1-1-2024

Selection and validation of release sites for conservation translocations of temperate-zone snakes

Jonathan D. Choquette
Université Laurentienne

Ali I. Mokdad
Great Lakes Institute for Environmental Research

Trevor E. Pitcher
Great Lakes Institute for Environmental Research

Jacqueline D. Litzgus
Université Laurentienne

Global Ecology and Conservation 49 (2024) e02765

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: www.elsevier.com/locate/gecco

Selection and validation of release sites for conservation translocations of temperate-zone snakes

Jonathan D. Choquette[a,b,c], Ali I. Mokdad[d], Trevor E. Pitcher[e], Jacqueline D. Litzgus[a,*]

[a] School of Natural Sciences, Laurentian University, Sudbury, ON P3E 2C6, Canada
[b] Wildlife Preservation Canada (WPC), 5420 Highway 6 N., Guelph, ON N1H 6J2, Canada
[c] SCC Ecological, Windsor, ON N9A 6K1
[d] Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada
[e] Department of Integrative Biology & Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada

ARTICLE INFO

Keywords:
Viperidae
Resilience zone
Artificial hibernaculum
Brumation
Overwintering
Reintroduction

ABSTRACT

Poor habitat quality is one of the most important reasons for reintroduction failure of reptiles; therefore, release site suitability ought to be evaluated prior to conducting conservation translocations. In temperate zone snakes, translocations have failed due to high overwinter mortality, so practitioners have recommended that release sites be located near suitable hibernacula. The presence of a Life Zone (LZ), the underground space above the groundwater table and below the frost line, may indicate the presence of suitable hibernation habitat. Identification and validation of sites with LZ, however, is challenged by the dynamic nature of groundwater and frost levels, coupled with the secretive nature of hibernating snakes. In this study, our goals were to 1) identify potential reintroduction sites for the globally imperiled Eastern Massasauga (*Sistrurus catenatus*) in southern Canada based on the presence of a LZ, 2) to validate the suitability of those reintroduction sites by hibernating a surrogate species (Eastern Gartersnake, *Thamnophis sirtalis*) in constructed hibernacula, and, 3) to determine if two separate measures of LZ were associated with the survival of individual gartersnakes to the end of hibernation. Four 1-ha study grids, each consisting of multiple groundwater and frost monitoring stations, were surveyed from 2015 to 19. Release sites were identified in each grid where a LZ of $\geq$ 10 cm was observed consistently for at least 2 full winters, and gartersnakes were successfully hibernated therein and at 2 reference sites over 3 winters. Overall, survival of subadult/adult snakes was very high (100 %; n = 20), regardless of site, whereas juvenile survival was lower (78 %; n = 93). A Kaplan-Meier test indicated that juvenile survival differed significantly among sites, and ranged from 60 % to 100 %. GLMM analysis indicated that mass at ingress had a significant positive effect on snake survival during hibernation. The temporary loss of a LZ did not impact snake survival. Contrary to our expectations, snake survival in hibernacula was negatively associated with both minimum LZ size and LZ frequency (i.e., the % of sampling occasions when LZ size $\geq$ 10 cm). Hibernating snakes can tolerate periods with a very small or non-existent LZ, but a large LZ may become unsuitable to juveniles, possibly due to a lack of moisture. Our results will guide the selection of release sites for Massasauga reintroductions, and for the development of a rigorous release site selection process for temperate zone snakes.

* Corresponding author.
E-mail address: jlitzgus@laurentian.ca (J.D. Litzgus).

https://doi.org/10.1016/j.gecco.2023.e02765
Received 5 September 2023; Received in revised form 9 November 2023; Accepted 6 December 2023
Available online 7 December 2023

1. Introduction

Reptiles are declining globally (Reading et al., 2010; Böhm et al., 2013), nationally (Lesbarrères et al., 2014), and locally (Choquette and Jolin, 2018), which has generated a growing interest in translocations as a tool to augment or reintroduce populations of some species. Prior to conducting conservation translocations, however, release site suitability ought to be properly evaluated (IUCN/SSC, 2013). Poor habitat quality and lack of specific habitat characteristics are two of the most important reasons for translocation failure of herpetofauna (Germano et al., 2014); therefore, successful establishment of populations will depend upon releasing animals into high quality habitat (Kingsbury and Attum, 2009). For temperate zone snakes, the identification of suitable hibernacula prior to translocation is critical given their dependence on these features for approximately half of their lives, high site fidelity to previously used hibernacula (Sage, 2005; Harvey and Weatherhead, 2006; Smith, 2009), and the high over-winter mortality rates previously observed in translocated snakes (Sainsbury et al., 2021; Choquette et al., 2022). Furthermore, investigators have recommended that conservation translocations of Eastern Massasaugas (*Sistrurus catenatus*; hereafter Massasaugas), an endangered pit viper, occur in close proximity to suitable hibernacula because they are known to hibernate near release sites (Bieser, 2008; Kingsbury and Attum, 2009; Harvey et al., 2014).

Suitability of hibernation habitat for snakes may be dependent on a combination of factors. These factors include subterranean humidity (Costanzo, 1989a), depth of frost penetration (Shine and Mason, 2004), groundwater depth and variability (Smith, 2009; Todd et al., 2009), and presence of an aerobic space and thermal buffering (Yagi et al., 2020). Recent work, however, has focused on the interaction between two main factors, groundwater and frost depth, in an effort to advance the identification and characterization of snake overwintering habitat (Smolarz et al., 2018; Markle et al., 2020a, 2020b, 2020c; Yagi et al., 2020). The concept of the "life zone" (LZ; also known as the "resilience zone") has thus emerged, which is defined as the underground space above the groundwater table and below the frost line which occurs during the reptile hibernation period (Markle et al., 2020b; Yagi et al., 2020). Although circumstantial evidence exists to support the hypothesis that snake overwinter survival increases with LZ size in peat bog habitats (Markle et al., 2020a; Yagi et al., 2020), survival experiments that control snake hibernacula coupled with LZ measures are needed to provide direct evidence that the presence of a LZ supports overwinter survival (Yagi et al., 2020). Furthermore, the relationship between LZ size, snow pack, thermal buffering and snake survival, among other factors, remains unclear and requires additional research (Sage, 2005; Yagi et al., 2020). Finally, the successful application of LZ monitoring in habitats and soil types outside of peat bogs would support the generality of the LZ concept (Yagi et al., 2020).

Our goals were to 1) identify reintroduction sites for the globally imperiled Eastern Massasauga at a provincial park in southern Canada, based on the presence of a LZ by monitoring a grid of groundwater wells and frost tubes at a location unoccupied by Massasaugas, and at a nearby reference location occupied by Massasaugas, 2) validate the suitability of those reintroduction sites by hibernating individuals of a surrogate species (Eastern Gartersnake, *Thamnophis sirtalis;* hereafter gartersnake) in constructed hibernacula, and 3) determine whether or not the presence of a LZ supports a high overwinter survival rate of hibernating gartersnakes, and if survival rate is impacted by LZ size. If release sites are suitable (i.e., they maintain a LZ over winter), then the survival rate of hibernated gartersnakes should be similar to published overwinter survival rates.

2. Materials and methods

2.1. Study location and study grids

Our study took place within the Ojibway Prairie Complex and Greater Park Ecosystem, which is a complex of parks and natural areas in the Windsor-Essex region of extreme southern Canada (42.26 N, 83.07 W). The Ojibway Prairie Provincial Park is central to the park complex (Woodliffe, 1997; Ontario Parks, 2015) and was previously identified as an appropriate location for Massasauga conservation translocations (Ontario Parks, 2005). The Park was historically occupied by Massasaugas and was the site of a reintroduction attempt (Harvey et al., 2014), but recent intensive surveys suggest the species is extirpated (Choquette et al., 2023 in review). To identify suitable sites for conservation translocations, four 1 ha (100 m x 100 m) study grids (A, B, C, and D) were established in October 2015 using publicly available data in a GIS (Fig. 1). Grid locations were based on presence of some or all of the following characteristics: 1) Maximum possible distance from roads and residential areas, 2) Moist or water-saturated upland areas adjacent to wetlands but not flood-prone, 3) Presence of dense vegetation for shelter from harsh winter conditions, 4) Proximity to a previous release site (Harvey et al., 2014), and 5) Proximity to suitable Massasauga foraging and gestation habitat (Choquette et al., 2020) (Appendix A).

Two reference grids (E and F) were established in 2016 ~2.5 km from the study grids at a location that was still occupied by Massasaugas. Both reference grids were placed in presumed Massasauga hibernation areas, identified using early spring observation data (Choquette and Fournier, *in prep*). Grid E was smaller than all other grids, partially due to constraints imposed by a natural gas pipeline and stormwater pond, and funding availability (25 m x 50 m in 2016; expanded to 50 m x 75 m in 2018). Unfortunately, Massasaugas may have ceased overwintering within reference grids by the 2019–20 winter due to a population decline (Choquette and Fournier, *in prep*). At all reference and study grids, a handheld GPS unit was used to inventory and map all animal burrows (i.e., potential hibernacula) during the 2016–17 and 2017–18 field seasons, and the perimeter of all pools of surface water during early April 2018.

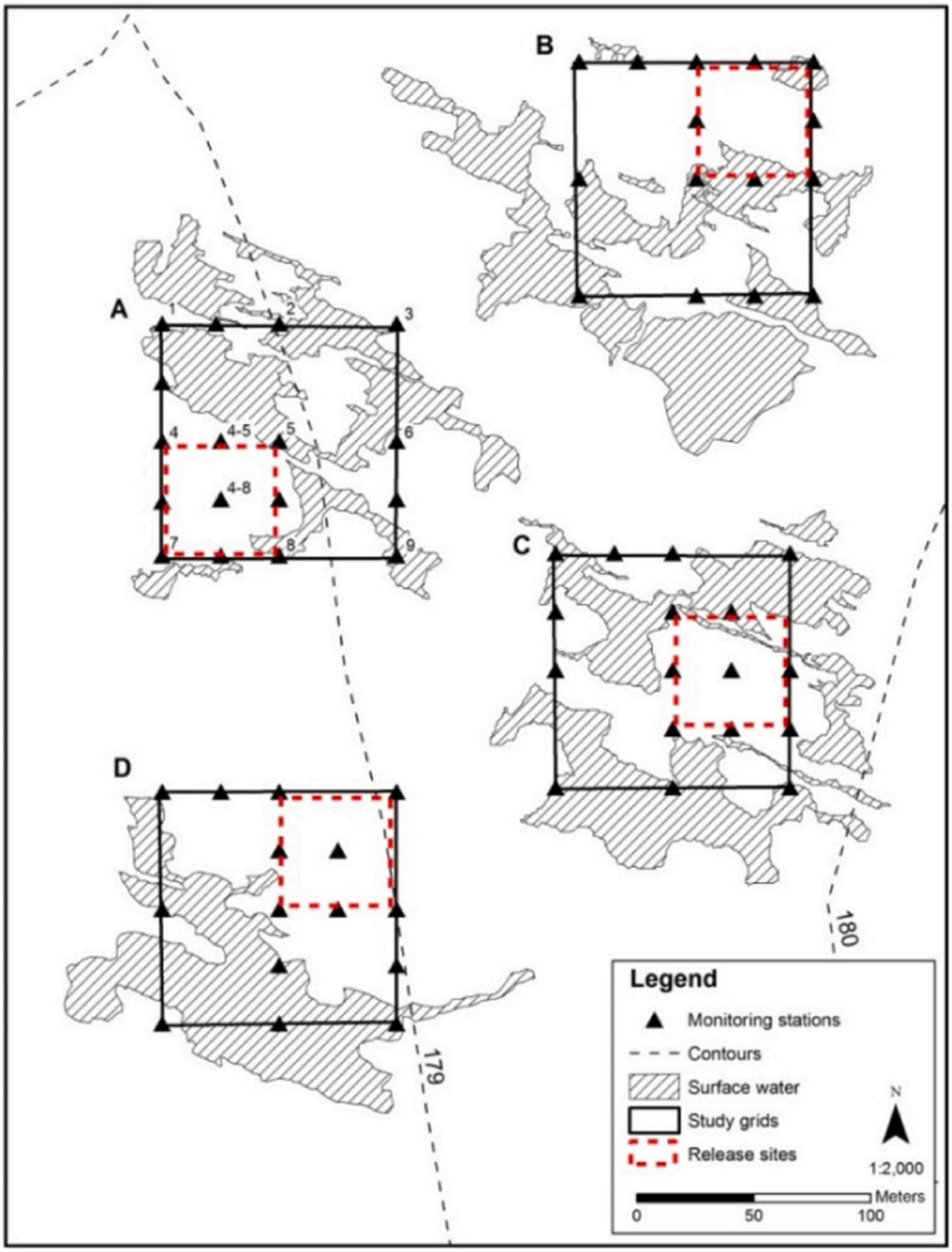

Fig. 1. Four 1 ha (100 m x 100 m) study grids established at Ojibway Prairie Provincial Park, Ontario, Canada, and monitored from 2015 to 2019 for the purpose of identifying release sites for Eastern Massasaugas (*Sistrurus catenatus*). Grids were situated in areas dominated by thicket or savanah based on Ecological Land Classification data (Chambers, 2010), and which included a mix of uplands and wet areas. Wet areas were initially identified by overlaying and merging in a GIS all wet meadows, marshes, swamps, waterbodies and ecosites with fresh/moist soils, which were previously mapped by others (OMNRF, 2002, 2009; Chambers, 2010). The dominant soil type at study and reference grids is Berrien Sand (Richards et al., 1949). Surface water was inventoried in spring 2018. See study grid A for an example of the numbering system used for initial monitoring stations (A1 – A9), and stations installed afterward (e.g., A5-8 and A4-8).

2.2. Monitoring stations

Within each grid, nine monitoring stations were initially installed 50 m apart in 3 equal rows of 3 stations each (with the exception of grid E, where only 6 stations were initially installed 25 m apart). A handheld GPS unit was used to mark the centre of each station in the field. A station consisted of one groundwater well and one frost tube placed 1 m apart (Fig. 2), used to sample groundwater depth and frost depth, respectively. Stations were labelled digitally with a GPS and physically with flagging tape.

Groundwater wells consisted of ~1.8 m-long ABS pipes (4.0 cm inner diameter [ID]) installed 1 m below grade, with the bottom 50 cm perforated to allow water entry (Appendix B). Previous work at Ojibway Prairie Provincial Park recorded gleysol soil from 50 to 70 cm below surface, an indication of the presence of groundwater (Chambers, 2010). Frost tubes consisted of an outer plastic pipe ~1.5 m long (1.5–2.0 cm ID) installed 1 m below grade, and an inner removable polyethylene tube of slightly shorter length filled with dyed water (McCool and Kok, 1990; Appendix B). Frost tube length was based on presumed maximum frost depth (<1.0 m: OPS, 2010) and snow pack (20 cm) for our study area. Similar frost tubes provided accurate measurements of soil frost depth (to within 1.3–5.0 cm), and were presumed to provide a more accurate measurement of frozen soil depth than excavation (Patric and Fridley, 1969; Hanson and Flerchinger, 1990). Frost depths recorded using our tubes were within 1.8 cm, on average (range = 0–5.0 cm), of frost depths recorded using manual excavations, based on a comparison of the two methods at 13 frost tubes in February 2017.

Monitoring stations were labelled (e.g., A1, A2, A3, etc.) and characterized based on their distance to nearest drainage channel, elevation, Ecological Land Classification vegetation type (Chambers, 2010), % canopy cover, and presence of surface water. In each fall following the initial winter of study, new monitoring stations were established in between existing stations in response to surface flooding and to increase data resolution (e.g., A1-2 was established halfway between A1 and A2, 25 m from each). Monitoring stations that were characterised by surface water in winter or early spring were presumed to support low hibernation habitat suitability; therefore, groundwater and frost monitoring equipment were moved to an alternate station, and the well was replaced by a wooden stake. Regardless, 9 paired wells and tubes were maintained in each grid annually (except at grids E and F, where the number of paired wells and tubes ranged annually from 6 to 9, and 8 to 10, respectively). We continued to record presence and depth of surface water at monitoring stations with wooden stakes.

2.3. Groundwater and frost depth data collection

Groundwater depth (GWD) and frost depth (FD) were manually recorded weekly at each monitoring station over 7 winters (from 2015–16 to 2021–22; only surface water level was recorded at wooden stakes). Start dates ranged from 2 to 21 November, and end dates ranged from 27 March to 27 April. An effort was made to standardize manual data collection to the same weekdays each week;

Fig. 2. Example of a monitoring station consisting of a groundwater well (black pipe, left), and frost tube (white pipe, right) used to measure groundwater and frost depth at the Ojibway Prairie Complex and Greater Park Ecosystem, Ontario, Canada. Groundwater wells marked the centre of each station, and frost tubes were placed ~1 m due west of each well, and both were installed 1 m below ground.

however, monitoring days were shifted in response to poor weather conditions (e.g., precipitation and winter storms) or scheduling conflicts. Data collection began at opposite ends of the grid system on alternate weeks. Data collection occurred over 2–3 visits each week, and each visit began at a similar time of day, and within ~2.25 h of solar noon. During each visit, the date, surveyor, start/end air temperature and start/end time were recorded. To reduce impact on snow cover conditions surveyors made an effort to follow the same paths to each monitoring station, and to stand, work, and place gear away from each frost tube (snow cover insulates the soil, reducing frost penetration depth: Vermette and Kanack, 2012).

At each monitoring station we recorded: station #, time of day, depth of groundwater from top-of-well with a water level meter (Little Dipper, Heron Instruments), frost depth, status of frost tube (frozen or liquid), height of groundwater well from ground to top-of-well, height of frost tube from ground to top-of-tube, depth of snow within 1 m of frost tube (3x), and surface water depth at base of groundwater wells and wooden stakes. All measurements were recorded to the nearest 0.5 cm using a metre stick, unless otherwise noted. Missing FD or GWD data (due to leaking frost tube, broken equipment, etc.) were replaced with the average FD recorded within the same grid on the same day, or the average GWD recorded during the previous and following week, respectively (Yagi et al., 2020).

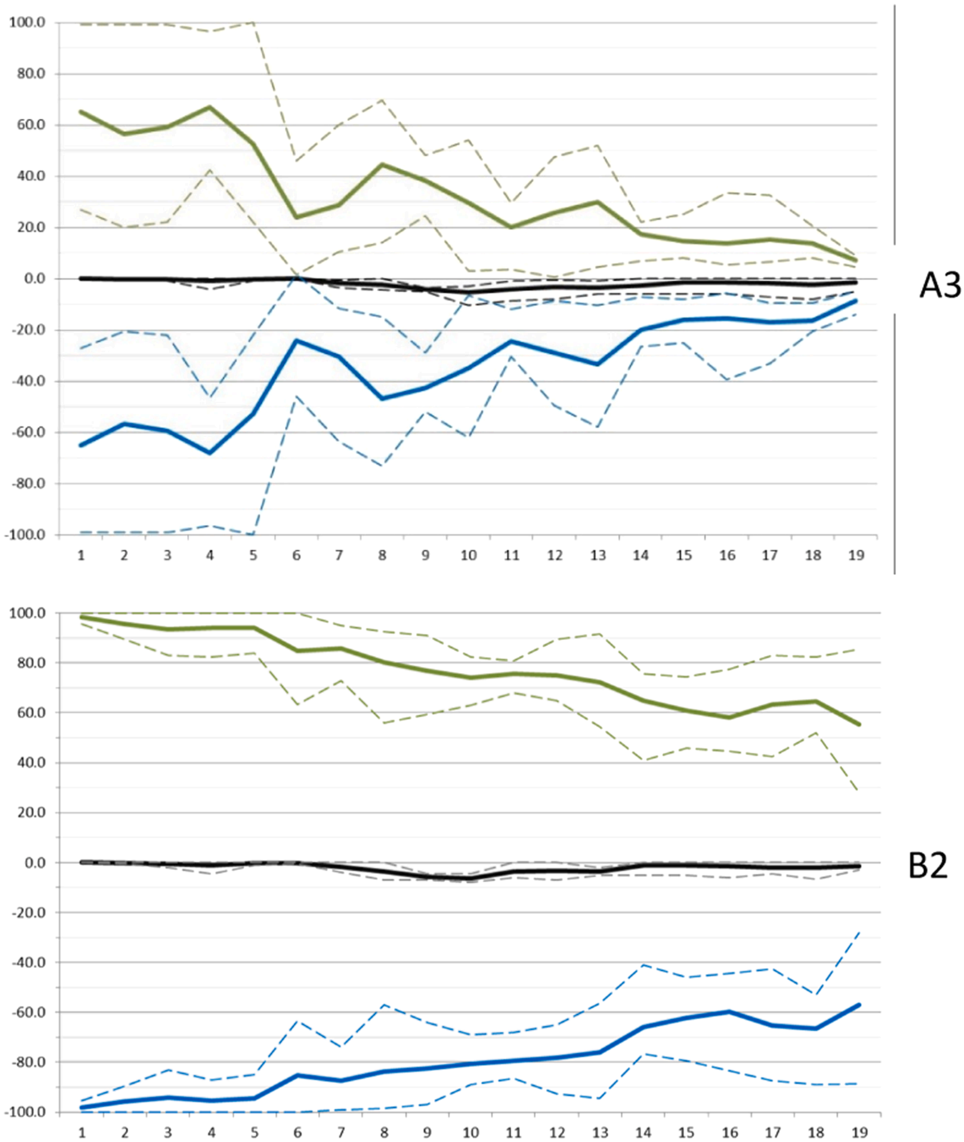

Fig. 3. A comparison between the average groundwater depth (blue line) and frost depth (black line) recorded at monitoring station A3 (above) and B2 (below) at Ojibway Prairie Provincial Park, Ontario, Canada, based on data collected from Nov 2015 to Mar 2019. Average life zone (LZ) size (green line) is depicted as the difference between groundwater and frost depths. Hatched lines are the minimum and maximum values recorded during the study period. Week 1 in each year was the 3rd week of Nov and week 19 in each year was the 4th week of Mar. Station A3 had a minimum LZ of 0 cm (not chosen as a station for artificial hibernation), whereas B2 had a minimum LZ of 28 cm (chosen as a station for artificial hibernation).

From 2015 to 18, missing FD and GWD data accounted for <1 % of data points, and from 2019 to 21 (at stations where snakes were hibernated, see below), missing data accounted for <2 % of data points.

To record groundwater temperature and to estimate measurement error in manual readings, one automatic water level data logger (Solinst, Levelogger Junior Edge, model 3001) was installed at each study grid and one reference grid (Appendix B). Leveloggers (n = 5–6) were deployed for 6 winters, starting from 14 November to 13 December, and ending from 28 March to 30 April (none deployed during winter of 2021–22). A subsample of levelogger readings (n = 335) was compared to manual groundwater measurements from 4 to 5 grids in each of 4 years (from 2015–16 to2018–19); manual measurements at each grid in any given year were within 1.9 cm (range = 0.3–5.1 cm), on average (excluding 9 measurements deemed erroneous due to discrepancies >10 cm).

2.4. Release site identification and validation

After four winters of data collection, four release sites were identified (one at each of grids A to D; Fig. 1; Appendix C). Each release site was 50 × 50 m in size (2500 m^2; one quarter of a study grid), which is similar in size to naturally-occurring hibernation areas (Appendix A). Release sites were situated where a LZ of ≥10 cm was observed at three adjacent monitoring stations (25–50 m apart) for at least two winters (Fig. 3). Life zone was computed as the difference between GWD and FD (Yagi et al., 2020). For example, if a GWD of 65 cm and a FD of 10 cm were recorded, then:

LZ = GWD - FD = 65–10 cm = 55 cm·

In addition, release sites were located where groundwater fluctuations were synchronous, surface flooding was mostly absent, and animal burrows were present. We assumed the absence of a LZ if at any point during monitoring (i.e., a 19-week period from late Nov to late Mar in each year) the groundwater table rose to the point of meeting or rising above either the frost level or the ground surface. Monitoring ended in late March to coincide with the general timing of soil temperature inversion, which signals the start of Massasauga egress (Smith, 2009). Furthermore, and to account for the possibility of combined measurement error in frost and groundwater depths, a space of <10 cm measured between the groundwater and frost depths was equated with the absence of a LZ.

Release sites were validated using an established technique at a subset of monitoring stations: "artificial" hibernation of snakes in constructed hibernacula (Gillingham and Carpenter, 1978; Todd et al., 2009; Bryan, 2015). We used hibernacula designs similar to those developed and tested by others (*S. catenatus* and *T. sirtalis*: Yagi pers. comm., 2018; *Vipera ursinii*: Halpern et al., 2013; Soorae, 2013; Péchy et al., 2015); however, we altered those designs to make use of readily available ABS plumbing supplies and to better replicate the shape and structure of natural hibernacula such as crayfish burrows (Choquette et al., 2023 in review). One artificial hibernaculum was installed at each of three monitoring stations per release site (Appendix C) and reference grid, for a total of 18 artificial hibernacula. Gartersnakes were used as a surrogate species for Massasaugas because the genus *Thamnophis* demonstrates many characteristics common to pit vipers, such as: seasonal hibernation, communal overwintering, long term sperm storage, birthing live young, and multiple paternities of litters (Taylor and Booth, 2017). Gartersnakes are known to communally hibernate with Massasaugas (King et al., 2004; Sage, 2005; Smith, 2009; Wylie et al., 2011; Harvey et al., 2014), and their presence has been considered a useful indicator of suitable hibernacula for Massasaugas (Sage, 2005; Smith, 2009). We presumed release sites suitable for gartersnake overwintering would be suitable for Massasauga overwintering.

A total of 113 gartersnakes of various ages were hibernated during 3 hibernation seasons (21 – 54 snakes in each year; Table 1), from fall 2019 to spring 2022. Snakes were hibernated at each of 6 grids (release sites in grids A – D; reference grids E – F) for 2 or 3 winters, with an average of 19 snakes (range = 13 – 29) hibernated at each grid. Wild snakes from within ~4 km of the study grids were captured in fall of each year, and in 2020 and 2021, these were supplemented with captive-reared individuals. Captive-reared snakes were born in the summer to wild-caught gravid females held in captivity until parturition. All snakes, regardless of source, were held temporarily in captivity for at least two weeks prior to hibernation to purge their digestive systems. Snakes held for a longer period were fed in captivity until 2 weeks prior to hibernation. Snakes were hibernated from mid to late November until late April in each winter, for an average of 158 days in hibernation (range 155 – 162 days). Our live animal protocol was approved by an authorized ethics committee (Laurentian University AUP # 6020448) and by the Province of Ontario (Wildlife Scientific Collector's Authorizations 1094523, 1096334, 1098752).

In all years, snakes were randomly assigned to a release grid (Haahr, 2023). Captive-reared snakes were first grouped based on litter (to facilitate later return to their mother's site of capture) and were then randomly assigned as a group to a release grid. Snakes were assigned to a grid until the maximum number was achieved for that year (based on availability of hibernacula). Each subgroup of snakes assigned to a release grid was further subdivided randomly into hibernation groups of 1 – 5 snakes (mean = 3), and these groups were randomly assigned to an artificial hibernaculum. Some snakes were reassigned non-randomly to a different hibernation group to achieve a minimum snake mass of ~20 g or 40 g in each group (equivalent to 1 or 2 yearling Massasaugas, respectively: Choquette and Fournier, *in prep*). Hibernation group mass ranged from 18.0 to 172.0 g (mean = 41.4 g); however, in each year an effort was made to evenly spread the largest groups across grids to reduce potential bias due to group size. Also, in years when both captive-reared and wild-caught snakes were used, individuals from both sources were spread evenly among release grids. To prevent mortality from predation or temperature fluctuations, artificial hibernacula remained capped until snakes were removed in the spring. Health status of snakes was checked with a borescope camera (Extech Instruments Corp., model BR250) on relatively warm days (≥ –2 °C) once every 1 – 2 weeks. Following the study, surviving snakes were released at their or their mothers' original sites of capture.

Table 1
Characteristics of Eastern Gartersnakes (*Thamnophis sirtalis*) artificially hibernated at four study grids (A – D) and two reference grids (E – F) at the Ojibway Prairie Complex and Greater Park Ecosystem, Ontario, Canada, from 2019 to 2022. Each grid was studied for two winters (2020–21 and 2021–22) or three winters (2019–20 to 2021–22). Reference grids were situated at a separate location from study grids, within hibernation areas recently used by Massasaugas. Mass of snakes based on weights at ingress. JU = Juvenile, CR = Captive-reared.

Grid	Total # of snakes hibernated	% JU	% CR	# of winters studied	Mean # of days in hibernation (range)	Total snake mass (g)	Median snake mass (range) (g)	Mean # of snakes per hibernation group (range)	Median mass of hibernation groups (range) (g)
A	17	76	24	3	158 (155–162)	374.5	6.5 (2.5–172.0)	2 (1–4)	44.5 (19.5–172.0)
B	21	71	33	3	158 (155–162)	334.0	7.0 (2.5–130.0)	3 (1–5)	45.0 (20.0–103.0)
C	13	85	69	2	160 (156–162)	108.5	4.5 (3.0–30.0)	3 (1–5)	23.0 (19.5–43.0)
D	29	79	55	3	158 (156–162)	465.4	5.0 (3.0–125.5)	3 (1–5)	23.5 (18.0–132.0)
E	16	94	25	2	159 (156–160)	124.5	6.3 (4.5–26.0)	3 (3–4)	20.0 (20.0–43.5)
F	17	88	53	2	158 (156–160)	125.0	5.5 (3.5–40.5)	3 (2–5)	20.5 (19.0–44.0)
A – F Combined	113	81	43	3	158 (155–162)	1531.9	6.0 (2.5–172.0)	3 (1–5)	23.5 (18.0–172.0)

2.5. Statistical analysis

The Kaplan-Meier method (Kaplan and Meier, 1958; Goel et al., 2010) was used to estimate gartersnake survival rates in grids A–F. Snakes were pooled by grid regardless of year hibernated. The total number of days in hibernation was used, regardless of the calendar date of ingress/egress. Snakes were considered censored if they survived until the end of hibernation, and the event of interest was the confirmed or suspected death of individuals at each grid. Date of snake death was either presumed based on unresponsiveness during regular borescope inspections of hibernacula, or confirmed when hibernacula were removed in the spring. Proportional hazards were assumed if survival curves were approximately parallel over time. We assumed that the censoring process did not bias survival estimates (i.e., non-informative censoring) because ingress and egress dates for all snakes within each year occurred on the same dates or within 2 days. Kaplan-Meier survival curves were initially computed and visualized using an online tool (Statistics Kingdom, 2017), and differences in survival rates between groups were tested for significance using a log-rank test in program R (v. 4.1.3). Two sample 2-tailed t-tests were performed in Microsoft Excel to compare mean mass of groups of juvenile snakes.

We fit two generalized linear mixed effects models (GLMM) with logit as a link function in program R to further investigate if two separate measures of LZ were associated with the survival of individual snakes to the end of hibernation (response variable). Model 1 included minimum LZ size (cm) as a predictor variable, while model 2 included % of sampling occasions with LZ size $\geq$10 cm (i.e., LZ frequency) as a predictor variable. Covariates for both models included year of study (2019, 2020, or 2021), snake mass (g) at ingress, and age class (juvenile [mass $\leq$12 g] or subadult/adult [mass $\geq$ 23 g]), and random factors included hibernation grid and artificial hibernaculum identifier.

Minimum LZ size was based on the smallest LZ recorded at a particular monitoring station over the course of a monitoring period in a given year (19 – 20 weeks), as described above (incl. negative values recorded when the groundwater level rose above the frost line). Minimum LZ size was corrected for any elevation differences between each artificial hibernaculum and its associated groundwater well, as measured using a surveyor's transit level in spring 2023. The transit was set up level within viewing distance of 1 – 3 artificial hibernacula, and a 1.75 m long measuring rod was used to record three surface elevations at each hibernaculum entrance and one surface elevation at the base of its associated groundwater well, to the nearest 0.5 cm. The difference between the elevation at the groundwater well and the average elevation at the hibernaculum determined the correction factor used (mean = –2.0 cm; range = –11.5 to 10.0 cm). Percent of sampling occasions with LZ size $\geq$ 10 cm was determined for a monitoring station in any given year by dividing the number of sampling occasions when the LZ was $\geq$10 cm (to the nearest 1 cm, after applying the correction factor) by the total number of sampling occasions at that station in that year.

3. Results

Study grids were comparable to the reference grids occupied by Massasaugas in that study grids contained a mix of open-canopy herbaceous and closed-canopy woody vegetation communities, contained animal burrows and surface water in spring, were situated within relatively flat terrain, and had similar groundwater temperatures (Table 2). Study grids differed from reference grids in that study grids were generally farther from open municipal drains and had smaller average LZs (Table 2). Prior to artificial hibernation of

Table 2
Characteristics of four study grids (A – D) and two reference grids (E – F) at the Ojibway Prairie Complex and Greater Park Ecosystem, Ontario, Canada. Reference grids were situated at a separate location from study grids, within hibernation areas recently used by Massasaugas. Ecological Land Classification (ELC) communities follow Chambers (2010): ME = Meadow, SV = Deciduous savanna, TH = Deciduous thicket, WO = Deciduous woodland, FO = Deciduous forest. Average values based on data from each of 9 initial monitoring stations at each grid (e.g., A1, A2, A3 … A9). Groundwater (GW) temperature was recorded from mid-Dec 2016 to end of Feb 2017. Average minimum life zone (LZ) size was based on data collected at each monitoring station from late Nov 2016 to late Mar 2017 (19 weeks). A thorough surface water inventory was completed in Apr 2018. Elevations and distances are from Google Earth. The locations of municipal drains were determined using publicly available City of Windsor and Town of LaSalle maps. *ELC communities at these grids were not characterised as part of a formal ELC study.

Grid	ELC communities present	Avg. index of canopy cover (range)	Avg. distance to nearest open municipal drain (range) (m)	Avg. elevation (range) (m)	Surface water present?	Animal burrows present?	Avg. GW temp (range) (°C)	Avg. min. LZ size (range) (cm)
A	ME & TH	6 (0–9)	71 (14–24)	179 (178–179)	Yes	Yes	5.5 (4.3–8.1)	12.3 (0–69.5)
B	ME, SV & WO	6 (0–9)	229 (173–297)	180 (179–181)	Yes	Yes	5.2 (3.9–7.8)	17.3 (0–52.0)
C	ME, TH & FO	5 (0–9)	252 (188–313)	179 (179–180)	Yes	Yes	5.7 (3.5–8.7)	12.2 (0–59.0)
D	ME, TH & FO	5 (0–9)	105 (49–156)	179 (177–181)	Yes	Yes	4.9 (3.1–7.7)	29.3 (0–89.0)
E*	ME & TH	3 (0–9)	39 (13–65)	179 (178–180)	Yes	Yes	4.5 (3.2–7.1)	31.0 (1.5–67.5)
F*	ME, TH, WO & FO	6 (0–9)	60 (15–108)	180 (179–181)	Yes	Yes	No data	55.8 (11.0–89.0)

snakes, and within each study grid, a stable LZ was identified at the same 4 – 7 monitoring stations for at least two consecutive winters (i.e., where LZ size never dropped below 10 cm during monitoring); 3 – 5 of the monitoring stations with a stable LZ were included within each release site (Appendix C). At each reference grid, a stable LZ was identified at the same 2 – 5 monitoring stations for at least two consecutive winters.

Survival rate of gartersnakes pooled by release site in grids A through D ranged from 0.65 to 0.95 (Table 3). Survival rate of all subadults/adults was 1.00 (20 / 20; mean mass at ingress = 50.2 g; range = 23.0–172.0 g), whereas survival of all juveniles was 0.78 (73 / 93; mean mass at ingress = 5.7 g; range = 2.5–12.0 g). For juveniles only, survival rate at release sites ranged from 0.60 at grid D to 0.93 at grid B, which was similar to juvenile survival at reference grids (0.63–1.00; Table 3; Fig. 4). Juvenile survival rate differed significantly between grids ($p = 0.03$). Survival rate of juveniles that were wild-caught (0.86) and captive-reared (0.71) did not differ significantly ($p = 0.1$), nor did survival rate of juveniles released in different years (range = 0.68–0.89; $p = 0.3$). Surviving juveniles weighed more at ingress, on average (mean = 6.0 g; SD = 1.8; range = 3.0–12.0 g), than those that died (mean = 4.5 g; SD = 1.4; range = 2.5–7.0 g; $p < 0.001$); however, there was no difference between mean body mass of juveniles hibernated at the two grids with the lowest survival (5.3 g; SD = 1.8; n = 39; D and F) and the mean mass of juveniles hibernated at all other grids (5.9 g; SD = 1.8; n = 54) ($p > 0.05$).

A Life Zone (LZ) was recorded at monitoring stations with hibernating snakes during 97 % (697 / 721) of LZ sampling occasions from 2019 to 2022 (Table 4). A LZ was recorded on 100 % of sampling occasions across monitoring stations and monitoring periods at two grids (D and F; Table 4), and these were the two grids with the lowest snake survival rates. In contrast, the grid with the highest survival rate (E) was also the grid where a LZ was least often recorded (Table 4). Across all winters combined, a total of 20 snakes out of 113 were found dead by the end of hibernation, and the majority of deaths (15 / 20; 75 %) occurred between the end of LZ monitoring (31 Mar – 1 Apr) and egress (20 – 30 Apr). All of the snakes that died (n = 20), including five that died prior to the end of LZ monitoring, were hibernated at stations where the minimum LZ was ≥10 cm during 100 % of sampling occasions. By contrast, 22 % of all snakes that survived hibernation (20 / 93) were overwintered at stations where the minimum LZ was < 10 cm during monitoring. Both GLMM indicated that mass at ingress had a significant positive effect on snake survival to end of hibernation ($p < 0.005$), while age class and year had no significant effect ($p > 0.3$) (Table 5). Model 1 indicated that minimum size of LZ had a marginally significant negative effect on survival ($p = 0.05$), and model 2 indicated that % of sampling occasions with a LZ size ≥ 10 cm had a significant negative effect on survival ($p = 0.006$).

4. Discussion

Based on how we quantified LZ, our results did not indicate a positive relationship between minimum LZ size and overwinter survival in gartersnakes hibernated in artificial hibernacula. One possible explanation is that the artificial hibernacula we used influenced snake survival by capturing small air pockets during flood events (Choquette et al., *in review*), thereby providing a temporary source of O_2 when the LZ was reduced to zero. An alternative, but not necessarily contradictory, explanation is that hibernating snakes can tolerate periods when LZ is small or absent, which is consistent with what has been demonstrated by others. For example, Markle et al. (2020a) found that at 16 peat hummock hibernacula occupied by Massasaugas, the life (i.e., resilience) zone was lost an average of 2 – 79 times per hummock during two study winters, but the LZ was lost for an average of only 41 min to 4 h 48 min per event. By contrast, at 2 hummocks where Massasaugas did not overwinter, LZ was lost for an average of over 6.5 days per event. Also, Costanzo (1989b) found that gartersnakes voluntarily submerged in cold water for prolonged periods while hibernating in both natural and artificial conditions; in natural conditions snakes hibernating 80 cm below water did not surface to breathe for up to 2 h (Costanzo, 1986). Furthermore, Costanzo (1989b) submerged gartersnakes to measure cutaneous O_2 consumption for periods of 20 h and observed no mortality. Therefore, to predict survival rate of snakes at hibernacula, the minimum LZ size (or loss of LZ) in and of itself may be less important than the duration of LZ absence.

Table 3
Survival rate (S_t) of Eastern Gartersnakes (*Thamnophis sirtalis*) artificially hibernated at four study grids (A – D) and two reference grids (E – F) at the Ojibway Prairie Complex and Greater Park Ecosystem, Ontario, Canada, from 2019 to 2022. Reference grids were situated at a separate location from study grids, within hibernation areas recently used by Massasaugas. JU = Juveniles (i.e., snakes ≤ 12 g). * 100 % of subadults/adults (i.e., snakes ≥ 23 g) survived hibernation; mortalities occurred only within the juvenile age class. S_t = Survival rate after 160 – 162 days of hibernation. See Appendix D for S_t for each artificial hibernaculum.

Grid	# of winters studied	# of snakes hibernated (# JU)	# of snakes surviving hibernation*	S_t (all ages)	S_t (JU)* , 95 % CI
A	3	17 (13)	15	0.88	0.85 (0.51–0.96)
B	3	21 (15)	20	0.95	0.93 (0.61–0.99)
C	2	13 (11)	11	0.85	0.82 (0.45–0.95)
D	3	29 (23)	20	0.65	0.60 (0.37–0.77)
E	2	16 (15)	16	1.00	1.00 (1.00–1.00)
F	2	17 (16)	11	0.65	0.63 (0.35–0.81)

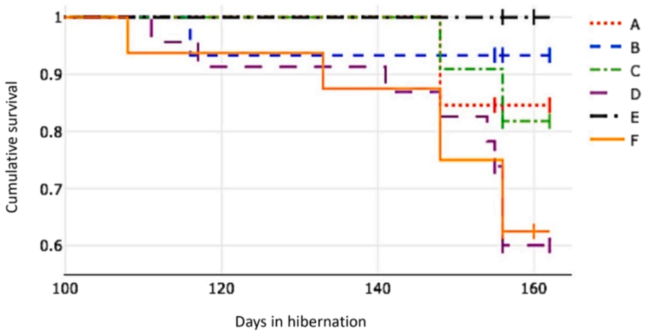

Fig. 4. Survival rate (S_t) of juvenile Eastern Gartersnakes (*Thamnophis sirtalis*) after 160 – 162 days of being artificially hibernated at four study grids (A – D) and two reference grids (E – F) at the Ojibway Prairie Complex and Greater Park Ecosystem, Ontario, Canada, from 2019 to 2022. Vertical bars indicate days when surviving snakes were censored (removed from hibernation). Note that the x-axis begins at 100 days in hibernation and the y-axis begins at ~0.57 cumulative survival. The first snake death was recorded on day 108. See Table 3 for 95 % confidence intervals.

Table 4
Results of Life Zone (LZ) monitoring at stations where Eastern Gartersnakes (*Thamnophis sirtalis*) were artificially hibernated across four study grids (A – D) and two reference grids (E – F) at the Ojibway Prairie Complex and Greater Park Ecosystem, Ontario, Canada, from 2019 to 2022. Reference grids were situated at a separate location from study grids, within hibernation areas recently used by Massasaugas. To account for the possibility of combined measurement error in both frost and groundwater depth, a LZ of < 10 cm was equated with the absence of a LZ. Number of dry groundwater well (GW) events indicates the number of pooled sampling occasions when no water was recorded in a monitoring well at each grid (wells were installed 1.0 m below ground).

Grid	# of winters studied	# of pooled LZ sampling occasions	Proportion of pooled sampling occasions with LZ	Smallest proportion of sampling occasions with LZ, by station	Smallest LZ size recorded (cm)	# of dry GW events
A	3	136	0.96	0.84 (A4–8 in 2019–20)	- 1 (A4–5 in 2019–20)	25 (18 %)
B	3	136	0.98	0.84 (B3–6 in 2021–22)	5 (B3–6 in 2021–22)	20 (15 %)
C	2	79	0.99	0.95 (C5–8 in 2020–21)	4 (C5–8 in 2020–21)	4 (5 %)
D	3	174	1.00	1.00	14 (D5–6 in 2021–22)	62 (36 %)
E	2	98	0.85	0.53 (E5 in 2021–22)	- 9 (E5 in 2021–22)	0 (0 %)
F	2	98	1.00	1.00	10 (F1–4 in 2021–22)	9 (9 %)

Table 5
Outputs from two generalized linear mixed effects models used to investigate the association between two measures of life zone and survival of individual Eastern Gartersnakes (*Thamnophis sirtalis*) that were artificially hibernated across four study grids (A – D) and two reference grids (E – F) at the Ojibway Prairie Complex and Greater Park Ecosystem, Ontario, Canada, from 2019 to 2022. The predictor variables were LZAH in model 1 (minimum life zone size [cm] at each snake's artificial hibernaculum) and %LZ in model 2 (the percent of sampling occasions with an LZ size ≥ 10 cm at each snake's artificial hibernaculum). See main text for additional details on predictor variables. The parameter estimates (Estimate), standard error (SE), and p-values for all variables are presented. Means (with SE) for the following variables are: mass = 13.6 g (SE 23.6), LZAH = 26.0 cm (SE = 1.8), %LZ = 97.0 (SE = 0.8).

	Estimate	SE	p-value
Model 1			
Year 2020	1.15	1.33	0.388
Year 2021	–0.58	1.24	0.638
Mass	0.78	0.25	0.002
Age class	–3.08	9251.09	1.000
LZAH	–0.04	0.02	0.050
Model 2			
Year 2020	0.48	1.27	0.702
Year 2021	–0.50	1.25	0.689
Mass	0.70	0.24	0.004
Age class	–5.67	533.15	0.992
%LZ	–3.75	1.37	0.006

Others have stressed the importance of using high temporal resolution data to accurately identify periods of LZ absence (e.g., sampling every 10 – 30 min: Markle et al., 2020a). Our manual groundwater and frost sampling was coarse (i.e., weekly), precluding our ability to estimate the length of time the LZ was lost at certain stations during monitoring. Works cited above suggest that a LZ can be lost for periods up to 20 h, but likely not longer than 6 days, without causing mortality in hibernating snakes. Additional study is therefore needed to identify temporal criteria for LZ identification, determine whether they are species-specific, and recommend minimum data collection frequencies and associated methods (e.g., manual vs. automatic sampling). It is also important to determine how sensitive LZ estimates are to different sampling frequencies, to guide selection of an optimal sampling regime within a particular study landscape. For example, extreme water-level fluctuations could occur between manual groundwater readings, and risk being overlooked without the use of data collected by automatic leveloggers (USGS, 2010), particularly at times of year when saturated soils are very responsive to rain events (e.g., late Mar – Apr: Guiton, 1978).

In addition to its minimum size, the maximum size of a LZ is important to consider when investigating hibernacula suitability. In our case, all of the snakes that died were hibernated at stations where the LZ size was always $\geq$ 10 cm during sampling, a result we did not anticipate. An overly large LZ (e.g., due to a lowering of the water table), therefore, could be detrimental to hibernating snakes. To overwinter successfully, snakes must locate and occupy the most humid and coldest tolerable microsites available to provide a buffer from extreme temperatures, reduce oxygen demands, and prevent desiccation (Costanzo, 1989a). For example, *T. sirtalis* hibernated underwater conserved more energy (>55 % reduction in energy expenditure) and experienced higher survival rates (e.g., 100 % vs. 6 %) than individuals hibernated in air, most of whom died from dehydration (Costanzo, 1989a; b). In our study, juveniles hibernated at grid D experienced the lowest survival rate, and this also happened to be the study grid where we recorded the greatest number of dry well events. Furthermore, Sage (2005) reported that Massasaugas in southeastern Michigan spent most of the winter fully submerged in groundwater, yet the water table at hibernation sites was lower than at random sites, and periodic flooding occurred at random sites but never at hibernation sites. Presumably, both the presence of a LZ and a high water table are necessary to optimize overwinter survival in temperate zone snakes.

Our release sites A, B and C maintained a LZ for the majority of the hibernation season (96 – 99 % of sampling occasions) and supported high overwinter survival of subadult/adult (100 %) and juvenile (82 – 93 %) Eastern Gartersnakes. Survival rates recorded by us are similar to published overwinter survival rates for both artificial and natural hibernation in gartersnakes, including *Thamnophis radix* (83 % and 90 % in various ages: Bailey, 1949), *T. sauritus* (80 % in unknown ages: Todd et al., 2009), and *T. sirtalis* (98 % in various ages: Costanzo, 1986; 74 %, 80 % and 93 % in adult males: Larsen and Gregory, 1989; 71 % in juveniles: A. Yagi unpub. data). Release sites A – C supported suitable hibernation habitat for gartersnakes, therefore, they may also provide suitable overwintering habitat for Massasaugas. Overwinter survival of wild adult Massasaugas was ~ 66 – 73 % in nearby southern Michigan (Sage, 2005; Hileman et al., 2018), therefore, if our sites are suitable for Massasauga hibernation, we would not expect as high of an overwinter survival rate as we observed in gartersnakes. Also, because Massasaugas are on average larger than gartersnakes of the same age class (Choquette and Fournier, *in prep*) release site selection based on survival of juvenile gartersnakes would provide a conservative assessment of suitability for juvenile Massasaugas. Massasaugas, however, would presumably require wider, larger and deeper hibernacula than similar-aged gartersnakes to be able to physically access suitable conditions below ground. Although the subterranean conditions may appear suitable at our proposed release sites based on LZ, we did not systematically assess or compare the adequacy (size, depth, number, etc.) of potential natural hibernacula at release sites. Investigations into the use of our artificial hibernacula as a habitat enhancement tool may be warranted where natural features of an appropriate size are lacking.

Our release site identification process was probably too lengthy to be practical for some translocation practitioners. The duration resulted from two of our initial assumptions, which led us to abandon some monitoring stations prematurely if the LZ dropped below 10 cm, while continuing to invest time monitoring other stations with repeatedly large LZs. To accelerate the process, future research should aim to refine release site identification criteria based on maximum LZ size and duration of extreme events (flooding or drought) tolerable to hibernating snakes. Regarding our release site validation process, that phase benefited from the use of all gartersnake age classes. Only juveniles perished in our study, which allowed us to detect differences we otherwise may have overlooked; no subadults/adults died even at grids where juvenile mortality was highest. Juvenile snakes were more sensitive to poor quality hibernacula than subadults/adults, presumably because they were smaller and thus more susceptible to desiccation. Release site validation would benefit from including members of the smallest age class to avoid survival rates biased toward larger, more desiccation-resistant individuals.

5. Conclusions

We used four years of data to identify Massasauga reintroduction sites in a sand plain ecosystem, validated these for three years using artificial hibernation of gartersnakes, and compared survival rates to two measures of LZ. Our results will guide the selection of reintroduction sites for an endangered snake in the study landscape (sites A, B and C appear most suitable), and will contribute to future applications of the LZ theory. We encourage the wider adoption of a rigorous release site selection process for translocations of temperate zone snakes to improve conservation translocation outcomes.

CRediT authorship contribution statement

JDC: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing – Review & Editing, Visualization, Project administration, Funding acquisition, Supervision. **AIM:** Methodology, Software, Formal analysis. **JDL:** Conceptualization, Resources, Writing – Review & Editing, Supervision, Funding acquisition. **TEP:** Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jonathan Choquette reports financial support was provided to WPC by Ganawenim Meshkiki and the Eastern Georgian Bay Initiative. Jonathan Choquette (through SCC Ecological) reports a relationship with Blazing Star Environmental, Environment and Climate Change Canada, Gouvernement du Québec, Piroli Construction Inc., and 8Trees Inc. that includes: consulting or advisory.

Data Availability

R code and raw data used in the GLMM analyses are included as supplementary materials.

Acknowledgements

Funding was provided by Ontario Ministry of Natural Resources and Forestry grants to WPC (SAR-00097; 109-18-WPC3), Ontario Ministry of the Environment Conservation and Parks grants to WPC(117-20-WPC3; 79-23-WPC2), a Ganawenim Meshkiki and the Eastern Georgian Bay Initiative grant to WPC (EGBI-2021-08), Environment and Climate Change Canada grants to WPC (2015HSP6988; 2017HSP8062; GCXE24C276), a Natural Sciences and Engineering Research Council of Canada CREATE grant to Laurentian University (481954-2016 [ReNewZoo]), a Colleges and Institutes Canada grant to SCC Ecological (Eco C6591), Employment and Social Development Canada grants to WPC and Georgian Bay Turtle Hospital (Canada Summer Jobs Internships), and an Eco Canada grant to WPC (sh14594). No funders played a direct role in the design, development or undertaking of this study. This project has received funding support from the Government of Ontario; such support does not indicate endorsement by the Government of Ontario of the contents of this publication.

Seven field technicians collected and entered most of the data: A. Hand, E. Jolin, J. Van Poucke-Choquette, J. Barden, K. Antaya, D. Wright, and I. Fergusson. Many other field technicians and volunteers assisted with the construction/installation/maintenance of monitoring equipment, data collection, or snake collection/care/hibernation: M. Cairns and the Ontario Stewardship Rangers, A. Choquette, L. Choquette, E. Dinel, D. Dunlop, L. Gagnon, J. Gui, A. Lavictoire, T. Lourie, S. Marks, H. McCurdy-Adams, Ojibway Nature Centre staff, S. Paul, L. Savi, C. Trowbridge, and S. Yong. A. Yagi assisted with and advised on set up of grids and monitoring stations, and data collection. A. Yagi and three anonymous reviewers provided comments on an earlier version of this manuscript. Advice and supervision also provided by: R. Heide, A. Lentini, A. Shulte-Hostedde, J. Steiner, E. Williams, L. Woolaver Jr., and members of the Ojibway Prairie Reptile Recovery Working Group. K. Woodhouse assisted with climate and levelogger data manipulation. C. Fournier assisted with manuscript formatting, French translation of abstract (Appendix E), and figures. Ontario Parks and Town of LaSalle provided land access.

Appendix A. Important characteristics of Massasauga (*Sistrurus catenatus*) hibernation habitat

Description of Characteristic	Sources
Moist or water saturated upland areas that are adjacent to wetlands but not flood-prone and with several animal burrows allowing access below the frost line	Reinert and Kodrich, 1982; Seigel et al. (1998);Johnson et al. (2000);Dreslik (2005);Sage (2005);Bissell (2006);Moore and Gillingham (2006);Smith (2009); COSEWIC, 2012
Presence of dense vegetation for shelter from harsh winter conditions	Bissell (2006);Moore and Gillingham (2006); COSEWIC, 2012
Proximity to suitable basking, foraging and gestation habitat	Dreslik (2005);Sage (2005); Durbian et al., 2008;Smith (2009);DeGregorio et al. (2011)
Hibernation areas are non-random and generally occur at the scale of 1000 – 8000 m^2	Johnson et al. (2000);Sage (2005);Bissell (2006);Smith (2009); COSEWIC, 2012

Appendix B. Fabrication, installation, and data collection methods for groundwater wells and frost tubes

B.1 Groundwater wells and leveloggers

Groundwater wells were built from 1.8 m (6′) lengths of 4.0 cm inner diameter [ID] ABS pipe. Wells were capped at the bottom end with an ABS test cap, and the top end with either a test cap or a cleanout cap. Holes 0.6 cm in diameter (0.25″) were drilled in the bottom 50 cm of each well in 4 rows equally spaced around its circumference (48 holes per well). The bottom 50 cm of each well was wrapped using one layer of a synthetic fibre cloth as a screening to prevent entry of soil particles, which was affixed to the well using fiberglass tape. Wells were marked with fiberglass tape at the 1.0 m mark and installed into the ground to a depth of 1.0 m in a hole bored using a manual auger (and manually backfilled). A small notch was made near to the top of each well using a handsaw, to allow the pressure inside the wells to equalize with atmospheric pressure. Groundwater depth (GWD) was calculated as the difference between the depth of groundwater from top-of-well and the height of the groundwater well above ground.

One automatic water levelogger was installed at a central monitoring station of each grid (A5, B5, C5, D8, and E5; none installed at grid F). Loggers were configured with a pre-programmed start time prior to installation in the field. Each logger was suspended in an existing groundwater well using steel wire, with a metal carriage bolt used to secure the levelogger to the side of the well near the cap. Loggers were installed at a different depth at each well to ensure they would remain submerged under water during the study and in some cases as low as possible without sitting on the bottom of the well. Loggers did not interfere with the weekly manual data collection at the wells. Wells with water leveloggers were fitted with locking caps to reduce risk of theft. Deployment depth and time of deployment were recorded during each installation. Three leveloggers were reinstalled at alternate monitoring stations beginning in the second and/or third year of deployment in response to low ground water levels (levelogger at A5 moved to A3; levelogger C5 moved to C1, then to C1–2; levelogger D8 moved to D5–8, then to D5–6), and in some cases the wells with leveloggers were reinstalled deeper than the others (e.g., 1.4 m below ground) to ensure leveloggers would remain submerged.

Leveloggers were programmed to record water temperature ($\pm$ 0.1°C) and water level ($\pm$ 0.005 m) at 15-minute intervals. Leveloggers recorded absolute pressure in a well (equal to combined barometric and water pressure) which was later converted to water level depth after accounting for barometric pressure recorded hourly at a nearby weather station (Windsor Airport, $<$ 10 km away and within an elevation change of ~12 m). Levelogger readings were automatically temperature-compensated (Solinst Canada Ltd, 2013). Deployment depth for all loggers in each year was measured by adding the "hanging depth" to the distance between the "hanging point" and the top of the well for each unit at time of installation (see USGS, 2010 for definitions). A manual water level reading and measurement of groundwater well height were recorded within 0 – 2 days of levelogger installation.

B.2 Frost tubes

Frost tubes were constructed following the CRREL-Gandahl design (McCool and Kok, 1990) and consisted of an outer plastic casing and an inner plastic tube. The outer casing was constructed from ~1.5 m long (1.5 – 2.0 cm ID, 2.1 – 2.7 cm outer diameter [OD]) PVC pipe, with a removable ABS cap installed at both ends. Outer casings were marked with electrical tape at the 1.0 m mark and installed into the ground to a depth of 1.0 m in a hole bored using a manual auger (and manually backfilled). The inner tube was made of low density polyethylene tubing (1.3 cm OD, 1.0 cm ID), which was inserted into the outer casing and was cut flush with the outer casing at the top end. A mono-filament line was passed through the inner tube and taped to the exterior of both ends. The inner tube was plugged at the bottom with exterior vinyl caulking and/or natural cork, as well as a plastic cap to prevent leakage. Inner tubes were marked 1.0 m from the tube bottom using electrical and/or fiberglass tape such that the mark on the inner tube lined up exactly with the 1.0 m mark on the outer casing. The 1.0 m mark on the removable inner tube was used in the field to gauge frost tube depth, so it was necessary for this to accurately represent ground level as marked on the outer casing. A red dye solution was created by mixing 100 drops of generic red food colouring in 1.0 L of water, which was used to fill each inner tube to the 1.0 m mark. A small loop of cotton string was suspended across the top of the inner tube, which was then capped with a plastic cap. The inner tube could then be slid out of the outer casing by gently pulling up on cotton loop. The ID of the outer casing was between 0.2 and 0.7 cm wider than the OD of the inner tube.

Frost depth (FD) was recorded by pulling out the inner tube from the outer casing of a frost tube far enough to measure the length of frozen liquid in the inner tube, based on the distance from the soil surface to the lowest ice dendrite visible inside the tube. The boundary between the uncoloured frozen portion of the frost tube and the dyed unfrozen liquid was distinct. If the uncoloured portion of the liquid was not frozen, presumably due to a recent thaw event, the liquid was mixed to redistribute the dye.

Appendix C. Summary of results from life zone (LZ) monitoring completed at multiple monitoring stations within four study grids at the Ojibway Prairie Provincial Park, Ontario, Canada, from late 2015 to early 2019. Number of years with data is based on combined years with a paired groundwater well and frost tube, or monitoring stake, if applicable. Minimum LZ size is based on the smallest measured LZ in any of the monitoring years, and is based only on data collected from late Nov to late Mar. Only stations with at least two winters of data are shown. Dashed boxes depict the monitoring stations contained within the boundaries of each release site. Each artificial hibernaculum (AH) was installed 1 m to the east of the groundwater well at that particular monitoring station. At each release site, three artificial hibernacula (AH) were installed no more than ~60 m from each other

(*continued on next page*)

(*continued*)

Monitoring Station	No. of years with data	Min. LZ size (cm)	AH installed
Grid A			
A1	2	0	
A1-2	3	0	
A1-4	2	6	
A2	3	0	
A3	4	0	
A4	4	27	
A4-5	3	24	X
A4-8	2	24	X
A5	4	0	
A5-8	2	16	X
A7	2	2	
A8	4	0	
A6	3	0	
A6-9	3	26	
A9	3	0	
Grid B			
B1	3	0	
B1-2	2	5	
B2	4	28	X
B2-3	2	14	X
B3	2	0	
B3-6	3	10	X
B5	4	8	
B6	4	0	
B4	4	4	
B7	3	0	
B8	4	36	
B8-9	3	9	
B9	4	0	
Grid C			
C1	3	0	
C1-2	2	7	
C1-4	2	32	
C2	4	18	
C3	4	0	
C4	4	45	
C5	3	0	
C5-6	2	14	X
C5-8	3	10	X
C6	4	4	
C6-9	3	62	X
C7	2	1	
C8	4	0	
C9	3	0	
Grid D			
D1	4	13	
D1-2	2	40	
D2	4	39	
D2-6	2	39	X
D3	4	15	
D5	4	9	
D5-6	2	13	X
D6	4	42	X
D4	2	6	
D5-8	3	0	
D7	4	0	
D8	4	0	
D9	4	0	

Appendix D. Survival rate (S_t) of Eastern Gartersnakes (*Thamnophis sirtalis*) hibernated within 18 artificial hibernacula at the Ojibway Prairie Complex and Greater Park Ecosystem, Ontario, Canada, from 2019 to 2022. JU = Juveniles. *Only juvenile snakes died during hibernation; no snakes ≥23 g died during winter (i.e., subadults/adults). S_t = Survival rate after 160–162 days of hibernation

Artificial hibernaculum identifier	# of winters studied	# of snakes hibernated (# JU)	# of snakes surviving hibernation*	S_t (all ages)	S_t (JU)
A4–5	3	5 (3)	5	100	100
A4–8	2	7 (6)	6	86	83
A5–8	2	5 (4)	4	80	75
B2	2	4 (2)	4	100	100
B2–3	2	5 (2)	4	80	50
B3–6	3	12 (11)	12	100	100
C5–6	1	3 (2)	3	100	100
C5–8	1	4 (4)	4	100	100
C6–9	2	6 (5)	4	67	60
D2–6	3	11 (8)	8	73	63
D5–6	3	12 (11)	7	58	55
D6	3	6 (4)	5	83	75
E5	2	6 (6)	6	100	100
E7	2	7 (7)	7	100	100
E10	1	3 (2)	3	100	100
F1–4	2	7 (6)	4	57	50
F2	2	6 (6)	4	67	67
F4	1	4 (4)	3	75	75

Appendix E. Supporting information

Supplementary information associated with this article (Abstract in French / Résumé en français, R code, and raw data used in GLMM analyses) can be found in the online version at doi:10.1016/j.gecco.2023.e02765.

References

Bailey, R.M., 1949. Temperature toleration of Gartersnakes in hibernation. Ecology 30 (2), 238–242. https://doi.org/10.2307/1931190.

Bieser, N.D., 2008. Spatial ecology and survival of resident juvenile and headstarted Eastern Massasauga (*Sistrurus catenatus catenatus*) in northern Michigan (PhD Thesis). Purdue University, Fort Wayne, Indiana, USA.

Bissell, K.M., 2006. Modeling habitat ecology and population viability of the Eastern Massasauga rattlesnake in southwestern lower Michigan (M.Sc. Thesis). Michigan State University, East Lansing, Michigan, USA.

Böhm, M., Collen, B., Baillie, J.E., Bowles, P., Chanson, J., Cox, N., Zug, G., 2013. The conservation status of the world's reptiles. Biol. Conserv. 157, 372–385. https://doi.org/10.1016/j.biocon.2012.07.015.

Bryan, D.L., 2015. Survival and natural history of the timber rattlesnake (*Crotalus horridus*) at Center Hill Lake in Dekalb County, Tennessee (Ph.D Thesis). Tennessee Technological University, Cookeville, Tennessee, USA.

Chambers, J., 2010. Ecological land classification of Ojibway Prairie Provincial Nature Reserve. Ontario Parks, Ontario Ministry of Natural Resources.

Choquette, J.D., Jolin, E.A., 2018. Checklist and status of the amphibians and reptiles of Essex County, Ontario: a 35 year update. Can. Field-Nat. 132 (2), 176–190. https://doi.org/10.22621/cfn.v132i2.2053.

Choquette, J.D., Savi, L., Fournier, C., *in preparation*. An inexpensive artificial snake hibernaculum built using readily available plumbing supplies. MethodsX.

Choquette, J.D., Macpherson, M.R., Corry, R.C., 2020. Identifying Potential Connectivity for an Urban Population of Rattlesnakes (*Sistrurus catenatus*) in a Canadian Park System. Land. 2020 (9), 313. https://doi.org/10.3390/land9090313.

Choquette, J.D., Litzgus, J.D., Gui, J.X.Y., Pitcher, T.E., 2022. A systematic review of snake translocations to identify potential tactics for reducing postrelease effects. Conserv. Biol. 37 (1), 14016. https://doi.org/10.1111/cobi.14016.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC), 2012. COSEWIC Assessment and status report on the Massasauga *Sistrurus catenatus* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa.

Costanzo, J.P., 1986. Influences of hibernaculum microenvironment on the winter life history of the garter snake (*Thamnophis sirtalis*). Ohio J. Sci. 86 (5), 199–204.

Costanzo, J.P., 1989a. Effects of humidity, temperature, and submergence behavior on survivorship and energy use in hibernating garter snakes, *Thamnophis sirtalis*. Can. J. Zool. 67 (10), 2486–2492. https://doi.org/10.1139/z89-351.

Costanzo, J.P., 1989b. A physiological basis for prolonged submergence in hibernating garter snakes *Thamnophis sirtalis*: evidence for an energy-sparing adaptation. Physiol. Zool. 62 (2), 580–592. https://doi.org/10.1086/physzool.62.2.30156186.

DeGregorio, B.A., Manning, J.V., Bieser, N., Kingsbury, B.A., 2011. The spatial ecology of the Eastern Massasauga (*Sistrurus c. catenatus*) in Northern Michigan. Herpetologica 67 (1), 71–79. https://doi.org/10.1655/09-049R1.1.

Dreslik, M.J., 2005. Ecology of the Eastern Massasauga (*Sistrurus catenatus catenatus*) from Carlyle Lake, Clinton County, Illinois (PhD Thesis). University of Illinois Urbana-Champaign, Champaign, Illinois USA.

Durbian, F.E., King, R.S., Crabill, T., Lambert-Doherty, H., Seigel, R.A., 2008. Massasauga home range patterns in the Midwest. J. Wildl. Manag. 72 (3), 754–759. https://doi.org/10.2193/2007-710.

Germano, J., Ewen, J.G., Mushinsky, H., McCoy, E., Ortiz-Catedral, L., 2014. Moving towards greater success in translocations: recent advances from the herpetofauna. Anim. Conserv. 17 (S1), 1–3. https://doi.org/10.1111/acv.12172.

Gillingham, J.C., Carpenter, C.C., 1978. Snake hibernation: construction of and observations on a man-made hibernaculum (Reptilia, Serpentes). J. Herpetol. 12, 495–498. https://doi.org/10.2307/1563354.

Goel, M.K., Khanna, P., Kishore, J., 2010. Understanding survival analysis: Kaplan-Meier estimate. Int. J. Ayurveda Res. 1 (4), 274–278.

Guiton, R.S., 1978. A Groundwater Study of the Ojibway Prairie. University of Waterloo Research Institute, Waterloo, Ontario, Canada.

Haahr, M., 2023. RANDOM.ORG: True Random Number Service. [ONLINE] Available at: ⟨https://www.random.org⟩ [Accessed 6 November 2023].

Halpern, B., Péchy, T., Takacs, G., Sós, E., Szovenyi, G., 2013. Conservation of Hungarian Meadow Viper. Layman's report of the Hungarian Meadow Viper LIFE+ project. Hungarian Ornithological and Nature Conservation Society, Budapest, Hungary. ⟨https://www.rakosivipera.hu/en/downloads/⟩.

Hanson, C.L., Flerchinger, G.N., 1990. Comparison of the three methods for measuring depth of soil freezing, in: Cooley, K. R. (Ed.), Proceedings from the International Symposium of Frozen Soil Impacts on Agricultural, Range and Forest Lands. USDA Agricultural Research Service, Boise, ID, USA, pp. 257–262.

Harvey, D.S., Weatherhead, P.J., 2006. Hibernation site selection by Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*) near their Northern range limit. J. Herpetol. 40 (1), 66–73. https://doi.org/10.1670/89-05A.1.

Harvey, D.S., Lentini, A.M., Cedar, K., Weatherhead, P.J., 2014. Moving Massasaugas: insight into rattlesnake relocation using *Sistrurus c. catenatus*. Herpetol. Conserv. Biol. 9 (1), 67–75.

Hileman, E.T., King, R.B., Faust, L.J., 2018. Eastern Massasauga demography and extinction risk under prescribed- fire scenarios. J. Wildl. Manag. 82 (5), 977–990. https://doi.org/10.1002/jwmg.21457.

IUCN/SSC, 2013. Guidelines for reintroductions and other conservation translocations. IUCN Species Survival Commission, Gland, Switzerland.

Johnson, G., King, R., Parent, C., Seigel, R., Szymanski, J., Kingsbury, B., 2000. The Eastern Massasauga: A Handbook for Land Managers. US Fish and Wildlife Service, Fort Snelling, MN, USA.

Kaplan, E.L., Meier, P., 1958. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53 (282), 457–481.

King, R., Berg, C., Hay, B., 2004. A repatriation study of the Eastern Massasauga (*Sistrurus catenatus catenatus*) in Wisconsin. Herpetologica 60 (4), 429–437. https://doi.org/10.1655/03-01.

Kingsbury, B.A., Attum, O., 2009. Conservation strategies: captive rearing, translocation, and repatriation, in: Mullin, S. J. and Seigel, R. A. (Eds.), Snakes: Ecology and Conservation. Cornell University Press, Ithaca, New York, USA, pp. 201–220.

Larsen, K.W., Gregory, P.T., 1989. Population size and survivorship of the common garter snake, *Thamnophis sirtalis*, near the northern limit of its distribution. Ecography 12 (2), 81–86. https://doi.org/10.1111/j.1600-0587.1989.tb00825.x.

Lesbarrères, D., Ashpole, S.L., Bishop, C.A., Blouin-Demers, G., Brooks, R.J., Echaubard, P., Govindarajulu, P., Green, D.M., Hecnar, S.J., Herman, T., Houlahan, J., Litzgus, J.D., Mazerolle, M.J., Paszkowski, C.A., Rutherford, P., Schock, D.M., Storey, K.B., Lougheed, S.C., 2014. Conservation of herpetofauna in northern landscapes: threats and challenges from a Canadian perspective. Biol. Conserv. 170 (2014), 48–55. https://doi.org/10.1016/j.biocon.2013.12.030.

Markle, C.E., Moore, P.A., Waddington, J.M., 2020a. Temporal variability of overwintering conditions for a species-at-risk snake: Implications for climate change and habitat management. e00923 Glob. Ecol. Conserv. 22. https://doi.org/10.1016/j.gecco.2020.e00923.

Markle, C.E., Moore, P.A., Waddington, J.M., 2020b. Primary drivers of reptile overwintering habitat suitability: integrating wetland ecohydrology and spatial complexity. BioScience 70 (7), 597–609. https://doi.org/10.1093/biosci/biaa059.

Markle, C.E., North, T.D., Harris, L.I., Moore, P.A., Waddington, J.M., 2020c. Spatial heterogeneity of surface topography in peatlands: assessing overwintering habitat availability for the Eastern Massasauga Rattlesnake. Wetlands 40 (6), 2337–2349. https://doi.org/10.1007/s13157-020-01378-2.

McCool, D.K., Kok, H., 1990. Field and laboratory techniques for frozen and thawing soil, in: Cooley, K.R. (Ed.), Proceedings from the International Symposium of Frozen Soil Impacts on Agricultural, Range and Forest Lands. USDA Agricultural Research Service, Boise, ID, USA, pp. 271–278.

Moore, J.A., Gillingham, J.C., 2006. Spatial ecology and multi-scale habitat selection by a threatened rattlesnake: the Eastern Massasauga (*Sistrurus catenatus catenatus*). Copeia 2006 (4), 742–751. https://doi.org/10.1643/0045-8511(2006)6[742:SEAMHS]2.0.CO;2.

Ontario Ministry of Natural Resources (OMNRF), 2002. Southern Ontario Land Resource Information System, Final Release 2000–2002, Version 1.2. Land Information Ontario, Ontario Ministry of Natural Resources and Forestry, Peterborough, ON, Canada.

Ontario Ministry of Natural Resources (OMNRF), 2009. Southern Ontario Wetland Evaluation - Ojibway Prairie Wetland Complex. Ontario Ministry of Natural Resources, Aylmer District, Chatham, Ontario.

Ontario Parks, 2005. Ojibway Prairie Resource Stewardship Plan Amendment. Appendix F: Repatriation of Eastern Massasauga Rattlesnakes at Ojibway Prairie Nature Reserve. Ontario Parks, London, ON, Canada.

Ontario Parks, 2015. Ojibway Prairie Management Plan. Ontario Parks, Wheatley, ON, Canada.

Ontario Provincial Standards (OPS), 2010. Foundation frost penetration depths for Southern Ontario (OPS drawing No. OPSD 3090.101), Rev. 1.

Patric, J.H., Fridley, B.D., 1969. A device for measuring soil frost (No. Research Note NE-94). USDA Forest Service, USA.

Péchy, T., Halpern, B., Sós, E., Walzer, C., 2015. Conservation of the Hungarian Meadow Viper *Vipera ursinii rakosiensis*. Int. Zoo. Yearb. 49 (1), 89–103. https://doi.org/10.1111/izy.12088.

Reading, C.J., Luiselli, L.M., Akani, G.C., Bonnet, X., Amori, G., Ballouard, J.M., Rugiero, L., 2010. Are snake populations in widespread decline? Biol. Lett. 6 (6), 777–780. https://doi.org/10.1098/rsbl.2010.0373.

Reinert, H.K., Kodrich, W.R., 1982. Movements and habitat utilization by the Massasauga, *Sistrurus catenatus catenatus*. J. Herpetol. 16 (2), 162–171. https://doi.org/10.2307/1563809.

Richards, N.R., Caldwell, A.G., Morwick, F.F., 1949. Soil survey of Essex County. Report No. 11 of the Ontario Soil Survey. Experimental Farm Service, Dominion Department of Agriculture and the Ontario Agricultural College, Ottawa, ON, Canada.

Sage, J.R., 2005. Spatial ecology, habitat utilization, and hibernation ecology of the Eastern Massasauga (*Sistrurus catenatus catenatus*) in a disturbed landscape (M.Sc. Thesis). Purdue University, West Lafayette, IN, USA.

Sainsbury, K.A., Morgan, W.H., Watson, M., Rotem, G., Bouskila, A., Smith, R.K., Sutherland, W.J., 2021. Reptile Conservation: Global Evidence for the Effects of Interventions for reptiles, in: Conservation Evidence Series Synopsis. University of Cambridge, Cambridge, pp. 1–662.

Seigel, R.A., Sheil, C.A., Doody, J.S., 1998. Changes in a population of an endangered rattlesnake *Sistrurus catenatus* following a severe flood. Biol. Conserv. 83 (2), 127–131. https://doi.org/10.1016/S0006-3207(97)00070-0.

Shine, R., Mason, R.T., 2004. Patterns of mortality in a cold-climate population of garter snakes (*Thamnophis sirtalis parietalis*). Biol. Conserv. 120 (2), 201–210. https://doi.org/10.1016/j.biocon.2004.02.014.

Smith, C.S., 2009. Hibernation of the Eastern Massasauga rattlesnake (*Sistrurus catenatus catenatus*) in northern Michigan (M.Sc. Thesis). Purdue University, Fort Wayne, Indiana, USA.

Smolarz, A.G., Moore, P.A., Markle, C.E., Waddington, J.M., 2018. Identifying resilient Eastern Massasauga Rattlesnake (*Sistrurus catenatus*) peatland hummock hibernacula. Can. J. Zool. 96 (9), 1024–1031. https://doi.org/10.1139/cjz-2017-0334.

Solinst Canada Ltd., 2013. User Guide Levelogger Series - Software Version 4. Canada.

Soorae, P. S. (ed.), 2013. Global Re-introduction Perspectives: 2013. Further case studies from around the globe. Gland, Switzerland: IUCN/ SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. xiv + 282 pp.

Statistics Kingdom, 2017. Kaplan Meier Survival Analysis [web application]. ⟨https://www.statskingdom.com/kaplan-meier.html⟩. [Accessed May 2023].

Taylor, E.N., Booth, W., 2017. Rattlesnakes as Models for Reproductive Studies of Vertebrates, in: Schuett, G.W., Feldner, M.J., Smith, C.F., Reiserer, R.S. (Eds.), Rattlesnakes of Arizona. ECO Publishing, Rodeo, NM, USA, p. 520.

Todd, J., Amiel, J., Wassersug, R., 2009. Factors influencing the emergence of a northern population of Eastern Ribbon Snakes (*Thamnophis sauritus*) from artificial hibernacula. Can. J. Zool. 87 (12), 1221–1226. https://doi.org/10.1139/Z09-114.

United States Geological Survey (USGS), 2010. Measuring water levels in wells and piezometers by use of a submersible pressure transducer (No. GWPD 16). United States Geological Survey, USA.

Vermette, S., Kanack, J., 2012. Modeling Frost Line Soil Penetration Using Freezing Degree-Day Rates, Day Length, and Sun Angle, in: Langlois, A., Frankenstein, S. (Eds.), Proceedings of the 69th Annual Eastern Snow Conference. Presented at the 69th Annual Meeting of the Eastern Snow Conference, Eastern Snow Conference, Hanover, New Hampshire, USA, Claryville, New York, USA, pp. 143–156.

Woodliffe, P.A., 1997. Resource Management Plan for Ojibway Prairie Provincial Nature Reserve (Ontario Parks). Ontario Ministry of Natural Resources, Chatham, ON, Canada.

Wylie, S.J., Dreslik, M.J., Wylie, D.B., Philips, C.A., 2011. Feasibility of reintroduction and translocation of neonate Eastern Massasauga Rattlesnakes as a tool for population recovery - Year 2. Illinois Natural History Survey, Champaign, IL, USA.

Yagi, A.R., Planck, R.J., Yagi, K.T., Tattersall, G.J., 2020. A long-term study on Massasaugas (*Sistrurus catenatus*) inhabiting a partially mined peatland: a atandardized method to characterize snake overwintering habitat. J. Herpetol. 54 (2), 235–244. https://doi.org/10.1670/18-143.