion dominated by invasive plants between two most recent surveys	Increasing	Stable	Decreasing	
Occupied fields: proportion with mean DIVA score <60	<100%		100%	
Occupied fields: change in proportion with mean DIVA score <60	Decreasing	Stable	Increasing	
Genetics				
Effective population size (θ_{Ne})	<1		>1	
Bottleneck Mode Shift Test	Shifted Mode		L-shape	

Metric	Measure			Values and Comments
	Negative	**Neutral**	**Positive**	
Radiotelemetry				
Habitat use	Snakes regularly leave protected area.	Snakes complete entire life cycle on protected site, but cross road(s).	Snakes complete entire life cycle on protected site, w/o crossing road(s).	
Home range size for males, non-gravid females	MCP >14 ha		MCP <14 ha	

Appendix F. Illustrated disinfection protocol

http://northeastparc.org/disinfection-protocol/

DISINFECTION OF FIELD EQUIPMENT TO MINIMIZE RISK OF SPREAD OF CHYTRIDIOMYCOSIS AND RANAVIRUS[1]

NORTHEAST PARC — PARTNERS IN AMPHIBIAN AND REPTILE CONSERVATION

IMPORTANCE OF DISINFECTION

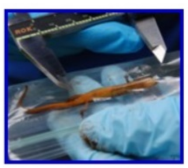
The spread of pathogens is a major threat to amphibians and reptiles worldwide.[2-5] This is particularly true for Ranavirus (RV) and *Batrachochytrium dendrobatidis* (Bd) responsible for chytridiomycosis. Humans can transmit diseases from one place to another and from one organism to another in a short amount of time and over distances the organisms cannot traverse. With the increasing spread of pathogens and reports of die-offs among amphibians and select reptiles worldwide, it is imperative that field biologists, researchers, hobbyists, and anyone interested in recreational herpetology-related field activities employ basic disinfecting procedures to prevent the spread of pathogens.

BEFORE LEAVING FOR THE FIELD

Although other chemicals are effective (see table), NEPARC recommends a 3% bleach solution to inactivate Bd and most RV's.[3-7] Concentrated bleach is inexpensive and readily available. However, diluted bleach solutions lose their potency if exposed to air, sunlight, or organic material, and should be discarded after 5 days if exposed.[8] To ensure maximum efficacy, prepare only as much solution as you will need for the sampling event.

Suggested equipment:

- Brushes for scrubbing and/or removing mud and vegetation from equipment.
- Hand sanitizers and antiseptic alcohol wipes.
- Handheld bottles and/or pump sprayers for applying bleach and water. Bring clean rinse water.



- Gloves for handling animals. These should be disinfected or discarded between animals.
- Plastic bags of different sizes: examining animals in bag minimizes contact.
- Prepare additional sets of equipment if sampling at multiple locations.
- Trash bags.

INSTRUCTIONS FOR LARGE EQUIPMENT

Brush off mud, wash with biodegradable soap, disinfect with bleach and rinse all exterior surfaces of boats, canoes, vehicles or trailers and their tires that may have come in contact with potentially affected water (e.g. stream or wetland).

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AFTER EACH SAMPLING EVENT AND BEFORE MOVING TO THE NEXT SITE

1. Brush off mud and vegetation from field equipment (e.g., nets, buckets, boots). Soil or mud can reduce the effectiveness of the disinfection process.
2. Generously spray or immerse all items in bleach solution.
   - Bleach is highly toxic to aquatic organisms; stand at least 50 m from any natural water source.
   - Lab studies indicate 1 minute contact time to be sufficient to inactivate pathogens but NEPARC recommends 5 minutes in field situations.
3. Rinse bleached items with water to minimize damage to the equipment and to prevent exposing the next wetland to residual bleach.
4. Use alcohol wipes to disinfect calipers, measuring boards, and other sensitive equipment.

END OF THE DAY

After returning from the field, all equipment should be washed and thoroughly disinfected. If available, set up 2 buckets or large tubs: one with soapy water and one with 3% bleach solution.

Brush or scrub off any soil or vegetation. Immerse into soap, wash then rinse.

- Immerse in bleach and leave for 5 minutes. Rinse thoroughly with water.
- Hang equipment and gear, and allow them to air dry completely.

DISINFECTION OPTIONS FOR RANAVIRUS (RV) AND *BATRACHOCHYTRIUM DENDROBATIDIS* (*Bd*)

Although these chemicals were not developed specifically for RV or Bd, these recommendations represent the minimum concentration and contact time demonstrated as effective

	Clorox Bleach®	Nolvasan®	Virkon S®	Ethanol
Active Ingredient (AI)	Sodium hypochlorite	Chlorhexidine	Potassium peroxymonosulfate	Ethyl alcohol
Concentration of AI	6.0%	2.0%	20.4%	70.0%
Relative cost	$4.99/gal	$65.95/gal	$76.50/10 lb or $1.60/gal	$23.45/L or $88.83/gal
Min. Contact Time RV[9]/Bd[10]	1 min / 30 sec	1 min / not determined	1 min / 20 sec	1 min[11] / 20 sec
Min. Concentration RV[9]/Bd[10]	3.0% / 1.0%	0.75% / not determined	1.0% / 1.0%	70% / 70%
Effective dilution ratio for both RV and Bd	1:32 dilution (bleach:water) for 3% solution using 6% concentration of household bleach.	1:127 (Nolvasan®: water) for 0.75% solution (RV only)	1 scoop (1.3 oz) or 1 tablet per gal of water	Effective when applied undiluted (70%)
Toxicity to Humans	• Vapor may cause severe irritation or damage to eyes and skin • Harmful if swallowed	• May be fatal if inhaled • Avoid breathing spray mist • Causes irreversible eye damage • Harmful if swallowed	• Harmful if swallowed • Irritating to respiratory system and skin • May cause serious eye damage	• May be fatal if swallowed or inhaled • Can damage liver, kidneys and nervous system by repeated or prolonged exposure • May be absorbed through skin. Repeated or prolonged contact can cause eye irritation or dermatitis[12]
Toxicity to Amphibians	• Fatal at high concentrations	• Safe for short durations [13]	• Non-toxic [14]	• May destroy mucus and wax resulting in dehydration and microbial infection[11]
Effects on Equipment	• Corrodes metals • Will fade colors and break down cloth fibers	• None reported	• Safe on fabric • May cause pitting on galvanized or soft metal if not rinsed with water	• May damage rubber and plastics • May cause deterioration of glues[12]

Special Instructions:

• Remove debris from equipment prior to treatment.[15] • Wear safety glasses and gloves when handling chemicals. • Water pH can affect chemicals; all information in this table assumes the use of tap or municipal water. • Keep out of lakes, streams, or ponds; stand at least 50 m from any natural water source. • Do not clean equipment or dispose of waste solutions at field sites. • For disposal, follow local, state, and federal guidelines.

Bleach: Inactivated by organic material. • Inactivated by sunlight. • If in an opaque container, diluted bleach will last 1 month[16]. If exposed to sunlight or air, it will only last 5 days.

Nolvasan: Can be inactivated by organic material.[15] • Store at room temperature in sealed container.[17] • Dilute concentrate with water of pH 5-7.[18] • Remains stable for 1 week if dilute with tap water, and for up to 6 weeks if diluted with deionized water.[17] • Use concentrate within 36 months.[17] • Toxic to fish.[18]

Virkon-S: Store at room temperature.[19] • Keep solution away from extreme cold or heat. • Shelf life for tablets is 2 years and for powder is 3 years. • Remains stable for 1 week if diluted with tap water.

Ethanol: Highly flammable. • Use and store in a well ventilated area. • Evaporation may diminish effective concentration.[12,18]

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Citations for Disinfection of Field Equipment to Minimize Risk of Spread of Chytridiomycosis and Ranavirus

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