Eastern Massasauga Rattlesnake: Ohio Population Survey and Survey Technique Development

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16. Abstract

The Eastern Massasauga Rattlesnake (*Sistrurus catenatus*; EMR) has declined through most of its range and is endangered in Ohio and federally threatened. Areas that EMR may inhabit must be surveyed prior to approving development projects. Therefore, comprehensive, fast, and affordable survey techniques for the EMR are of interest to government and non-government groups. This is particularly evident in Ohio where all known EMR populations are near maintained or expanding road networks. Traditional EMR surveying in Ohio uses visual encounter surveys and artificial cover object arrays. The purpose of this research is to test and refine a new survey technique, the Adapted-Hunt Drift Fence Technique (AHDriFT) and compare it to current EMR survey methods using a cost-benefit analysis. We deployed AHDriFT arrays at 66 fields across seven Ohio Counties over the course of four years, yielding 351 EMR detections at 57 fields. AHDriFT was the most effective and consistent method for detecting massasaugas in Ohio across a broad range of environmental and temporal variables. In this report, we generated relatively precise estimates for deploying these arrays to achieve high confidence in survey results.

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Table of Contents

List of In-Text Figures and Tables

Problem Statement

The Eastern Massasauga (*Sistrurus catenatus*; EMR) was once found throughout much of the Great Lakes region, including unglaciated portions of Ohio, but has declined drastically during the last few decades due largely to habitat loss and fragmentation (Szymanksi, 1998; Szymanksi et al., 2016). The EMR 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. On September 30, 2015, the USFWS proposed listing the EMR as threatened under the ESA; this subsequently took effect on September 30, 2016.

The required habitat of the EMR is commonly referred to as "early-successional" and includes a variety of herbaceous-dominated habitats: prairies, grasslands, savannas, and fallow fields. The presence of wetlands within or adjacent to these habitats is required, as the species typically overwinters in burrows (usually those made by crayfish) that allow access to groundwater. These wetlands include wet prairies, sedge meadows, bogs, fens, and swamp forests. Areas with suitable habitat where EMR may still occur must be surveyed prior to approving development projects. However, massasaugas are cryptic and inhabit densely vegetated habitats, leaving a very narrow and weather-dependent window during early spring where visual surveys are effective.

The low detectability of snakes (Steen, 2010; Durso and Seigel, 2015), combined with the somewhat common occurrence of apparently suitable habitat for the EMR, can complicate environmental reviews of projects meant to determine potential impacts to this state endangered and federally threatened species. The currently accepted techniques for determining the presence or absence of the species (discussed below) are effective, but time consuming and costly. This can be especially problematic for transportation corridors, which are often constructed and maintained in non-forested habitats, and particularly for Ohio, which has an extensive road network and roads near all known sites of EMR occurrence.

Development of alternative, less-costly, and less-time consuming means for determining the presence or absence of the EMR would permit for much greater efficiency of projects, if the alternative(s) was shown to be as effective as the current protocol. Therefore, comprehensive, fast, and affordable survey techniques for EMR are of great interest to government and non-government groups. This is particularly evident in Ohio where all known EMR populations are near maintained or expanding road networks. The purpose of this research is to test and refine a new survey technique, the Adapted-Hunt Drift Fence Technique (AHDriFT) and compare it to current EMR survey methods using a cost-benefit analysis. The AHDriFT system uses modified drift fences and camera traps to remotely and continuously survey for small animals, minimizing researcher field effort (Martin et al., 2017). The application of AHDriFT to survey for EMR presents an opportunity to potentially increase survey effectiveness and consistency, while lowering long-term costs.

Research Background

Current methods for EMR surveys

USFWS recommended standard survey protocol for the EMR calls for "visual searches" as the main technique for determining the species' presence (Casper et al., 2001). Searches are recommended to focus on the early-spring to capture emerging snakes and mid-summer to detect gravid females. A minimum accumulation of 40 person-hours distributed over a standard (April–October) field season is recommended, with 10 years of surveying occurring before evaluating the likelihood of population extirpation at a site. This can be supplemented with drift fence sampling; a commonly used method to sample for reptiles that is both time and labor intensive (Enge, 1997; Fitzgerald, 2012; Fitzgerald and Yantis, 2012; Metts and Graeter, 2013; Willson, 2016) and can result in mortality if not diligently monitored (Wilson, 2016)

The use of artificial cover objects has become increasingly common for surveying reptiles (Scheffers et al., 2009; Godley, 2012; Mills et al., 2013; Willson, 2016). The currently accepted survey technique for the EMR in Ohio uses artificial cover objects consisting of corrugated metal barn roofing material placed in transects within the field being surveyed (Lipps and Smeenk, 2017; **Figure 1**). Roofing tin density usually ranges from 5–7 tins/ha (2–3/ac). Tins are generally placed prior to snake emergence (early-April). Snakes take refuge under these objects to remain hidden and take advantage of the warmth that they provide. Surveys (checks of tin) take place during the activity period of the EMR (mid-April through mid-September, depending on latitude and local conditions). Weekly checks (~25 surveys) without detecting EMR has been interpreted as evidence of its absence. Compared to simple visual encounter surveys, employing artificial cover objects can increase snake detections by an order of magnitude (Lipps and Smeenk, unpublished data) while reducing the harm and stress to animals that traps can cause.

Other techniques for snake surveying and detection

Over the last decade, environmental DNA (eDNA) has been utilized to confirm the presence of hard to detect species (Thomsen and Willerslev, 2015). Use of eDNA is most effective when water samples can be collected and filtered (Goldberg et al., 2016), but eDNA can suffer from false positives and negatives (Thomsen and Willerslev, 2015, Wilson et al., 2016). Such errors or inconsistency have the potential to produce biased estimates of occurrence (Lahoz-Monfort et al., 2016). The use of eDNA with EMR would be challenging. Presumably, the most effective means of obtaining samples containing EMR eDNA would be to extract water samples from crayfish burrows. Baker et al. (2018) attempted this technique but were largely unsuccessful, likely due to the many different variables that affect the quantity and quality of eDNA present at a given time and location (Strickler et al., 2015).

Figure 1. EMR observed during a tin survey in Ohio. *Photo credit: Gregory J. Lipps, Jr*

The Adapted-Hunt Drift Fence Technique (AHDriFT)

Martin et al. (2017) combined features of the COAT method (Welbourne, 2013) and the Hunt trap (McCleery et al., 2014) to develop AHDriFT for surveying reptiles and small mammals with camera traps (see the full literature review in Appendix VII for more details on the COAT method and Hunt trap). With AHDriFT, reptiles and small mammals are guided along a drift fence to inverted buckets at the ends of the fence (**Figure 2**), each containing a camera trap with a fixed focal length lens similar to the Hunt trap (**Figure 3**). The thermal profile within the bucket is much more homogenous, and the camera much closer to the target, meaning the passive infrared sensors can be triggered by a variety of ectothermic animals, even large invertebrates. Animals can move freely through the trap, removing the need to check traps frequently.

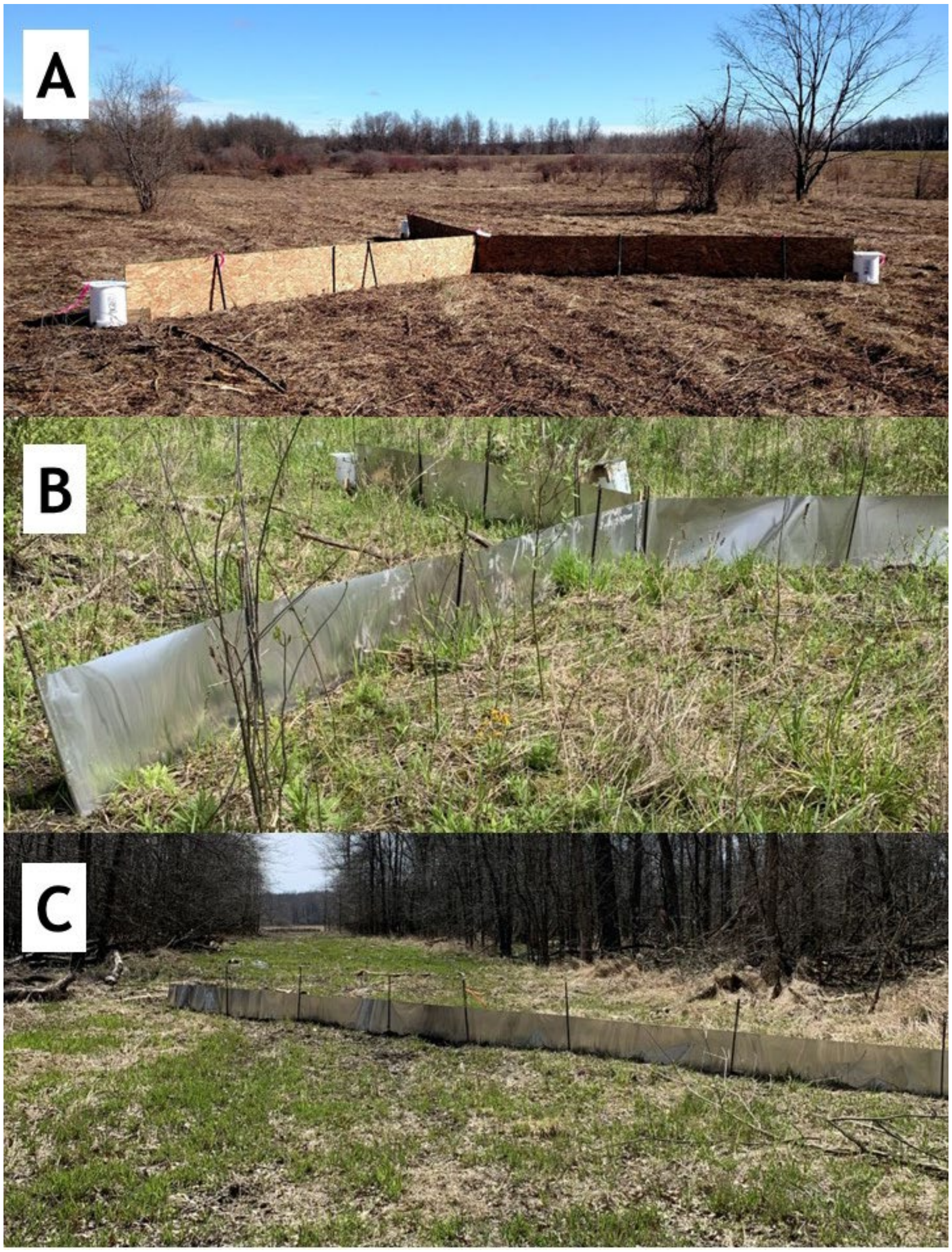

Figure 2. Evolution of AHDriFT array construction from Y-shaped strand board fences (A: 2019) and Y-shaped aluminum flashing fences (B: 2021) to our final linear aluminum flashing fence design (C: 2021–2022). Inverted buckets contain the camera trap units, with wooden guide boards on the inside and outside of the entrances. Buckets have exits to allow free movement of animals without trapping.

Figure 3. Adapted-Hunt Drift Fence Technique (AHDriFT) camera trap housing unit adapted from Martin et al. (2017). A 5-gallon bucket is inverted with the base removed, and entrance and exit openings cut for animals to pass through. A fixed focal-length camera trap is attached to an acrylic plate, aimed downwards towards the lid. Animals move along the drift fence and are coaxed into the trap and directly under the camera IR sensor by wooden guide boards. The small image frame and short distance of the camera to the lid (about 28 cm) allows for high quality images that can be used to identify small mammal, herpetofauna, bird and invertebrate species.

Research objectives

The goal of the proposed project is to test an alternative method for detecting the presence of the EMR in Ohio using AHDriFT. We deployed AHDriFT systems in fields where EMR are known to occur, and capture-mark-recapture surveys have been conducted concurrently (**Figure 4**). Objectives to meet this goal include:

1) Identify any technical or mechanical issues with the use of the AHDriFT system to detect EMR in a variety of habitats across Ohio.
2) Test any modifications to the AHDriFT system implemented to overcome these issues, or that could generally improve survey efficiency and effectiveness.
3) Compare the number and frequency of EMR detections captured with the AHDriFT system to the detection rates of previous surveys and to estimated population size and density where data are available.
4) Determine covariates related to detection of EMR using the AHDriFT system, including date, temperature, humidity, precipitation, length of deployment, and population density. Determine the amount and timing of survey effort required using the AHDriFT system to achieve desired confidence of EMR absence from a site.
5) Determine within-field spatial covariates that might help increase AHDriFT detection rates to optimize fence placement at sites where populations are unknown or poorly studied.
6) Create a comparison matrix summarizing the results of the AHDriFT system and the currently accepted survey protocol, providing details on the effectiveness, limitations, and cost (materials, time, and labor) of each.

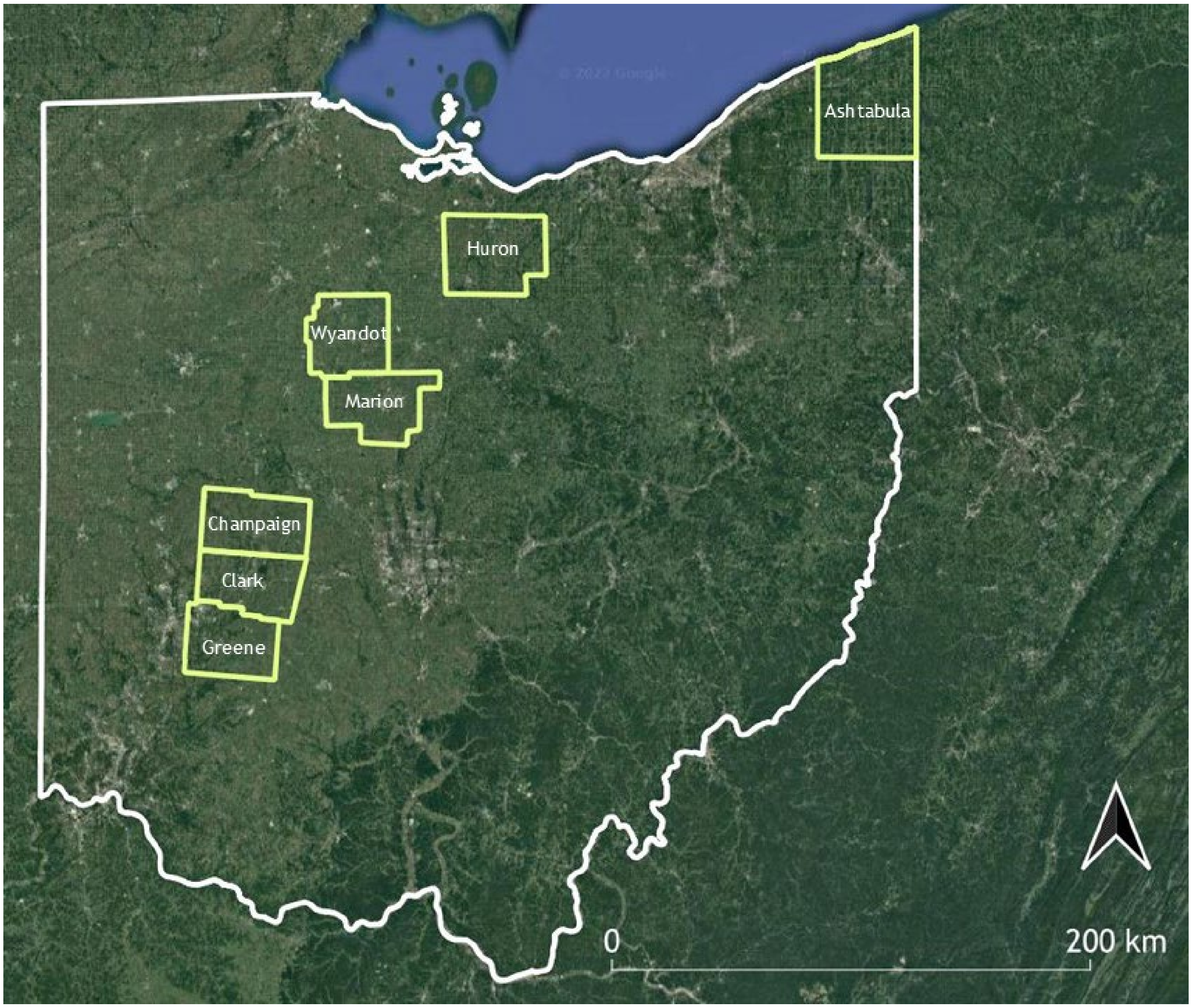

Figure 4. Map highlighting the seven counties where we deployed AHDriFT arrays to survey for EMR from 2019–2022 in Ohio. We surveyed 47 fields in Wyandot and Marion Counties (2019–2022), 12 fields in Ashtabula County (2019), one field in Huron County (2019), two fields in Greene County (2020), two fields in Clark County (2021), and two fields in Champaign County (2021–2022).

Research Approach

Work plan

There were six stages to our research approach:

I. Review relevant literature related to AHDriFT and other EMR survey methods.
II. Test the published AHDriFT design (Martin et al. 2017) at fields with well-studied massasauga populations.
III. Analyze data collected from AHDriFT arrays and adjust materials used, construction protocol, and deployment strategy accordingly.
IV. Compare AHDriFT results to traditional methods and generate comparative cost-benefits matrices.
V. Deploy AHDriFT arrays to fields with small, remnant massasauga populations in relatively degraded habitats to test the approach under adverse conditions.
VI. Develop models to predict massasauga detection probability under varying spatial and temporal conditions and refine deployment protocol.

Stage I – Literature Review

We completed a comprehensive literature review on EMR survey methods, including grey literature across state agencies, with an emphasis on detection probabilities. We submitted this review as an independent document to ODOT on 1 March 2019. The review is included in this report as Appendix VII.

Stage II – Construction and Initial Testing

We built and tested prototype AHDriFT arrays and deployed these arrays in 15 wet prairie fields in northern Ohio across three Counties during 2019. We selected fields that had multiple years of EMR mark-capture-recapture data (Lipps and Smeenk, 2017), as well as two fields where the species has not been detected. We deployed arrays at locations chosen *a priori* by using GIS to determine the geometric centers of each field. We built drift fences using 1/2" oriented strand board. Each arm of the array used two 2' x 8' boards bolted together, and zip-tied to the inverted bucket camera trap housing unit. We placed boards into the ground within a shallow trench and supported them with 3' steel garden fence posts (Lowes #493051). We then backfilled all along the arrays to ensure that there were no gaps for snakes to move through or under. We experienced no camera or technical difficulties during this early deployment phase and checked arrays every one to two weeks to swap SD cards and batteries, and to check for any needed repairs.

We processed camera trap images using the R package 'camtrapR' (Niedballa et al. 2017, version 1.1; R Development Core Team 2019, version 3.6.1) and considered all cameras at an array as one sampling unit. We defined AHDriFT detections as Massasauga images at a single array that were taken at least 60-min apart. The interval reduced the likelihood that Massasauga detections were inflated by one individual moving around an array within a short timeframe (Martin et al. 2017).

Stage III – Design Alterations

We tested potential alterations to the original AHDriFT design throughout the study, including the use of 7-gallon buckets in place of 5-gallon buckets for housing cameras (McCleery et al., 2014, Martin et al., 2017), the use of white acrylic, rather than clear plastic, the use of aluminum flashing instead of strand board for fences, and the use of linear (2-camera) arrays in place of Y-shaped (3-camera) arrays.

We determined that the 5-gallon buckets produced clearer species-level images, triggered more reliably, and were much easier to transport and deploy than the bulkier 7-gallon buckets. We also spray painted the blue lids with matte grey paint for better image contrast. We found that a fixed camera trap focal length from the acrylic to the lid is about 28 cm, and produced clear images. The use of acrylic instead of clear plastic made the system more durable and held together well during the initial AHDriFT deployments despite ice, sleet, rain and snow. Further, while others have attempted to alleviate camera overheating by using shade cloths, the white acrylic appears to have resolved this issue. During this period, we also determined and selected the most effective settings on the cameras (Reconyx HyperFire 2 Professional Covert IR Camera; SKU # HP2XODG). We set custom fixed focal-length cameras at the highest sensitivity, 3-round burst, fast shutter speed and lowest flash settings, and equipped them with 32-GB SD cards and rechargeable NIMH batteries. These materials and settings became the standard for constructing and deploying AHDriFT arrays in our study from 2020 through 2022.

During 2021, we concurrently deployed six two-camera linear arrays and six three-camera Y-shaped arrays in a field with a robust massasauga population to directly compare the two designs. We found that linear arrays yielded similar detection rates to the Y-shaped, arrays at a much lower cost and with easier installment. We therefore switched to exclusively using a linear setup in 2022. Additionally, switching from strand board (2019) and corrugated plastic (2020) to aluminum flashing held up by steel rebar (2021–2022) drastically increased the ease and speed at which fences could be transported and deployed.

Stage IV – Comparison with Tin Surveys

During 2020, we concurrently tested traditional visual and tin surveys alongside AHDriFT arrays in two fields with well-studied massasauga populations separated by approximately 500-m of developed or agricultural land. We deployed 15 Y-shaped AHDriFT arrays from 16 March – 03 October and colleagues deployed 181 corrugated tin sheets (2.4 x 0.6-m) from 17 May – 07 October 2020 (Douglas Wynn, unpubl. data). We established a tin density between 1.5 – 2 tin/ha, in accordance with accepted Ohio protocols for Massasauga (Lipps Jr and Smeenk 2017).

We selected only the first survey of each week to include in method comparison analyses, constraining the comparison analyses to 20 equal weeks of concurrent surveys for both methods from 14 May – 07 October 2020. To compare the two methods, we defined a "survey" as one week of camera trapping or one weekly check of all available tin. Since no single definition of a survey can perfectly compare methods that operate on different time schedules (i.e., camera traps are continuous, while tin surveys are single time points), we recognize that our framework is a subjective attempt to compare these methods using a practical and equitable survey definition. In order to compare costs associated with both methods, we maintained detailed logs of project effort and expenses. We calculated catch-per-unit-effort based on the field time spent checking tin and the field time spent servicing AHDriFT arrays plus the image processing time specifically for Massasaugas.

To equitably compare detection probabilities of tin and arrays, we generated detection accumulation curves for both methods by creating weekly detection/non-detection (1/0) datasets from one to the maximum number of units deployed. For instance, we first assumed that only a single unit of tin was deployed and fit a binomial model for the 20-week detection history for each tin. Next, we assumed two units of tin were used, and looked at all possible combinations of two units of tin. We repeated this process until all units of tin were included. We conducted the same process for AHDriFT arrays. When the number of tin or array combinations exceeded 5,000, we randomly selected 5,000 combinations.

We fit maximum likelihood binomial models to all generated datasets in R and calculated the mean and 95% confidence intervals. We then plotted the AHDriFT accumulation curves along with the mean detection probability estimate per survey for tin densities of 1–2 tin/ha. We determined the number of arrays that produced a survey detection probability equivalent to that of the maximum tin density in each field. We then computed the number of weeks needed for that number of arrays to be deployed to achieve a 95% and 99% confidence of EMR absence.

Stage V - Surveying Low-density Sites

During 2021, we selected three sites in western Ohio with well-studied EMR populations that were known to be in decline or near extirpation and deployed five Y-shaped AHDriFT arrays in open habitats near previous massasauga records at each site. We also conducted weekly checks of established tin lines at all three sites to serve as a methodology comparison for low density sites and to potentially inform us if AHDriFT had failed to detect massasaugas that were present during the survey period. Neither AHDriFT arrays nor tin surveys yielded sufficient EMR detections to allow for a formal analysis of data from these three western Ohio sites.

Given the relatively small, disjunct openings present at these sites, it was difficult to define a “field” and sometimes impossible to deploy multiple arrays into a single, connected area of open habitat as is necessary to achieve a high level of confidence that non-detections are indicative of EMR absence. Therefore, we redeployed three linear arrays at one of these sites following habitat management efforts to open and connect field habitats in 2022. This was the only site where our tin surveys yielded massasauga detections but AHDriFT arrays did not, and we revisited this site specifically to test whether adherence to our deployment recommendations might improve EMR detection.

Stage VI – Assessing Variation in AHDriFT Placement

During 2019, we assessed eight covariates to better understand the influence variation in AHDriFT placement might have on EMR detection at the 15 fields where we first deployed AHDriFT arrays. We quantified vegetation height and density at each array using a Digital Imagery Vegetation Analysis (DIVA; Jorgensen et al. 2013) in mid-July 2019. We extracted elevation, slope, and hydrologic flow rate using U.S. Geological Survey (USGS) 3x3 m digital elevation models (DEM) in ArcMap (version 10.0). Colleagues created a GIS polygon layer of the fields in ArcMap and we input the layer into the R package ‘landscapemetrics’ (Hesselbarth et al. 43 2019, version 1.2.2) to determine field total areas, field edge habitat percentage, and distance of the arrays to forest edges. For each array, we recorded the dominant land cover as either herbaceous or mixed vegetation, the distance to the nearest predator perch tree, and the distance to the nearest known or suspected hibernacula area. We also deployed Hygrochron iButton Temperature/Humidity Loggers (DS1923; **Figure 5**) at each array to measure temperature variation over time and between fields. These data were complemented by precipitation data from nearby NOAA weather stations and vegetation height and density data collected using the Digital Imagery Vegetation Analysis (DIVA) approach (Jorgensen et al., 2013).

Figure 5. Deployment unit for the Hygrochron iButton Temperature/Humidity Loggers.

We fit generalized linear mixed effects models (GLM) to test the effects of spatial and weather covariates on AHDriFT detection rates. We built GLMs using a Bayesian framework to account for small sample sizes using the R package 'brms' (Bürkner 2018, version 2.9). We modelled total Massasauga captures per array using a spatial model, and weekly detection probability per array using a weather model. The spatial model fit landscape covariates and Massasauga total population size estimate (both as a fixed effect term and as a random slope) with geographic region as random intercept using a Poisson GLM. The weather model used a Bernouilli GLM to predict weekly detection probability from averaged weather covariates and the sampling season. We defined the season that each capture occurred in by evenly dividing the 30-week study period into three survey sessions. We considered the first 10-week survey session as spring (10 March–19 May, 2019), the second as summer (20 May–28 July, 2019) and the last as fall (29 July–06 October, 2019). We binned weather covariates and Massasauga captures by week to account for infrequent daily detections. We set a random intercept of field nested within geographic region and a random slope of Massasauga population estimate. We scaled and centered all continuous predictors to have a mean of zero and standard deviation of one. We manually set all models with normally distributed priors with a mean of zero and standard deviation of ten. We visually inspected model chains for mixing and used Gelman-Rubin statistics (Rhat) to confirm convergence (Cowles and Carlin 1996). We then assessed model fit with posterior predictive checks (Bürkner 2018).

We reduced our global models and selected our best-supported final models using the R packages ‘bayestestR’ (Makowski et al. 2019, version 0.3). Model selection analyses included Watanabe-Akaike information criterion (WAIC) and leave-one-out (LOO) model weights, with the largest weight attributed to the most supported model (Vehtari et al. 2017). We also used Bayes Factors to compare the likelihood of a model correctly capturing data variation relative to another (alternative) model. Large values (>100) can be interpreted as extremely strong evidence supporting the tested model over the alternative model (Lee and Wagenmakers 2014). We retained variables whose posterior distributions did not or only marginally included zero. We also checked if the variable had less than 11% of its posterior distribution within the Region of Practical Equivalence (Piironen and Vehtari 2017; ROPE). A small proportion of the distribution within ROPE suggests that the variable likely had a meaningful effect on the response.

During 2022, we deployed single, linear AHDriFT arrays in 43 randomly selected fields in a contiguous landscape managed partly for EMR in northern Ohio, and randomly positioned each array within their respective fields for a broader assessment of how spatial variation might impact EMR detection rates. We used aerial imagery to measure the distance from each AHDriFT array midpoint to the nearest road, tree, forest edge, permanent body of water, and agricultural field. We also used aerial imagery to delineate patches of vegetation dominated by two invasive species: reed canary grass (*Phalaris arundinacea*; RCG) and cut-leaf teasel (*Dipsacus laciniatus*; CLT), and habitat patches dominated by woody vegetation (shrubs and trees). We then calculated the distance from each fence midpoint to each of these three habitat types, and calculated the coverage of each habitat type within 20m of each fence midpoint.

We then fit a GLM with a binomial distribution to test the effects of spatial covariates on the number of weeks in which EMR were detected. We built GLMs using a Bayesian framework using the R package ‘brms’ (Bürkner 2018, version 2.9). We scaled and centered all continuous predictors to have a mean of zero and standard deviation of one and manually set all models with normally distributed priors with a mean of zero and standard deviation of ten.

Research Findings and Conclusions

Optimal Fence Construction and Maintenance

Initial testing showed that the 5-gallon buckets took clearer images than the 7-gallon buckets and were faster and easier to construct and deploy. However, the image frame is slightly smaller, so greater care needed to be taken to ensure that the camera lenses were directly over the internal wooden guide boards and that the IR sensor was right above the trap entrance. Cameras worked well on medium high sensitivity, lowest flash, fastest shutter speed and three-image burst.

We took 52 person-hours to construct our initial 45 AHDriFT camera trap housing units, although modifications to this original design reduced camera trap construction time to under 1 person hour per trap. Initial Y-shaped array deployment, not including transporting materials, averaged about one hour per array for two people, 45 minutes for three people and 35 minutes for four. However, switching to aluminum flashing for fence material and to a linear array setup reduced this construction time to 35-40 minutes for two people and 25-30 minutes for more than two people. We found that four or more people did not further decrease construction time.

We visited arrays every one to two weeks initially to swap SD cards and batteries. In this time, cameras typically took between 120-750 images. The batteries almost always read full, and the SD cards typically were 0-1% full. On rare occasion, false triggering led to upwards of 28,000 images. The batteries never dropped below 3/4 full, and the SD cards were never above 16% full. Lowering the sensitivity of cameras prone to false trigger from “very high” to “high” or “med-high” greatly reduced this issue. During 2021 and 2022, we reduced check frequency to once every four to five weeks and only experienced camera failure when cameras malfunctioned and took images almost constantly for no discernible reason, rapidly depleting the batteries.

Time spent to swap SD cards and batteries at an array by a single researcher averaged 13 minutes. Processing time for all 45 SD cards with images collected over a one to two-week period took about 10-15 hours. During the first few weeks of deployment, while sites still experienced snow, ice, hard winds and frequent water-level changes, some light repairs were needed. These repairs were only back-filling small gaps under the fences with mud and took a few minutes. Once the ground became more set, repairs were rarely needed. The strand board fences (2019) were sturdy and were never damaged or blown over, but the corrugated plastic fences (2020) were flimsy and required many posts for support. A small number of the metal flashing fences (2021, 2022) experienced damage from deer and heavy machinery. No fences were damaged by wind, water, or general weathering. We did not have any technical difficulties with the cameras deployed during 2019, 2020, or 2021 aside from some cameras triggering frequently, an issue which was usually remedied by turning down the sensitivity. In 2022, we had a single camera (out of 92) malfunction and stop working for unknown reasons. During this field season, we also had three cameras prone to frequent triggering that was not remedied by lowering the sensitivity setting. 2022 was also the only year we experienced camera theft (one instance) and equipment damage due to heavy machinery (one instance) and deer during rut (two instances).

Image Processing

We analyzed our data using the same 'camtrapR' settings used by Martin et al. (2017). Species images only count as a "unique-capture" if images of the same species at an array are at least one hour apart, with all cameras from an array counting as one. Many small mammal species and Gartersnakes frequently move between buckets, and this method prevents one individual from oversaturating the data set. Manual inspection of EMR images during our first year of operation showed that this method only removed one set of EMR images from the unique-capture data set, where one individual (clearly discerned by its dorsal patterning) moved between two cameras within 20 minutes. Processing two weeks' worth of images of 45 cameras (15 Y-shaped arrays) required 6–19 person-hours ($\bar{x}$ = 13.08 ± 3.84) for all species but could be done in two to four hours when only identifying Massasaugas (**Table 1**). The shortest processing times were in the spring (March – May) when animals were less active. The longest processing times resulted from when there were large amounts of false triggers.

Table 1. Typical effort breakdown for constructing and servicing arrays, and the image processing time for all three cameras used in an array for all species. Time range (minutes) minimums and maximums are approximated for array construction and for the final record table. Time range (minutes) minimums and maximums are exact for data acquisition and processing effort (mean ± standard deviation).

Task per array	Time (mins)
Array construction	
Build two camera trap housing units	60 – 90
Deploy one array (one person)	45 – 90
Deploy one array (two people)	30 – 50
Deploy one array (three people)	25 – 40
Data acquisition and processing	
Change batteries and SD cards	9 – 25 (*13.96 ± 3.21*)
Process two-weeks of images for all species	24 – 76 (*52.32 ± 12.84*)
Final record table ('camtrapR')	5 – 10

EMR Detections and Species Diversity

We obtained 351 EMR detections across 57 of the 66 fields we sampled. These observations spanned six of the seven counties sampled and included 17 of the 24 fields with previously well-documented EMR populations. We detected both adults and juveniles with observations occurring between 9am and 7pm, with a peak between 3-4pm. The first EMR images captured by the AHDriFT system were obtained on April 18, 2019, about one month after deployment. Most fields with EMR images detected one or two individuals, but three fences deployed during 2020 in fields with large EMR populations yielded 22 and 23 detections.

While the EMR is the objective of this study, the AHDriFT arrays documented a total of 51 vertebrate species. These included 15 reptile (12 snake) species, five amphibian species, 19 mammal species, 11 bird species, and a single fish species in a flooded bucket. Some of these are listed as Ohio Species of Concern, such as the Woodland Jumping Mouse (*Napaeozapus insignis*). The most common unique-captures were of Eastern Gartersnakes (*Thamnophis sirtalis*), followed by invertebrates, White-footed Deermice (*Peromyscus leucopus*), Masked Shrews (*Sorex cinereus*), Meadow Voles (*Microtus pennsylvanicus*) and Song Sparrows (*Melospiza melodia*). See Appendix III for detailed lists of species, unique-captures and diversity imaged by array.

Comparisons to Tin Surveys

In 2020, AHDriFT arrays outperformed concurrent tin surveys for Massasauga in terms of total captures (**Table 2**), detection rates (**Table 3** and **4**), and cost-efficiency after initial equipment investment (**Table 5**). Over 20 weeks of concurrent tin and AHDriFT surveys, 14 arrays (93%) cumulatively obtained 123 Massasauga detections, with a mean of nine per array and tin surveys obtained 34 detections from 33 units of tin (18%), with a mean of 0.2 detections per unit of tin. Array detection rate averaged 5.6 snakes per hour of effort, while tin averaged 1.2 snakes per hour of effort. AHDriFT arrays obtained 2-4 times more detections and 2-6 times greater catch per unit effort than tin and also recorded greater captures of other species that are traditionally difficult to observe or are species of interest in Ohio. We conducted double or nearly double array field visits than is strictly necessary for the method (Amber et al. 2020), so the array catch-per person-hour could have been even higher.

Table 2. Comparison of EMR captures and detection rates of concurrent AHDriFT and tin surveys. The field identifier is provided after the method type (in parentheses). Field A has a very high EMR density relative to most sites in Ohio while Field B has a moderately high EMR density relative to most sites in Ohio. We conducted double or nearly double array field visits than is strictly necessary for the method, denoted by an asterisk (*), which lowered our potential catch per-unit-effort (CPUE; captures/person-hours). I defined effort as the time spent in the field checking tin, and the time spent in the field servicing arrays plus image processing time specifically for EMR.

Metric	Tin (A)	Arrays (A)	Tin (B)	Arrays (B)
Total captures	19	91	15	32
Density (units/ha)	1.50	0.22	2.00	0.13
Effort hours (field visits)	13 (20)	10 (6*)	20 (20)	15 (6*)
Estimated CPUE	1.5	9.1	0.8	2.1

Table 3. Estimated error rate in failing to detect an EMR when present, independent of field size or population density, that a given number of tins will have when the desired confidence of absence threshold (90%, 95%, or 99%) is used and when tins are visited 10, 15, or 20 times. For instance, if 95% confidence in absence is desired and 20 tins are checked 10 times, there will be an error rate 0.43. This error rate is cut to 0.05 when the 20 tins are visited 20 times. Green highlighted values in the table indicate when the error rate is ≤0.10. Values are generated from analysis of tin data collected 2015–2017 at 15 NE Ohio fields where an EMR was detected at least once.

	10 site visits			15 site visits			20 site visits		
# Tins	90%	95%	99%	90%	95%	99%	90%	95%	99%
10	0.53	0.80	0.93	0.57	0.57	0.79	0.38	0.38	0.83
15	0.31	0.60	0.82	0.32	0.32	0.56	0.13	0.13	0.51
20	0.20	0.43	0.71	0.16	0.16	0.35	0.05	0.05	0.26
25	0.09	0.29	0.56	0.06	0.06	0.18	0.01	0.01	0.14
50	0.00	0.02	0.08	0.00	0.00	0.00	0.00	0.00	0.00

Table 4. Estimated error rate in failing to detect an EMR when present, independent of field size or population density, that a given number of arrays will have when the desired confidence of absence threshold (90%, 95%, or 99%) is used and when arrays are deployed for 12, 16, or 20 weeks. For instance, if 95% confidence in absence is desired and 3 arrays are deployed for 12 weeks, there will be an error rate 0.18. This error rate is cut to 0.08 when the 3 arrays are deployed for 20 weeks. Green highlighted values in the table indicate when the error rate is ≤0.10. Values are generated from analysis of 2019 AHDriFT data collected at 12 fields where an EMR was detected at least once. To reach an error rate of <10%, surveyors must deploy four AHDriFT arrays for 12 weeks or three AHDriFT arrays for at least 16 weeks.

	12 weeks			16 weeks			20 weeks		
# Arrays	90%	95%	99%	90%	95%	99%	90%	95%	99%
1	0.51	0.61	0.79	0.38	0.51	0.71	0.38	0.38	0.61
2	0.23	0.31	0.48	0.16	0.23	0.39	0.16	0.16	0.31
3	0.13	0.18	0.31	0.08	0.13	0.24	0.08	0.08	0.18
4	0.08	0.12	0.20	0.04	0.08	0.16	0.04	0.04	0.12
5	0.05	0.07	0.14	0.02	0.05	0.11	0.02	0.02	0.07
6	0.02	0.05	0.10	0.01	0.02	0.07	0.01	0.01	0.05

Table 5. Approximate dollar cost (USD) expense comparison of tin and AHDriFT surveys for EMR. Twenty field visits for tin surveys of 181 tin units resulted in 34 EMR captures. Only about five field visits of five arrays (three cameras per array) were needed to equate to the tin maximum detection probability, with a mean of nine EMR captures per array (five arrays would achieve an estimated 45 captures). I note that Ohio protocol typically calls for ~25 tin surveys, which would increase the travel and field expenses presented here. Estimates assume that each method employs a single researcher paid $15 (USD) per hour, who is based out of the Columbus, Ohio area (~120-mile roundtrip per field visit). Tin survey costs do not include miscellaneous equipment (i.e., snake tongs, bags) or time to process captured snakes.

Expense	**Estimated Cost (USD) - Tin**	**Estimated Cost (USD) - AHDriFT**
Equipment	$3,000 (181 tin sheets)	$8,750 (five arrays)
Mileage ($0.56/mile)	$1,344	$336
Travel time pay	$750	$165
Field time pay	$375	$95
Image sorting pay	NA	$150
Totals with equipment purchase		
Sum	$5,469	$9,496
Cost per snake	$160.85	$211.02
Totals without equipment purchase		
Sum	$2,469	$746
Cost per snake	$72.62	$16.58

We conducted this comparison at sites with EMR populations that are atypically high for Ohio. EMR population size positively correlates with captures using AHDriFT (Amber et al. 2021). During 2021, we detected only a single EMR at one of three low-density EMR sites in western Ohio. Although concurrent tin surveys failed to detect EMR at this site, tin surveys did detect multiple EMR at one of the other three low-density sites where concurrent AHDriFT surveys yielded no detections. However, the time and effort spent deploying five AHDriFT arrays to each of these sites was considerably lower than the effort that went into weekly tin surveys and most of the EMR detected during tin surveys were not in habitat sampled by our AHDriFT arrays. Further, we revisited this site in 2022 with more targeted deployment of three linear AHDriFT arrays and detected two EMR.

Overall, arrays matched the detection probability of all deployed tin using only about 1 array/15-ha and were the more cost-effective option. The dollar cost (USD) of 20 tin surveys was $5,469, equating to $160.85 per snake detection. Deploying 15 arrays for 20 weeks of surveys cost $9,496, equating to $211.02 per snake detection. However, 92% of the AHDriFT costs were the initial equipment purchases, particularly the camera materials (86% of total cost), which can be used for multiple seasons (detailed equipment cost breakdown provided in Amber et al. 2020; refer to Chapter 1). After removing equipment purchases, the tin surveys cost $72.62 per snake detection while arrays cost $16.58 per snake detection. These figures assume a consultant rate of only $15/hour for the purposes of comparing the methods equably, and likely do not reflect the true costs of a contracted survey or differences in specialist and generalist biologist consultant rates.

Optimizing Placement of AHDriFT arrays

Our 2020 analysis indicated that AHDriFT detection rates for Massasauga were greatest when arrays were set in dense, herbaceous vegetation, away from field edges and predator perch trees (**Figure 6**). Vegetation density (0.51, CI = - 0.04 – 1.12) and distance to a predator perch tree (0.51, CI = -0.12 – 1.11) were the best-supported spatial covariates for predicting EMR detections in 2020. Additionally, late summer and fall were the most productive seasons to detect EMR using AHDriFT arrays with 92% of total captures occurring from May–October and captures after 15 July accounting for >70% of all captures (**Figure 7**).

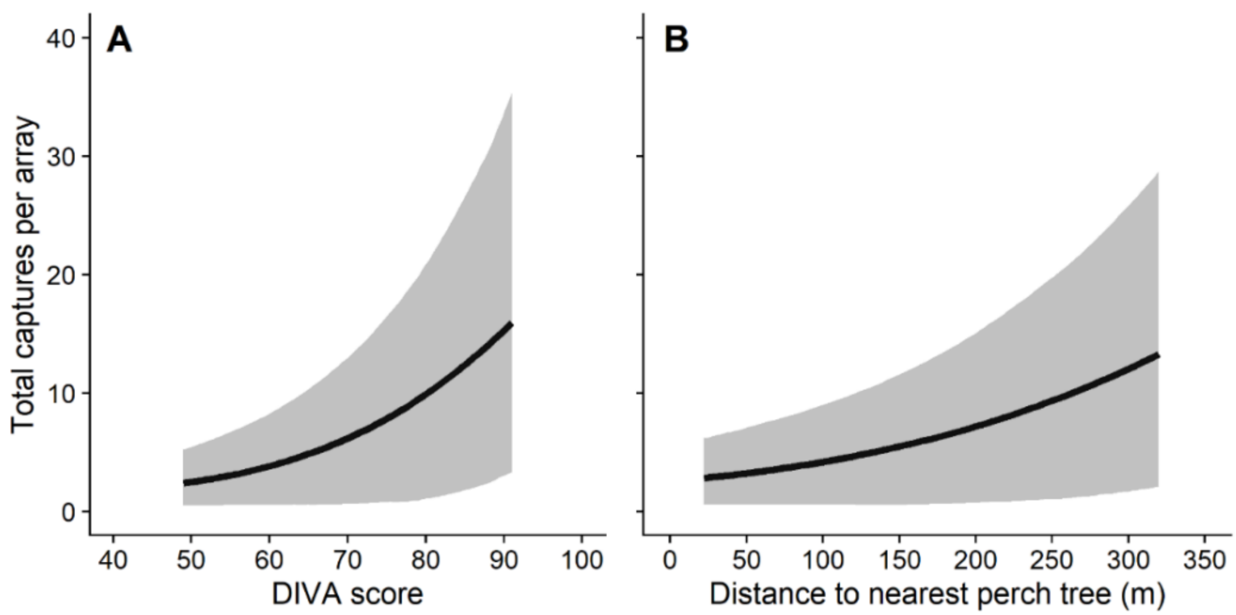

Figure 6. Influence on Massasauga weekly total captures per AHDriFT array of (A) vegetation density, with higher Digital Imagery Vegetation Analysis (DIVA) scores corresponding to denser wet meadow vegetation; and (B) the distance of the array from the nearest tree suitable for avian predators to perch on.

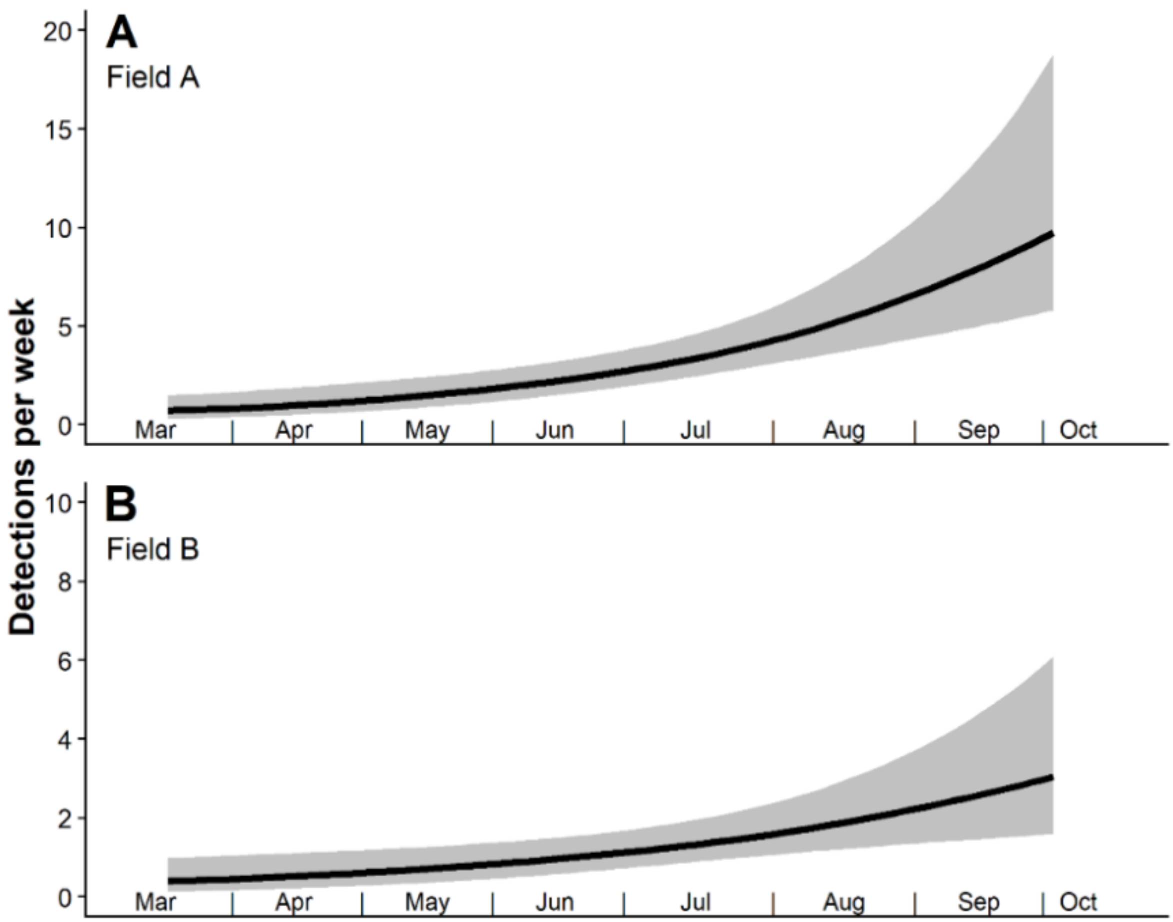

Figure 7. Influence of week of year on Massasauga weekly total captures per AHDriFT array. Field A has a higher Massasauga density relative to mean estimates for most sites in northern Ohio while Field B has a moderately high Massasauga density relative to most sites in northern Ohio.

Our 2022 analysis of spatial covariates influence on EMR detection rates across 43 fields yielded similar results with distance from tree being the single greatest predictor of weekly EMR detections for a given site (**Table 6**). Weekly EMR detections increased with increasing distance from the nearest tree but also increased with increasing woody vegetation cover within 20 m of the array (**Figure 8**). Distance to nearest road or agricultural field and cover of reed canary grass or cut-leaved teasel did not consistently affect EMR detections in our model.

Table 6. Estimated effects of environmental covariates on the probability of weekly EMR detection by AHDriFT arrays in northern Ohio during 2022. The lower and upper 95% highest density intervals (HDI low and HDI high, respectively) are presented alongside the probability of direction (PD), and percent of the posterior distribution inside of the region of practical equivalence (ROPE) using 89% of the posterior distribution. Parameters in boldface represent covariates with > 90% probability of direction.

Parameter	Estimate	HDI low	HDI high	PD	ROPE
Intercept	-1.47	-1.78	-1.17	1.00	0.00
Distance to nearest tree	**0.51**	**0.19**	**0.83**	**1.00**	**0.00**
Distance to agriculture	-0.16	-0.53	0.21	0.80	0.31
Distance to road	-0.22	-0.59	0.13	0.89	0.24
Woody cover	**0.26**	**-0.10**	**0.62**	**0.92**	**0.17**
Reed canary cover	0.08	-0.29	0.45	0.67	0.39
Teasel cover	-0.12	-0.59	0.25	0.72	0.35
TWI	0.16	-0.30	0.63	0.75	0.28

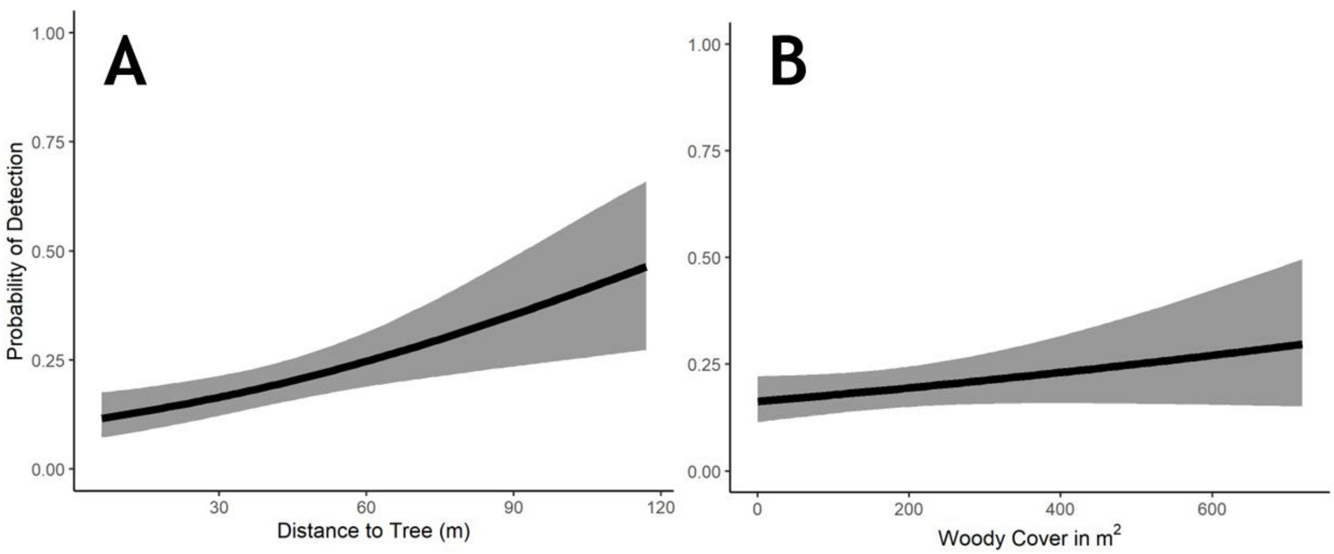

Figure 8. Influence on weekly EMR detections per AHDriFT array of (A) distance to nearest tree, and (B) woody vegetation cover within 20 m of the array at AHDriFT arrays in northern Ohio during 2022.

Recommendations for Implementation

Array construction and deployment

Much of the effort and costs needed for the AHDriFT method are upfront (**Table 7**), in the construction and deployment phases. Researchers seeking to implement AHDriFT should plan accordingly and ensure that they have enough people to help transport materials. Instructions on camera trap housing unit and array construction are in appendix VI. For EMR, we recommend using 5-gallon buckets rather than 7-gallon. If your species of interest is larger, a 7-gallon bucket may be necessary. Using semi-transparent acrylic plates instead of clear plastic is much more durable for holding the cameras. Further, we did not have any issues with the cameras overheating, as others found when using clear plastic. We also recommend investing in quality camera traps. Our cameras cost $400 each (with custom focal-lengths, after bulk-order discount). While this was more expensive than cameras tested in Martin et al. (2017), we had excellent image quality and few technical issues. Harsh spring environmental conditions in northern Ohio wetlands required the fences to be made of durable materials. Our use of 1/2" oriented strand board, held together with metal screws and upright by metal fence posts, allowed our AHDriFT arrays to withstand wind, ice, snow, rain and water-level changes without damage or falling over, though minor back-filling repairs were needed in the first few weeks.

However, these boards did not hold up well to repeated use, were difficult to sterilize (for limiting disease and seed transmission) and were labor intensive to transport and install. Aluminum flashing fences also proved durable, with few instances of damage and most fences being reusable over at least two seasons. High winds buffeting these aluminum fences did cause some trenching along the fence, but this usually did not compromise the fence integrity as a barrier. We recommend using aluminum flashing for fence construction for its ease of transport and installation, but caution against installing these fences in standing water or waterlogged soils, as they are not heavy enough to sink in firmly and nearly always required additional backfilling or re-burying once the water receded. This was not generally a problem when fences were installed in dry ground that later flooded.

For the most efficient array construction in the field, we recommend three people, equipped with two mattocks for digging trenches for the fence to sit in. We found installation to be the easiest when first digging the trench and laying out pairs of steel rebar 2–3 m apart. Then, erect rebar in place loosely and have one person hold the aluminum roll around one end post while another person unfurls the aluminum between the rebar all the way to the far end post. Both ends of the aluminum can then be tied to the end posts while the rebar posts are hammered in, and the trench is backfilled. Bring a cordless power drill to create holes for tying the fence to each end post, and use a metal mallet to drive rebar in. Use UV-resistant zip-ties to hold the bucket to the array, for tying the fence to the end posts, and to tie the other rebar posts together just above the array. Three people can construct and back-fill an array in about 30 minutes.

Array deployment schedule and density

We recommend 16 weeks of camera trapping, with a minimum of 12 weeks and up to 20 weeks if budget and time constraints allow (use **Table 4** to determine the number of arrays and weeks of deployment needed to obtain a desired confidence of species absence). Construct drift fences in late March, prior to Massasauga emergence and after the topsoil has begun to thaw. The majority of Massasauga captures with AHDriFT occur in summer and fall so activating cameras in early June would save field visits and image processing time while optimizing deployment during peak detection season. During the field visit to activate cameras, check the drift fences for any needed repairs or backfilling, and use hand shears to cut back any vegetation growing into the buckets. Service the arrays (change batteries and SD cards) every 4–8 weeks and deconstruct arrays in early October. Following this general schedule, an AHDriFT survey can require only five field visits, as opposed to the ~25 visits required for tin surveys in Ohio.

In northern Ohio, we found that arrays matched the detection probability of 2 tin/ha (typical upper end density of the current Ohio protocol) using about 1 array/15 ha. However, the site we surveyed had higher EMR population estimates than most other known sites in Ohio, and this framework may not be generalizable throughout the state. Overall, we recommend deploying a minimum of two arrays in any field. Thereafter, add one array per 10 ha (e.g., a 1 ha field will have two arrays, and a 10-ha field will have three arrays).

Table 7. Approximate equipment costs per linear (2-camera) array. Camera traps were customized by the manufacturer to a focal length of 28 cm. We include double the SD cards and batteries needed to set the cameras so that they can be swapped and allow for continuous camera operation. The total number of units needed of each piece of equipment is provided (in parentheses). Estimated costs represent the approximate total sum needed to purchase all the units needed of each piece of equipment.

Equipment	Estimated cost (USD)
Camera trap supplies	
Reconyx PIR custom cameras (2)	$920
Rechargeable AA batteries (48)	$60
SD cards (4)	$40
TOTAL	**$1,020**
Camera trap housing unit supplies	
5-gallon buckets and lids (2)	$14
Acrylic sheets (2)	$27
L-brackets (6)	$3
Machine screws / hex nuts (26)	$7
Washers (8)	$2
Wing nuts (6)	$2
Wood studs (2)	$5
TOTAL	**$60**
Drift fence supplies	
Aluminum flashing roll (1)	$70
Metal rebar (16)	$26
Zip-ties	$6
TOTAL	**$102**
TOTAL	**$1,182**

Image collection and processing

We visited our arrays every one to two weeks during 2019 but gradually switched to monthly visits. For the first few weeks of deployment, we recommend frequent visits in case any light repairs are needed, and to ensure that the arrays and cameras are functioning properly. However, the 32-GB SD cards and rechargeable NiMH batteries can last much longer than a couple of weeks. Martin et al. (2017) visited their arrays every 4-8 weeks. However, their arrays were built in Florida sand dunes. In Ohio wetlands, vegetation grows quickly, and soil is frequently washed away. We recommend visiting arrays once per month once the researcher is confident that arrays are functioning correctly. Visiting arrays at this frequency will allow the researcher to limit false triggering by cutting back vegetation near the bucket openings, removing paper wasp nests from the camera casings, and address any issues before too much data is lost. Image processing will also be less overwhelming than waiting to collect two months of images. Since average time spent at an array by one researcher is only 13 minutes, visiting once per month still significantly reduces travel and field time compared to traditional EMR survey methods.

We found the R package 'camtrapR' (Niedballa et al. 2017) to be simple and effective for processing AHDriFT image metadata. Prior to processing images, ensure that image folders are correctly organized and labelled for the 'camtrapR' workflow. We also recommend testing R code prior to collecting images. Completing these steps ahead of time will prevent spending a significant amount of time later to reorganize sorted images.

When viewing an SD card containing images, first change the SD card folder's name to match the unique camera name (e.g., "cam6A"). Use 'camtrapR' to rename the raw images in the folder, which will apply this camera name tag and the image metadata. Images will then all have unique file names pertaining to their specific camera and date and time of capture, allowing for easier downstream processing.

To process images, we recommend using a computer with large, dual screen monitors. View the raw images using the largest icon size on one screen with the screen set to maximum brightness. Have the specific camera folder that images will be sorted into available on the other screen. If only interested in Massasauga, simply scroll through the images and drag and drop species images into the appropriate folder. EMR are easy to see in the large icons of the images, so scrolling can be done fairly quickly. If interested in all species and data, we recommend to first sort out the false triggered images from images containing species, then returning and sorting species into their appropriate folders.

Obstacles

Underestimating time and effort of construction and deployment of AHDriFT arrays can be the biggest obstacle. When using aluminum flashing for fence material, two people can comfortably carry all needed materials to install a single fence, even over distances exceeding a kilometer. If needed, aluminum fence rolls can be held inside a large backpack. Digging the trenches in the field can be difficult if you run into root systems of woody vegetation, ant mounds or flooded areas, and can be a very physical task to dig through frozen topsoil. A mattock is much more efficient than a shovel. Ensure that all personnel are physically able for the task. While having more than 3–4 people install a fence does not noticeably increase the speed at which the fence is constructed, it can help prevent exhaustion when constructing multiple fences in a day. We recommend alternating the task of trench digging whenever possible in these circumstances. A small camp shovel can be used in the first couple of weeks of deployment to fill in any gaps that appear along the arrays.

Once deployed, we have experienced very few obstacles with the AHDriFT method. The biggest is false triggering caused by sunlight, flooding, vegetation, and paper wasps that build nests on the camera IR sensors and lenses. For buckets oriented in such a way that sunlight and flooding are issues, lowering the camera sensitivity can solve or greatly reduce the issue. Whenever possible, try to avoid fence orientations that expose bucket openings to sunrise/sunset. For vegetation, during visits quickly pull any vegetation near the bucket openings, taking care of hands of feet if in fields that may contain EMR. The buckets themselves prevent vegetation from growing under them. For the paper wasps, when visiting the traps, lift the acrylic slowly. If a wasp is there, let it fly out and brush the nest off of the camera. If you have a very inflexible schedule, this can also be an obstacle, since the cameras should not be opened to change SD cards and batteries in the rain.

One final obstacle may be storage space for images. Over the course of a single field season, we quickly accumulate tens of thousands of images containing species, and hundreds of thousands of images that false triggered. While an inexpensive external hard drive can easily hold the species images for use in ‘camtrapR’, also keeping all of the false trigger images may pose an issue for researchers with very limited storage space.

Costs

Excluding salaries, wages, travel and University administrative costs, our proposal budgeted $28,515 for supplies for the original 15 AHDriFT arrays (45 cameras). This equated to about $1,900 per array. Switching to linear AHDriFT arrays, we were able to reduce our cost per array to around $1,200 (see **Table 7**). The vast majority of the costs were the cameras, SD cards and batteries. However, we constructed our arrays under budget. While this upfront cost is more expensive than purchasing artificial cover objects, the cameras, SD cards, batteries and most materials can be used for multiple field seasons and visiting AHDriFT arrays requires significantly less person-hours than traditional survey methods.

Benefits

The AHDriFT system is capable of capturing EMR images and outperforms other methods for detecting EMR. While we did not detect EMR in every field we sampled, we know of only a single field with one EMR observation between 2019-2022 where our AHDriFT arrays failed to detect the snakes. AHDriFT vastly reduces field effort compared to traditional surveying and captures a surprising diversity of other small animal species in the process, including Species of Concern in Ohio. This appears to be particularly true for snake species, where many AHDriFT arrays imaged all snake species known to occur at a given site in one season of sampling. Additionally, some snake species have been imaged that are not easily found by visual surveys, such as Smooth Greensnakes (*Opheodrys vernalis*). This method also has a very low environmental impact as it does not require capturing or restraining the animals and minimizes the time surveys spend walking through the area. Although the initial construction of an AHDriFT array disturbs the vegetation and soil locally, once the fence is constructed it requires infrequent visits meaning surveyors create less paths and trample less vegetation than with other methods.

The AHDriFT method appears to be very effective at imaging small mammals such as mice, shrews, voles and moles. Larger mammal species have also been captured, such as weasels and minks. See Appendices II and III for detailed species information. AHDriFT may very well be the most efficient and effective method to survey for highly mobile, but cryptic small animals (including EMR). Although there is a relatively high upfront cost, the long-term cost efficiency is better than other survey methods, it is less invasive than most other methods, and is easy to deploy and maintain with little or no expertise or special training.

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Appendix

Appendix I *– Sample of EMR image captures using AHDriFT.*

Appendix II *– Species diversity captured by AHDriFT arrays during 2019. Invertebrates are a single lumped group and count as one 'species'. Most arrays are in Ashtabula County.*

**Wyandot County; **Huron County; ***No known EMR*

Station	n_species
FCM***	11
FCP	14
FCS	12
GRN	13
KPA*	14
KPC*	13
MLN	11
NOR***	10
RMM	17
RMN	14
RMW	17
STR	11
WIL**	16
YWN	16
YWS	18

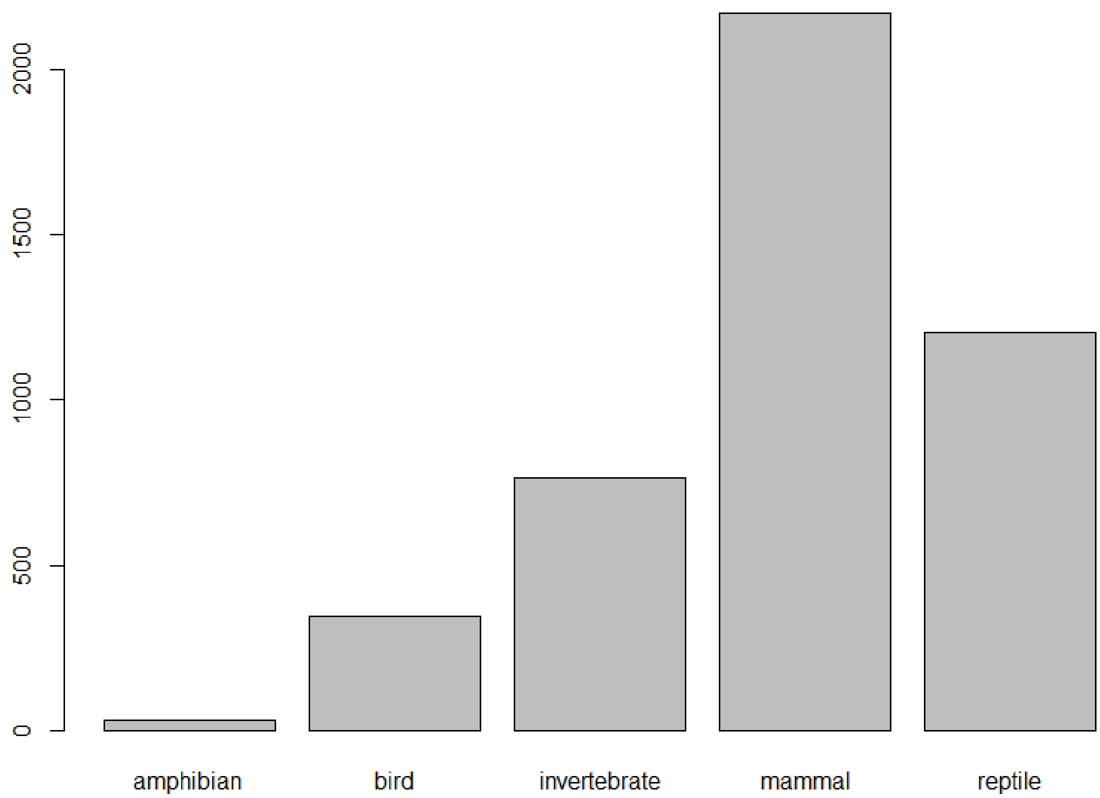
Appendix III – All species captures across 15 wet meadows in northern and northeastern Ohio in 2019 using AHDriFT. The number of fields that a species was imaged in (Fields) is followed by the total number of possible fields in which the species is known or expected to occur (in parentheses). For amphibians, lizards and mammals, total possible fields are based on prior opportunistic observations or inferred from species ranges and habitat requirements. For snakes, total possible fields are known from three years of prior visual encounter and artificial cover object (tin) surveys. Field values marked with an asterisk (*) indicate imaged species that are not known or expected to be in our fields but have been observed in or could potentially inhabit adjacent areas. Listed species have designations after their common names (E = Ohio Endangered; SC = Ohio Species of Concern; R = Rare in Ohio; LT = Federally Threatened).

Species	Common name / status	Captures	Fields
Amphibians			
Ambystoma texanum	Small-mouthed Salamander	1	1(2)
Anaxyrus americanus	American Toad	11	6(15)
Lithobates catesbeianus	American Bullfrog	1	1*
Lithobates clamitans	Green Frog	15	9(15)
Lithobates pipiens	Northern Leopard Frog	24	9(15)
Reptiles			
Chrysemys p. marginata	Midland Painted Turtle	3	3*
Clonophis kirtlandii	Kirtland's Snake	0	0(2)
Lampropeltis triangulum	Eastern Milksnake	10	5(8)
Nerodia s. sipedon	Northern Watersnake	9	8(8)
Opheodrys vernalis	Smooth Greensnake[E]	15	2(2)
Pantherophis spiloides	Gray [Black] Ratsnake	8	5(1)
Plestiodon fasciatus	Common Five-lined Skink	490	10(12)

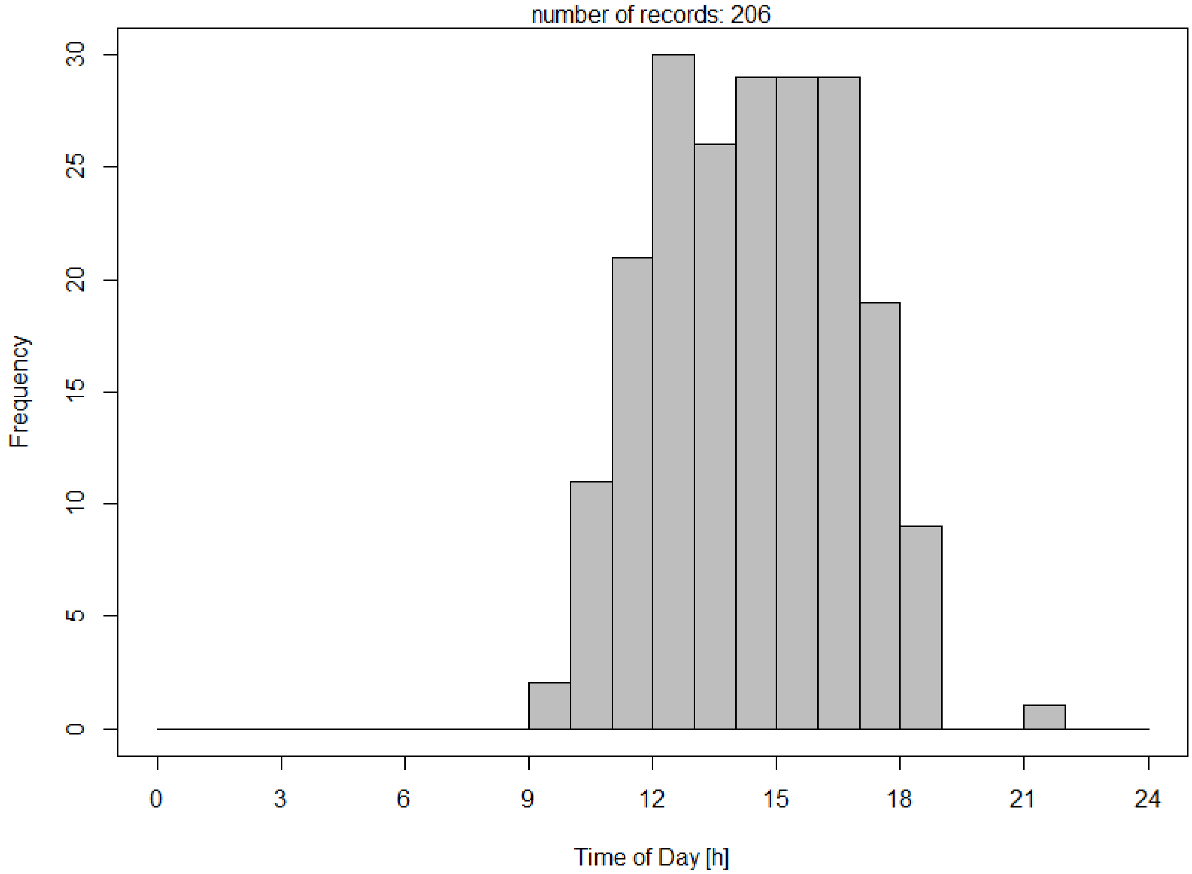
Species	Common name / status	Captures	Fields
Sistrurus catenatus	Eastern Massasauga Rattlesnake[E, LT]	72	12(13)
Storeia dekayi	Dekay's Brownsnake	69	12(15)
Storeia occipitomaculata	Northern Red-bellied Snake	3	2(4)
Thamnophis butleri	Butler's Gartersnake	24	1(1)
Thamnophis radix	Plains Gartersnake[E]	26	2(2)
Thamnophis sauritus	Eastern Ribbonsnake	21	6(1)
Thamnophis sirtalis	Eastern Gartersnake	1,745	15(15)
Mammals			
Blarina brevicauda	Northern Short-tailed Shrew	152	15(15)
Condylura cristata	Star-nosed Mole[SC]	16	9(12)
Cryptotis parva	Least Shrew[R]	0	0(15)
Didelphis virginiana	Virginia Opossum	58	13(15)
Marmota monax	Groundhog	7	5*
Mephitis mephitis	Striped Skunk	7	4*
Microtus spp.	Voles	1,390	15(15)
Mustela frenata	Long-tailed Iasel	97	12(15)
Napaeozapus insignis	Woodland Jumping Mouse[SC]	396	13(13)
Neovision vision	American Mink	11	4(12)
Peromyscus spp.	Deer Mice	1,031	15(15)
Procyon lotor	Raccoon	8	5*

Species	Common name / status	Captures	Fields
Rattus norvegicus	Brown Rat	1	1*
Sorex cinereus	Masked Shrew	1,135	14(15)
Sylvilagus floridanus	Eastern Cottontail	212	11(15)
Tamias striatus	Eastern Chipmunk	32	7(10)
Zapus hudsonius	Meadow Jumping Mouse[R]	41	3(3)
Birds and invertebrates			
Dumetella carolinensis	Gray Catbird	5	2
Geothlypis trichas	Common Yellowthroat	58	12
Melospiza melodia	Song Sparrow	633	14
Passerina cyanea	Indigo Bunting	5	1
Porzana carolina	Sora[SC]	1	1
Siala sialis	Eastern Bluebird	1	1
Troglodytes aedon	Northern House Wren	186	12
Invertebrate spp.	Invertebrates	987	15

Appendix IV – All species captures by class across 15 wet meadows in northern and northeastern Ohio in 2019 using AHDriFT.



***Appendix V** – Massasauga daily activity period derived from all captures from 2019 and 2020. Activity captured by the cameras is representative of when Massasaugas are moving through the environment.*



Appendix VI *– Instructions for construction of AHDriFT bucket camera trap housing units and drift fence arrays.*

Camera trap housing unit construction

1. Use a rotary tool to remove the base of the bucket, leaving about 0.64–1.3 cm (1/4–1/2”) for strength around the rim. Smooth with sandpaper. Alternatively, a jig saw may be used to remove the base after puncturing a hole in the base of the bucket.
2. Invert the bucket and mark 15 x 10 cm (6 x 4”) openings at the front and back. Use a rotary tool or jig saw and shears to cut the openings. Smooth with sandpaper. Snap on the lid.
3. Cut two wooden guide boards to length of 20 cm (8”). At one end of both guide boards, cut a 5 cm (2”) groove into the board perpendicular to the tall (4”) dimension of the wood and roughly 3 cm (1”) from the bottom of the piece.
4. Cut two ~ 2.5 cm (1”) wide sections of the outer lid ridges away on both ends of the front opening. The lid should now have two cut outs allowing the guide boards to slot in place securely.
5. Cut two, 2.5 cm (1”) wide openings extending up approximately 2.5 cm (1”) from the top corners of the front opening of the bucket. These are meant for the wood guide boards to slide into the bucket such that the tops are held in place by the bucket.
6. Place the acrylic sheet on top of the inverted bucket so that there is minimal overhang over the front of the bucket. With the front of the trap facing you, mark 0.64 cm [1/4”] equidistant holes around the top of the bucket for the three L-brackets at 12:00, 4:00 and 8:00 with the L-brackets flush to the acrylic. Drill the holes with and attach the L-brackets to the bucket with screws and hex nuts.
7. Rest the acrylic on top of the L-brackets and mark the three outmost openings. Use a drill to gently make 0.64 cm [1/4”] holes, careful to not crack the acrylic.
8. Make two small drill holes 30.5 cm [12”] above the front opening of the trap. These will be for the zip ties to attach the bucket to the drift fence; check for size.
9. Attach the camera trap to the underside of the acrylic so that the IR screen is closest to the entrance and the lens is directly over the internal guide boards. Attach by feeding two metal wires through four holes drilled into the acrylic. Double-check the camera orientation! You should be able to easily open the camera when it is attached to the acrylic to change SD cards and batteries.
10. Place the acrylic with the camera attached onto the L-brackets, and lock-in using screws and wing nuts. The acrylic should be easily removable in the field.

Fence construction

1. Select an array location with relatively even ground, so that the arms won’t go through impassable rocks or large shrubs. Mallet in a fence post for one end of the array, measure out 15 m (50’) and mallet in a fence post for the other end of the array.
2. Use a mattock to dig a shallow trench between the two posts for the fence to rest in. You can maximize efficiency by having multiple mattocks so that team members can dig trenches simultaneously, or by using a powered trencher.
3. Shallowly mallet in paired fence posts along the trench to stabilize the aluminum while placing it. Place the roll of aluminum down over one end post then have one team member hold the roll in place while another unfurls the roll between the posts from one end post to the other. Ensure the aluminum sits firmly down in the trench

and wrap both ends around the end posts such that ~ 20–40 cm (4–8”) of aluminum curls back and overlaps the main fence. Drill two holes where these layers overlap (one toward the top and one toward the bottom) and use zip-ties to tie the fence to the end posts.

4. Use zip-ties to hold the top of the posts together just above the fence.
5. At each end of the array, clear a flat space for each camera trap housing unit. Drill a hole into the aluminum even in height to the small holes on the front of the bucket and attach together using regular zip-ties.
6. Back-fill all along the aluminum flashing drift fence and the bucket wooden guide boards with dirt, to ensure that there are no gaps for animals to move under the boards or fence. Small snakes can move through pinky-sized holes.
7. Remove the acrylic from each bucket, open and set the cameras. Replace and lock-in the acrylic to the buckets, and the array is operational.

Appendix VIII – Protocol with detailed instructions for constructing and deploying AHDriFT arrays.

Adapted-Hunt Drift Fence Technique (AHDriFT): Protocol for use in detecting Eastern Massasauga (*Sistrurus catenatus*)

Andrew Hoffman, Evan Amber, Jennifer Myers, Gregory Lipps and William Peterman

Introduction: The eastern massasauga (*Sistrurus catenatus*) is endangered in Ohio and was listed as a threatened species under the Endangered Species Act in September 2016. When projects may impact potential massasauga habitat, surveys must be conducted to determine whether the snakes are present on site. USFWS recommended standard survey protocol for Eastern massasauga are visual searches of the area during spring, and the currently accepted survey technique for massasaugas in Ohio uses artificial cover objects consisting of corrugated metal barn roofing material placed in transects within the field being surveyed. Both methods can be effective but are costly, time-intensive, and weather-dependent. The Adapted-Hunt Drift Fence Technique (AHDriFT) has been proposed as a more efficient, less variable alternative to these survey methods for detecting massasaugas.

These protocols are designed to guide and instruct users in deploying AHDriFT arrays for detecting Eastern massasaugas in Ohio. This document covers the materials and construction process of AHDriFT arrays as well as image collecting and processing. These protocols are adapted from "Set AHDriFT: Applying Game Cameras to Drift Fences for Surveying Herpetofauna and Small Mammals, Martin et al., *Wildlife Society Bulletin, 2017*" and have been tested and modified for surveying Ohio habitats. This protocol is divided into five sections: A) Materials and Tools for Construction, B) Camera Trap Unit Construction, C) Array Construction and Deployment, D) Guidelines for Array Deployment, and E) Image Collection, Processing, and Storage.

Materials and Tools for Construction

CAMERA TRAP HOUSING UNITS

Materials Required:

- (1) 19 L (5-gallon) bucket with snap-on lid
- (1) 50 cm (20") of 2.5 cm (1") x 14 cm (5.5") outdoor wood
- (3) 3.8 x 3.8 cm (1.5 x 1.5") L-brackets (size 1/4-20)
- (13) machine screws (size 1/4-20) and hex nuts
- (4) 0.64 cm (1/4") washers
- (3) wing nuts (size 1/4-20)
- (2) 20 cm (8") lengths of 16-gauge steel rebar tie wire
- (1) 30.5 x 30.5 x 0.64 cm (12 x 12 x 1/4") acrylic sheet (US Plastics, Item 44671)
- (1) custom 28 cm (11") focal length Reconyx HyperFire 2 Professional Covert IR Camera (SKU # HP2XODG)

Tools Required:

- Drill (0.64 cm [1/4"] bit and a smaller bit for zip-tie holes)
- Screwdriver
- Hand shears
- Rotary tool (e.g., Dremel) OR Jig saw
- Sandpaper
- Multi-tool OR pliers

AHDRiFT ARRAYs

Materials Required:

- (2) fully constructed camera trap housing units (see above)
- (1) 15 x 0.5 m [50' x 2'] aluminum flashing
- (16) steel rebar posts (cut to 122 cm [4'] height)
- (11) 20 cm (8") lengths of 16-gauge steel rebar tie wire
- (2) UV-resistant zip ties (GTSE, Item 37048B)

Tools Required:

- Mattock or gas-powered landscape trencher (at least one)
- Metal mallet
- Cordless drill (0.64 cm [1/4"] spade and regular bits)
- Multi-tool OR pliers
- Screwdriver

Camera Trap Unit Construction:

1.

Use a rotary tool to remove the base of the bucket, leaving about 0.64–1.3 cm (1/4–1/2”) for strength around the rim.

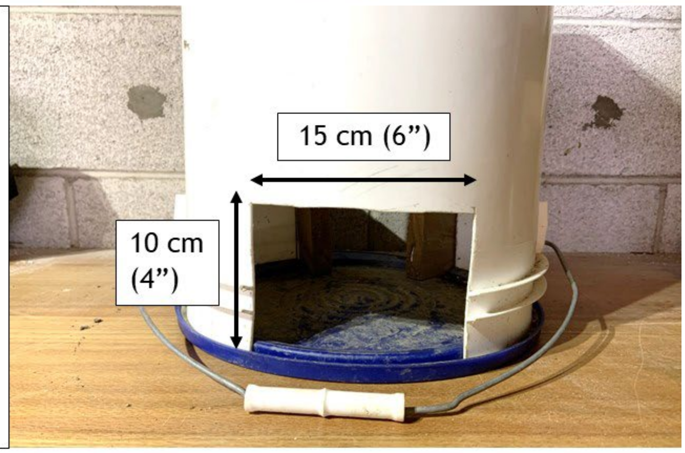
Smooth with sandpaper.

Alternatively, a jig saw may be used to remove the base after puncturing a hole in the base of the bucket.

2.

Invert the bucket and mark 15 x 10 cm (6 x 4”) openings at the front and back.

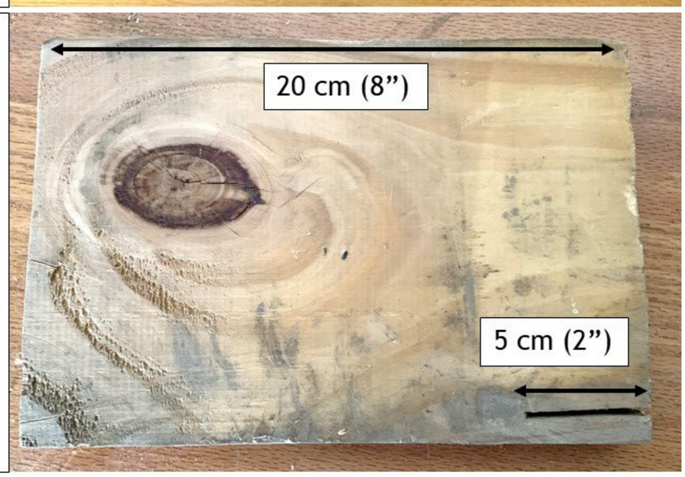
Use a rotary tool or jig saw and shears to cut the openings. Smooth with sandpaper. Snap on the lid.



3.

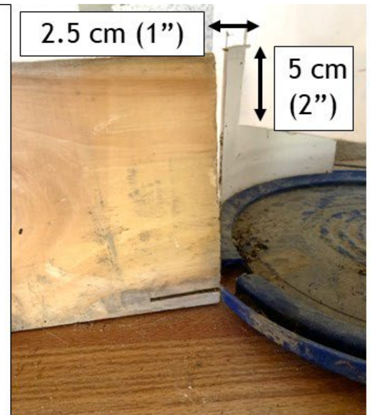
Cut two wooden guide boards to length of 20 cm (8”).

At one end of both guide boards, cut a 5 cm (2”) groove into the board perpendicular to the tall (4”) dimension of the wood and roughly 3 cm (1”) from the bottom of the piece.



4.

Cut two ~ 2.5 cm (1”) wide sections of the outer lid ridges away on both ends of the front opening. The lid should now have two cut outs allowing the guide boards to slot in place securely.



5.

Cut two, 2.5 cm (1”) wide openings extending up approximately 2.5 cm (1”) from the top corners of the front opening of the bucket. These are meant for the wood guide boards to slide into the bucket such that the tops are held in place by the bucket.

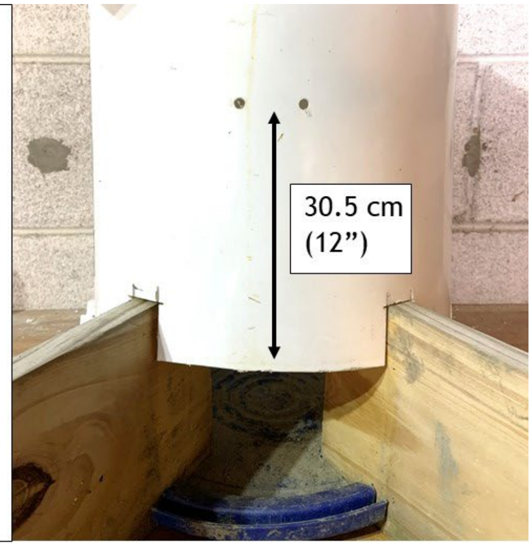
6.

Place the acrylic sheet on top of the inverted bucket so that there is minimal overhang over the front of the bucket. With the front of the trap facing you, mark 0.64 cm [1/4”] equidistant holes around the top of the bucket for the three L-brackets at 12:00, 4:00 and 8:00 with the L-brackets flush to the acrylic. Drill the holes and attach the L-brackets to the bucket with screws and hex nuts.

7.

Rest the acrylic on top of the L-brackets and mark the three outmost openings. Use a drill to gently make 0.64 cm [1/4”] holes, careful to not crack the acrylic.

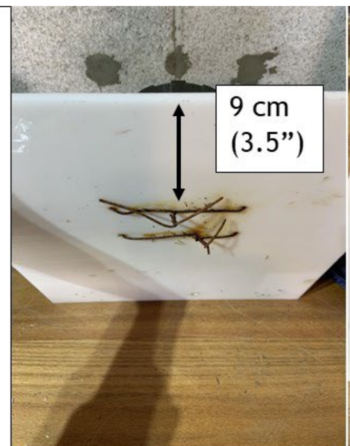
8.

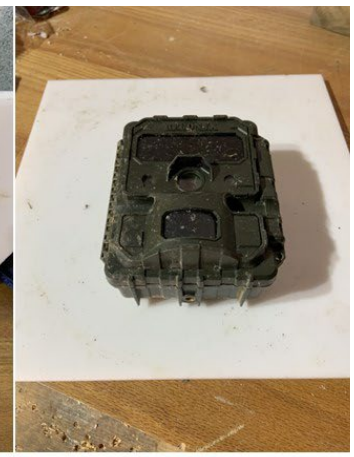


Make two small drill holes 30.5 cm [12”] above the front opening of the trap.

These will be for the zip ties to attach the bucket to the drift fence; check for size.

9.





Attach the camera trap to the underside of the acrylic so that the IR screen is closest to the entrance and the lens is directly over the internal guide boards.

Attach by feeding two rebar ties through four holes drilled into the acrylic.

Double-check the camera orientation! You should be able to easily open the camera when it is attached to the acrylic to change SD cards and batteries.

10. Place the acrylic with the camera attached onto the L-brackets, and lock-in using screws and wing nuts. The acrylic should be easily removable in the field.

Array Construction and Deployment:

1. Select an array location with relatively even ground, so that the arms won't go through impassable rocks or large shrubs. Mallet in a fence post for one end of the array, measure out 15 m (50') and mallet in a fence post for the other end of the array. **2.** Use a mattock to dig a shallow trench between the two posts for the fence to rest in. You can maximize efficiency by having multiple mattocks so that team members can dig trenches simultaneously, or by using a powered trencher.

3.

Shallowly mallet in paired fence posts along the trench to stabilize the aluminum while placing it. Place the roll of aluminum down over one end post then have one team member hold the roll in place while another unfurls the roll between the posts from one end post to the other. Ensure the aluminum sits firmly down in the trench and wrap both ends around the end posts such that ~ 20–40 cm (4–8”) of aluminum curls back and overlaps the main fence. Drill two holes where these layers overlap (one toward the top and one toward the bottom) and use rebar ties to tie the fence to the end posts.

4.

Use rebar ties to hold the top of the posts together just above the fence.

5.

At each end of the array, clear a flat space for each camera trap housing unit. Drill a hole into the aluminum even in height to the small holes on the front of the bucket and attach together using zip-ties.

6.

Back-fill all along the aluminum flashing drift fence and the bucket wooden guide boards with dirt, to ensure that there are no gaps for animals to move under the boards or fence. Small snakes can move through pinky-sized holes.

7.

Remove the acrylic from each bucket, open and set the cameras. Replace and lock-in the acrylic to the buckets, and the array is operational.

Guidelines for Array Deployment

Number of Fences and Duration of Deployment:

We recommend 16 weeks of camera trapping, with a minimum of 12 weeks and up to 20 weeks if budget and time constraints allow. Construct drift fences in March, prior to Massasauga emergence (typically in early April) and after the topsoil has thawed. During the first field visit to activate cameras, check the drift fences for any needed repairs or backfilling, and use hand shears to cut back any vegetation growing into the buckets. Service the arrays (change batteries and SD cards) every 4–5 weeks and deconstruct arrays in early October. Deploy a minimum two arrays per field, adding one array per 10 ha (e.g., a 1 ha field will have two arrays, and a 10-ha field will have three arrays).

Placement of Fences within a Field:

Place arrays as far away from mature trees and forest edges as possible, and within dense herbaceous vegetation. Placement within or near dense shrubs or young trees does not appear to hamper detection, but it can cause difficulty with installation due to tough and extensive root networks. If the site has notable topography, place fences near or adjacent to lower, wetter habitats as these may represent core massasauga habitat (e.g., overwintering sites). Installation on even ground is easier than on slopes, so avoid markedly uneven ground and steep slopes whenever possible. Also avoid installing fences in standing water, even temporary floodwaters, as this can result in the fences not being buried sufficiently and requiring more maintenance later on. Fences handle flooding well once installed.

Array Maintenance:

During the first few weeks of deployment, while sites still experience snow, ice, hard winds and frequent water-level changes, some light repairs may be needed. These repairs will likely include back-filling small gaps that appear under the fences or reburying fences initially installed in wet conditions. Once the ground is more set around the fences, repairs are rarely needed. Take care to regularly pull vegetation around the buckets to avoid false triggering from wind-blown grasses. Paper wasps (*Polistes* spp.) regularly build nests on the camera lens which can also lead to cameras over-triggering and may pose a risk to any workers with sting allergies. These nests can be easily removed early on when few wasps are present, especially in cool weather. Ants often build nests between the camera and acrylic or inside the cameras, resulting in dozens of ants scattering when the camera is removed. When this happens, the camera can be left open to allow the ants to remove their eggs and larvae. Typically, all ants will be gone within 5 mins. Ticks are apparently attracted to the camera trap units and may congregate on the underside of the acrylic, so check your hands and fingers after removing the acrylic.

Image Collection and Processing

We recommend using R package 'camtrapR' (Niedballa et al. 2017) for processing and analyzing AHDriFT image data. To use camtrapR, load the camtrapR package and library into R Studio. Additionally, ExifTool is needed to extract the metadata from the images.

To begin, insert an SD card. Rename the 100RECNX name to the camera ID. This is the name that was given to the individual camera when it was first installed and activated and it can be found by clicking on any of the images on the SD card and looking at the bottom left corner. If an additional, empty RECONYX folder appears as well, it can be deleted. Remember to be consistent with capitalization and naming throughout this process.

Prior to processing images, ensure that the folders the images will be sorted into are correctly organized and labelled for the 'camtrapR' workflow, which has a directory structure. For example: rawImages2022/stationA/camera1/speciesX. First, an "Images" directory is created. Within the "Images" directory, create a subdirectory for each station. A station is essentially an array. Within each station, create a separate subdirectory for each camera within the station. For a linear array, there will be 2 cameras per station. All cameras must be given unique names, even between stations. A subdirectory will then be created within each camera subdirectory for each species detected at that camera.

Once the directory is properly organized, code can be run to give each image a unique name and extract its metadata. This code will create a duplicate folder on the SD card containing the uniquely named images. Images will all have unique file names pertaining to their specific camera and date and time of capture, allowing for easier downstream processing. If only interested in Massasauga detections, simply scroll through the images, and drag and drop Massasauga images into the appropriate folder based on station, camera and species. Massasaugas are easy to see in the largest icons of the images, so scrolling can be done fairly quickly. If interested in all species and data, we recommend first sorting out the false triggered images from images containing species, then returning and sorting species into their appropriate folders.

Before a survey report can be generated or more extensive analyses completed using camtrapR, 2 Excel spreadsheets must be created. The first should be named "Species_Class". It will need to include a column for each of the following: Common Name, Species, Family and Class. This will be a cumulative list of all species found on all cameras. Once added to the list, a species does not need to be repeated.

The second sheet should be named "Schedule_captrapR_Year". It will need to include a column for each of the following: Station, Camera ID, utm_x, utm_y, Setup_Date, Retrieval_Date, Problemx_from, Problemx_to. Each row will contain information about one camera. Additional columns can be created for additional problems should the need arise.

Sample code to load the camtrapR library and ExifTool, name images and extract metadata, add species names to files, create a record table, account for camera operation problems and generate a survey report can be found below.

Example R Code

```
# LOAD LIBRARIES ------------------------------------------------------------
library(camtrapR)

# Make sure R can find exiftool
# Must rename the application (with extension .ece) to 'exiftool' and save in
C:/WINDOWS
# Info: https://cran.r-
project.org/web/packages/camtrapR/vignettes/ImageOrganisation.html#exiftool

Sys.which("exiftool")
# Output of "" means R can't find it. Should either be in C:/WINDOWS or
G:\\exiftool.exe

############## IMAGE MGMT ----------------------------------------------------
imageRename(inDir = "E:/DCIM",
            outDir = "E:",
            hasCameraFolders = FALSE,
            keepCameraSubfolders = FALSE,
            createEmptyDirectories = FALSE,
            copyImages = TRUE,
            writecsv = FALSE)

# Append species names to files, once in the correct folders
# THIS WILL ADD TO FILES ALREADY WITH NAMES, SO JUST DO AT THE VERY END
appendSpeciesNames(inDir = "G:/images_camtrapR_2019_SPECIES",
                   IDfrom = "directory",
                   hasCameraFolders = TRUE,
                   removeNames = TRUE,
                   writecsv = FALSE)

#Make new folder of all of 1 species images for easier quick viewing
# If you want all of the images of the species
getSpeciesImages(species = "storeia_occipitomaculata",
                 inDir = "G:/images_camtrapR_2019_SPECIES",
                 outDir = "G:",
                 createStationSubfolders = FALSE,
                 IDfrom = "directory")

############## MAKE RECORD TABLE ---------------------------------------------
# Import images, ONLY NEEDS TO BE RUN ONCE ON THE FINAL DATASET, THEN BLOCK
OUT
# Creates smaller file to that can be read in below as images2 for the final
table
# DELETE OLD FILES FROM DIRECTORY IF RUNNING WITH UPDATED DATA
images<- recordTable(inDir= "E:/images_camtrapR_2019_SPECIES",
                     IDfrom='directory',
                     cameraID='directory',
                     camerasIndependent = FALSE,
                     minDeltaTime= 60,
                     deltaTimeComparedTo='lastRecord',
                     timeZone='EST')

# Merge with class information
species_class <- read.csv("G:species_class.csv")
common_col_names <- intersect(names(images), names(species_class))
images2 <- merge(images, species_class, by=common_col_names, all.x=TRUE)
write.csv(images2,file="G:/images_camtrapR_2019_SPECIES/ImageTableR.csv")
# save as CSV to be re-read in

############## LOAD DATA -----------------------------------------------------
images3<-read.csv("G:/images_camtrapR_2019_SPECIES/ImageTableR2019.csv")
```

```
CTtable<-read.csv("G:/ahdrift_2019/Schedule_camtrapR_2019.csv") # Camera
Schedule Table

################### CAMERA OPERATION ----------------------------------------
# Create camera names
camnames<-c('KIL1','KIL2','KIL3','KIL4','KIL5','KIL6','KIL7','KIL8','KIL9',
'KIL10','KIL11','KIL12','KIL13','KIL14','KIL15','KIL16','KIL17','KIL18',
'KIL19','KIL20','KIL21','KIL22','KIL23','KIL24','KIL25','KIL26','KIL27',
'KIL28','KIL29','KIL30','KIL31','KIL32','KIL33','KIL34','KIL35','KIL36',
'CBG1','CBG2','CBG3','CBG4','CBG5','CBG6','CBG7','CBG8','CBG9','CBG10',
'CBG11','CBG12','CBG13','CBG14','CBG15','PRF1','PRF2','PRF3','PRF4','PRF5','P
RF6','PRF7','PRF8','PRF9','PRF10','PRF11','PRF12','PRF13','PRF14','PRF15','SP
V1','SPV2','SPV3','SPV4','SPV5','SPV6','SPV7','SPV8','SPV9','SPV10','SPV11','
SPV12','SPV13','SPV14','SPV15')

camopPlot <- function(camOp){
  which.tmp <- grep(as.Date(colnames(camOp)), pattern = "01$")
  label.tmp <- format(as.Date(colnames(camOp))[which.tmp], "%Y-%m")
  at.tmp <- which.tmp / ncol(camOp)

  image(t(as.matrix(camOp)), xaxt = "n",
        yaxt = "n", col = c("red", "grey70"))
  axis(1, at = at.tmp, labels = label.tmp)
  axis(2, at = seq(from = 0, to = 1,
                   length.out = nrow(camOp)), labels = camnames, las = 1)
  title(main='Camera Operation Periods',xlab='Date',ylab='Camera ID')
  box()}

camop_problem <- cameraOperation(CTtable      = CTtable,
                                 stationCol   = "Station",
                                 cameraCol='CameraID',
                                 byCamera=TRUE,
                                 setupCol     = "Setup_date",
                                 retrievalCol = "Retrieval_date",
                                 writecsv     = FALSE,
                                 hasProblems  = TRUE,
                                 dateFormat   = "%d/%m/%Y")
camopPlot(camOp = camop_problem)

################### SURVEY REPORT -------------------------------------------
# Can delete the old survey report file, but should make a report with a new
name
# Can't have NAs in the camera retrieval column

SurveyInfo<-surveyReport(images3,
                         CTtable,
                         stationCol="Station",
                         setupCol='Setup_date',
                         cameraCol = 'CameraID',
                         retrievalCol = 'Retrieval_date',
                         CTDateFormat="%d/%m/%Y",
                         CTHasProblems=TRUE,
                         Xcol='utm_x',
                         Ycol='utm_y',
                         sinkpath='G:/images_camtrapR_2019_SPECIES')
## --------------------------------------------------------------------------
```

For assistance or information on additional analyses that can be run with camtrapR, please visit: https://cran.r-project.org/web/packages/camtrapR/index.html

Appendix VIII – Complete literature review with figures. Submitted to ODOT as a separate document on 1 March, 2019.

INTRODUCTION

Wildlife populations are declining world-wide, leading to a continuous trend of global biodiversity loss (Butchart et al., 2010). Reptiles are not exempt from these losses (Gibbons et al., 2000). Reptile declines are largely attributed to habitat loss, degradation and fragmentation, as well as economic exploitation (Böhm et al., 2013). Direct morality from roads is also a strain on reptile populations (Andrews et al., 2008). In addition to these threats, snakes endure persecution due to people's deep-rooted antipathy towards them (Headland and Greene, 2011; Souchet and Aubret, 2016). Further, snake conservation is challenged by emerging pathogens, such as snake fungal disease (Lorch et al., 2016). Despite these threats, snake populations and ecology are largely understudied. There remains a need for more expansive surveying in order to develop stronger conservation benchmarks (Maritz et al., 2016).

The principle challenge of snake surveys is that snakes are very difficult to observe (Durso and Seigal, 2015). Snakes are secretive, cryptic and many species move slowly and infrequently (Greene, 2000). Therefore, even simple determination of presence or absence of snake species at a site can be difficult (Kéry, 2002). Infrequent snake encounters pose statistical challenges and force researchers to make inferences based on relative abundances from observations (Steen, 2010). However, relative abundances may not be correlated to actual population sizes or densities (Rodda et al., 2015). One resolution is to conduct longer-term studies of herpetofauna to ensure sizable datasets (Erb et al., 2015), though this is not always possible. Simply increasing the length of surveys may only increase the amount of data with wide confidence intervals (Steen, 2010).

Mark-recapture is another solution to estimate populations of cryptic species (Williams et al., 2002), and robust software packages are available (e.g. Program MARK, White and Burnham, 1999). However, snake ecologists still face difficulty in obtaining enough recaptures (Parker, 1987; Dorcas and Willson, 2009). Further, researchers must account for unequal capture probabilities of individuals within populations (Mazerolle et al., 2007).

To overcome these challenges takes concentrated sampling effort and application of careful study design (Kendall et al., 1995). For example, Willson et al. (2011) trapped for Banded Watersnakes (*Nerodia f. fasciata*) and Black Swampsnakes (*Liodytes [Seminatrix] pygaea*) at the Savannah River Site in South Carolina in 2005 and 2006. The authors placed 465 minnow traps spaced two meters apart around an isolated wetland. They checked the traps daily for a total of 69 'robust design' sampling days. With this exhaustive and carefully-planned effort, the study obtained 1,392 captures of 414 individual Banded Watersnakes and 1,286 captures of 495 individual Black Swampsnakes. While impressive, sampling of this intensity is not always possible, and may not be so successful when applied to less abundant snake species that are not concentrated in an isolated area. Further, Willson et al. (2011) showed that study methods, demographic and behavioral factors, and temporary emigration influences mark-recapture population estimates.

Thus, researchers must embrace new methods and analytical tools for dealing with species with low and variable detectability (Mazerolle et al., 2007). A significant advance is the development of low-detection occupancy models (MacKenzie et al., 2002; 2004; 2017), particularly using infrequent snake presence-absence data (Dorcas and Willson, 2009). Developments in computer software allows these analytical methods to be widely available (Hines, 2006). For example, the R package 'unmarked' allows for hierarchical modeling of wildlife occurrence and abundance (Fiske and Chandler, 2011).

Still, models need reliable input data in order to be effective. Traditional methods for snake surveys principally include visual encounter surveys, artificial cover objects and drift fence arrays. These methods are labor intensive, yield low detection rates and are highly variable in their efficiency (Dorcas and Willson, 2009; **Table 1**). Snake ecologists continue to look for more effective ways to conduct surveys. Further, the diversity of survey methods has led to variability rather than standardization in survey protocols.

These survey issues persist even for particularly well-studied species, such as the Eastern Massasauga Rattlesnake (*Sistrurus catenatus*; hereafter EMR). In this review, we discuss the different survey methods available to snake ecologists, with particular attention on survey methods to the EMR. Lastly, we conclude with how the survey protocols for the EMR vary between U.S. state agencies across its range.

THE EASTERN MASSASAUGA RATTLESNAKE (EMR)

The EMR is a small (<70cm) stout-bodied rattlesnake with populations centered around the North American Great Lakes region. Their historic range included parts of Ohio, Michigan, Indiana, Pennsylvania, New York, Illinois, Iowa, Minnesota, Missouri, Wisconsin and Ontario. The EMR has declined rapidly with remaining populations generally small and isolated, and is likely extirpated in Minnesota and Missouri (Lipps and Smeenk, 2017; Missouri Department of Conservation, 2018). The EMR is considered Endangered by all but one of the U.S. states encompassing its historic range. The exception is Michigan where the EMR is listed as Special Concern (Syzmanski et al., 2015). In 2016, the EMR was listed as Federally Threatened under the Endangered Species Act.

The EMR's decline led it to be the focus of extensive ecological research in the last couple of decades (Szymanski, 1998). The species requires open-canopy early-successional mixed-herbaceous grassland or prairie that encompasses or is adjacent to wetland. The EMR overwinters underground in burrow hibernacula, typically made by crayfish. While individual EMR return to the same area to hibernate, they don't show high fidelity to specific burrows (Ernst and Ernst, 2011). Still, burrows must meet particular conditions. For example, the winter water table must be high enough to keep the snake partially submerged to avoid freezing (Smith, 2009).

Historic EMR populations were decimated by loss of habitat due to destruction or conversion, as well as persecution. Today, the majority of known EMR populations are found on protected properties (Szymanksi et al., 2015), including in Ohio (Lipps and Smeenk, 2017). It is widely believed that the greatest threat to these populations is habitat loss through succession from herbaceous to woody-dominated vegetation and habitat degradation due to invasive plants (Szymanksi et al., 2015). The occurrence of snake fungal disease in some EMR populations also poses a significant risk (Tezlaff et al., 2015; Allender et al., 2016).

Robust spatial and habitat research on the EMR allowed for the development of numerous habitat suitability models (Bissell, 2006; Harvey and Weatherhead, 2006; Moore and Gillingham, 2006; Bailey et al., 2012; McCluskey, 2016). However, the predicted suitable habitat nearly always surpasses actual EMR occupancy. This may be due to historical effects not captured in the models (Lipps and Smeenk, 2017). Further, genetic differences between geographically close populations raises the possibility that the EMR faces dispersal barriers to potentially suitable habitats (Chiucchi and Gibbs, 2010). The inability of models to confidently predict EMR occurrence means that potential sites need to be surveyed (Fitzgerald, 2012; Willson, 2016). Although current methods for surveying the EMR are effective, they are time-consuming and costly (Lipps and Smeenk, 2017).

TRADITIONAL SURVEY METHODS

Visual Encounter Survey (VES)

The visual encounter survey (VES) entails walking sites or specified transects. This is the most commonly used method in snake surveys (Campbell and Christman, 1982). Visual 'active searches' were fairly unstandardized until the mid-1990s. In recent years herpetologists pay greater attention to survey transects, timing and personnel in order to quantify effort (Lacki et al., 1994; Fitzgerald, 2012; Willson, 2016, Crawford et al., 2020). Effort quantification ranges from reporting the number of site visits and time per visit (Kéry, 2002) to conducting correlations of sampling time to capture probabilities (Lind et al., 2005). Wagner et al. (2010) went further and assessed sampling effort with Bayesian regression models and bootstrapped species accumulation curves.

Nonetheless, VES is strongly biased by the skill of the observer or observers (Dorcas and Willson, 2009). In a controlled study, Albergoni et al. (2016) found that experienced observers detected two to three times more model herpetofauna than inexperienced observers. We have experienced similar intra-observer variation in detecting Northern Cottonmouths (*Agkistrodon piscivorous*) in North Carolina, EMR in Ohio and numerous snake species in Thailand (Amber, Lipps, pers. obs.).

The efficiency of VES for herpetofauna is highly variable. Success can depend on a range of factors including species, habitat, season and environmental covariates (Erb et al., 2015). Lind et al. (2005) investigated the population dynamics of the Aquatic Gartersnake (*Thamnophis atratus*) over 16 years. The authors found that VES capture probabilities varied from 0.089 to 0.288 (8.9–28.8% probability of detecting a snake where they occur at the sites), due to numerous confounding factors. They did clearly discern, however, that capture probability had a significant positive correlation with sampling effort. Effort was quantified using the number of surveys, length of surveys and the number of observers.

Another long-term VES study was published by Christy et al. (2010) on Brown Tree Snake (*Boiga irregularis*) populations in Guam. The Brown Tree Snake was introduced to the island after World War II. Its explosive population growth is a well-known threat to Guam's small vertebrate biodiversity (Rodda et al., 1992). Despite their prevalence, Christy et al. (2010) only obtained VES detection probabilities averaging 0.07, although they did record detection probabilities up to 0.18 under 'optimal conditions.'

Kéry (2002) conducted VES for five years in Europe for three snake species. Each survey was between 5–60 minutes, and he performed 645 site visits. The study generated detection probabilities ranging between 0.11 and 0.7, depending on the species, site and other covariates. The results highlight the difficulty in determining snake presence or absence by VES.

Still, VES does have its merits. Its simplicity allows for survey teams made of less trained individuals, such as research technicians or public volunteers. In groups, the efficiency gap between experienced and inexperienced observers decreases (Albergoni et al., 2016). Further, without the need of transporting equipment, VES is an efficient method in hard to reach areas. Additionally, tropical snakes are often arboreal, and tropical spatial and temporal temperature is more consistent than in temperate regions. Since artificial cover objects (see below) are less effective under these conditions, VES is perhaps of greater value in the tropics (Strine, pers. comm., 2017).

Indeed, Wagner et al. (2010) conducted VES for herpetofauna communities across land-use gradients in Indonesia. They walked pre-set diagonals of 31 equal-sized plots six times for about 25 minutes each. They detected 12 reptile species of which three species were forest specialists and four species were snakes, and a total of 118 individual reptiles. Unfortunately, the authors did not clarify the number of observers per survey. Under the assumption of one observer per survey, Wagner et al. (2010) detected ca. 1.52 reptiles per person-hour by VES. While they did detect some snakes, the majority of observations were lizards.

Though time-consuming and thus costly in person-hours, VES is effective to determine EMR occupancy (Casper et al., 2001; Lipps and Smeenk, 2017). At Carlyle Lake in Illinois, long-term EMR monitoring produced an average of 0.4 snake observations per person-hour of effort (Dreslik et al., 2016). This figure is in line with research in southwestern Michigan. Bartman et al. (2016) found an average of 0.41 EMR per person-hour by VES. In northeast Ohio, Lipps and Smeenk (unpublished data) found that the probability of detection by VES was best explained by search effort (hours/ha).

Therefore, the USFWS recommends extensive VES as the primary method for surveying the EMR (Casper et al., 2001). Their protocol suggests a minimum of 40 person-hours of VES over a field season (typically 3–5 months), although no guidance is provided to account for differences in the total area being surveyed. Further, 10 years of surveys are recommended prior to declaring the species absent from the site. This protocol continues to be the most commonly referenced by state and federal agencies in regard to EMR surveys. Still, it would seemingly take intensive effort in order to make meaningful population estimates by VES alone.

Artificial Cover Objects (ACO)

Setting arrays of artificial cover objects (ACO) is a frequently used method in conjunction with VES (Fitzgerald, 2012; Willson, 2016). Temperate snakes must seek areas of relative warmth or coolness to the ambient environment to thermoregulate (Greene, 2000). An ACO provides a shelter for snakes that allows them to hide from predators while thermoregulating. By creating an attractive refugia, ACO congregates snakes that are otherwise difficult to find. Surveyors then lift the ACO to see if snakes are sheltering underneath (Godley, 2012; Mills and Graeter, 2013).

Surveys by ACO can significantly increase snake observations while decreasing survey effort compared to VES. Further, ACO arrays capture biodiversity that VES may miss (Olson and Warner, 2003; Sewell, 2012). While ACO is still considered an 'active search' method, the method lessens observer bias and allows for standardization (Dorcas and Willson, 2009). For example, using ACO spatial capture-recapture provides a means to account for the lack of independence and overdispersion of observations (Sutherland et al., 2016).

Nonetheless, there are several significant considerations when designing an ACO study. First, type of ACO material matters. Different materials attract different species, and can be biased towards certain sexes or life stages (Scheffers et al., 2009; Halliday and Blouin-Demers, 2015). Sampling bias and unequal capture probabilities can skew biodiversity measures and/or population demographic information (Mazerolle et al., 2007; Willson et al., 2011). Typically, researchers choose wood or tin (Fitzgerald, 2012; Willson, 2016), but many other ACO including tarps, plastic and asphalt roofing have been tried (see an expansive list in Godley, 2012).

Which material to use is at the discretion of the researcher. Tin gets very hot when placed in direct summer sunlight. Heat can be problematic if too extreme, or may be very attractive to thermoregulating gravid females and diseased individuals (Greene, 2000). The choice of material can thus bias the dataset. Some species and demographics favor or disfavor ACO or different types of material (Parmelee and Fitch, 1995). For example, ACO is biased towards female EMR, which have increased thermoregulatory requirements when gravid (Bartman et al. 2016). Local experimentation is necessary to decide which material is most suitable for the purpose of the study.

The impact of environmental factors on the efficiency of ACO is largely unresolved. Joppa et al. (2009) examined how environmental variables affected plywood ACO capture rates of two Gartersnake species (*Thamnophis sp.*) in southeastern Wisconsin. The authors tested for temperature, time of day, humidity, wind speed and sky cover. The study found that time of day, sky cover and temperature (which are undoubtably correlated) had the greatest effects. Interestingly, they found that the highest capture rates occurred at 26°C with a large amount of cloud cover. However, such results are variable by species, site, material and time of year/season. Few studies have accurately discerned optimal ACO conditions, and "optimal" is likely to vary temporally, by species, and by the condition of individuals (e.g., gravid or non-gravid).

We note that ACO appear to become more effective if set for a long time and are infrequently disturbed (Parmelee and Fitch, 1995). Further, the temperature difference provided by ACO makes the method particularly effective in temperate and colder climates (Engelstoft and Ovaska, 2000). In tropical climates, dense vegetation and spatially uniform temperature makes ACO more difficult and less effective than in temperate habitats (Strine, pers. comm., 2017; Amber, pers. obs.).

Survey by ACO is not included in the USFWS recommendations for the EMR (Casper et al., 2001). There is some evidence that ACO is relatively ineffective in early-successional prairie compared to drift fences (Kjoss et al., 2001; Lind et al., 2017). Still, Wynn (2003) used ACO in northeastern Ohio to capture 36 EMR in 2002 and 51 EMR (41 individuals) in 2003. The method proved efficient enough to produce a population estimate of 106 individuals at the surveyed site.

Indeed, ACO is one of the currently accepted methods by the Ohio Division of Wildlife used to survey EMR (Lipps and Smeenk, 2017). In northeast Ohio, Lipps and Smeenk (unpublished data) found 37.5–100% of weekly EMR captures came from ACO (tin) as compared to VES. The tin was particularly effective later in the season, possibly due to increased vegetative cover reducing the ability of surveyors to visually locate snakes. Their EMR observations by ACO were best explained by the ACO density (tin/ha). Researchers in Ohio generally place one or two sheets of tin per hectare in linear transects through appropriate habitat, avoiding areas with tree canopy, which are then checked weekly for snakes. At their sites, Lipps and Smeenk (unpublished data) most often detected EMR within three visits when using ACO.

Drift Fences and Traps

Trapping by drift fence is one of the most widely-used methods in herpetological inventorying (Gibbons and Semlitsch, 1982; Bury and Corn, 1987; Fitzgerald, 2012; Metts and Graeter, 2013). Drift fence and trap arrays capture high percentage of herpetofauna biodiversity and a large number of individuals (Ryan et al., 2002). The method involves constructing a barrier fence, typically of tarp, metal or wood, that forces animals to move along it. The fence is constructed so that animals cannot go through, over or under. At either end of the fence are traps that hold the animals until the researcher releases them.

Two types of traps are used: pitfall and funnel traps. Pitfall traps are typically buckets dug into the ground that animals cannot escape from. Funnel traps are designed so that animals can enter through an opening into the holding area, but cannot exit. Funnel traps are frequently used when surveying for aquatic species and for snakes that can commonly escape from buckets (Fitzgerald, 2012; Willson, 2016). Trap choice is largely up to the researcher, despite early attempts at standardization (Enge, 1997).

Which trap type is the best has been an ongoing focus of research. Greenberg et al. (1994) surveyed the herpetofauna community in Florida pine scrub. They found that pitfall traps captured a higher number of individuals than funnel traps, but less biodiversity.

Further, the two methods demonstrated different species capture bias. For example, pitfall traps were largely ineffective for capturing snakes or estimating their relative abundance. Interestingly, type of funnel trap also mattered. Two-sided funnel traps captured large snake species, while single-sided funnel traps captured smaller species.

These conclusions are largely corroborated by Todd et al. (2007). The authors conducted a multi-trap type herpetofauna survey of a wetland in the southeastern US. They also found that pitfall traps yielded a high number of individuals but relatively low diversity compared to funnel traps. However, while funnel traps were most effective for large snakes, smaller species were mostly captured by large-sized pitfall traps. The difference in the studies may be attributed to different habitat types and/or different species assemblages. Thus, choice of trap type depends on the aim of the study. Still, both studies conclude that pitfall and funnel traps can be used alone or as complementary methods.

For example, Crane et al. (2018) sampled herpetofauna assemblages in Thailand using a combination of funnel and pitfall traps. They constructed 12 Y-shaped drift fence arrays in three forest types during May and June 2015. Each array had a total of 12 double-chambered funnel traps and three 40L pitfall traps. Each month, the authors set two arrays (to allow capture) in each forest type for three days. They then opened them (to not allow capture) and set the remaining arrays for another three days. Overall, the study captured 266 amphibians and 170 reptiles, representing 13 and 25 species respectively. The funnel traps caught 16 reptile and five amphibian species not found in the pitfall traps. The pitfall traps caught two reptile and two amphibian species not observed in the funnel traps. Snakes were exclusively found in funnel traps, with the exception of two species of Blindsnakes (*Indotyphlops sp.)* that were exclusive to pitfall traps.

Of course, there are also drawbacks to the drift fence and trap method. First is that the method requires considerable person-hours (Enge, 2001). Effort is not just in the construction and maintenance of the arrays, though rough terrain can add difficulty. Since the animals cannot escape the traps themselves the researcher must return frequently (typically daily). Although effective, extensive effort is still required to maximize detection and obtain reliable occupancy, abundance, or diversity estimates.

Halstead et al. (2011) needed 10–50 sampling days of 50 funnel traps checked daily to maximize detection probability for the Giant Gartersnake (*Thamnophis gigas*). Durso et al. (2011) took 150–1,890 trap nights and 5–63 site visits to obtain detection probabilities of 0.03–0.46 for seven aquatic snake species **(Figure 1).** Todd et al. (2007) took about 60 days of daily site visits to maximize lizard and snake species richness.

The time required to attend to drift fences with traps limits the number of arrays that a researcher can manage in a study. Further, the combined costs of materials and person-hours makes the method relatively expensive. To maximize efficiency, there have been attempts to increase snake capture rates that have had varying success. These attempts include baiting traps (Winne, 2005) or creating modified designs for target species (Burgdorf et al., 2005; Halstead et al., 2013).

An issue with trapping that has gathered great attention is that traps can lead to animal injury and mortality (Enge, 2001). Prolonged confinement and subsequent handling in traps can cause undue stress on the animals, sometimes leading to death (Gamble, 2003). Numerous possible issues with traps have been identified. These include rough handling by researchers, entanglement, predation, exposure, desiccation, drowning, starvation, disease and overcrowding of animals (Edwards and Jones, 2014). These issues raise ethical concerns among herpetologists regarding animal welfare and the purpose of conservation research.

Drift fences with funnel traps have been effective in surveying for EMR. Bartman et al. (2016) captured EMR in southwestern Michigan at an average of 2.64 snakes per person-hour. This figure is significantly higher than their comparative VES (0.41) and ACO (1.11) capture per person-hour rates. The USFWS recommends drift fence with funnel trap arrays as a 'supplemental method' to VES for the EMR (Casper et al., 2001).

Road Surveys

Roads fragment habitat, are a dispersal barrier and are a significant source of mortality of herpetofauna (Andrews et al., 2008). Still, roads can serve as an effective means of herpetological surveying. Animals that are road-killed can often be identified with reasonable confidence, or at least to the genus or family levels. Further, roads at night retain warmth relative to the environment, particularly in arid environments. Thus, ectothermic animals may be attracted to roads in the evenings to elevate their body temperature (Dodd et al., 1989; Rosen and Lowe, 1994; Andrews et al., 2008).

These traits make roads a very accessible means for researchers to sample herpetofauna assemblages in designated road sections (Langen et al., 2009; Colino-Rabanal and Lizana, 2012). However, there is evidence that sampling can be biased based on animal size and demographics (Steen and Smith, 2006). Most studies involving herpetofauna and roads have a road-mortality focus. However, we limit our discussion to case studies of road surveys as a means of snake detection.

One of the oldest published records of driving standardized road sections as a means of snake surveying was by Fitch (1949). He repeatedly drove a road transect in western Louisiana over the course of a year. On average, Fitch found one snake for every 240 miles of driving, with a peak in counts during 'snake season'. While this is a very low detection rate, Fitch chose his transect opportunistically. More recent studies with a purpose of finding reptiles have shown greater promise.

In a Wildlife Management Area in Texas, Coleman et al. (2008) road surveyed for herpetofauna in a bottomland hardwood forest. The study conducted routine driving and walking of road transects for a total of 122 hours. They found 130 reptiles, of which 40 were road-killed, equating to an average of 1.07 reptile observations per person-hour. Detection at the species-level was highly variable depending on species and location.

Köhler et al. (2016) obtained similar results for snake road surveys in Mexico. They conducted two night-time road surveys per month between February 2010 and June 2016 (ca. 156 surveys) along a 39km transect. Each survey lasted between 3–5 hours, depending on the number of snakes found, for a total of about 468–780 person-hours. The study produced 578 snakes, of which 433 were road-killed, for an average of ca. 0.74–1.24 snakes per person-hour.

Although the previous studies obtained similar detection rates, road survey efficiency is variable by location (Colino-Rabanal and Lizana, 2012). MacDonald (2012) surveyed a 77km road section in Australia over one year. He performed 77 drives consisting of two transect runs (one in each direction, East and West) at 1.3–1.9 hours per transect run, equating to 200–292 person-hours. The author observed 375 snakes, notably encompassing all 15-known species in the area, for a relatively high observation rate of 1.22–1.88 snakes per person-hour.

On the other end of the spectrum, Enge and Wood (2002) conducted 1,022 pedestrian walking surveys along a six kilometer road section. Assuming a conservative walking pace (not described in the published study), we approximate that each survey took an average of about 1.5 hours. Making another assumption of one surveyor per survey, this estimates ca. 1,533 person-hours. The study found 288 snakes of 18 species, 93% of which were road-killed, for a very low observation rate of ca. 0.15 snakes per person-hour.

However, the low detections of Enge and Wood (2002) may be due to the pedestrian walking survey design. Winton et al. (2018) examined the efficiency of road surveys for Western Rattlesnakes (*Crotalus oreganus*) in British Columbia, Canada. The authors surveyed a 11.7km section of road by walking, cycling and driving. They found that walking the roads (two observers per survey) only found about 8% of estimated road mortality, with a mean detection probability of 0.76. The study also highlights some of the shortfalls of road surveying in general. The authors estimate that actual rattlesnake road mortality at their site is 2.7 times the number of snakes detected across all road survey methods. A major reason for this discrepancy may be because 52% of the observed carcasses were removed by scavengers within two days. This can also partly explain the author's observations that consistent, standardized surveys detected more snakes per person-hour of effort compared to incidental snake observations while driving the same roads at other times.

Researchers should give considerable thought regarding their study species and site selection when developing a road survey study. The variability of detection among species and site poses a challenge to using road surveys for population estimates. However, these studies were successful in capturing a high diversity of herpetofauna, including snakes. Thus, repeated road surveying of potentially suitable habitat may serve as an effective means for determining snake presence.

To our knowledge, road surveys have not been specifically tested as a method for determining EMR occupancy of potential habitats. However, they have been used to determine road mortality rates of known populations (Shepard et al., 2008). Further, EMR are occasionally observed on roads (usually road-killed) adjacent to known sites in northeastern Ohio (Amber and Lipps, pers. obs.). These observations do raise the potential that routine road surveys around modelled suitable habitat may yield meaningful occupancy data.

Still, roads appear to serve as a dispersal barrier for the EMR, with genetic differences between geographically close populations (Chiucchi and Gibbs, 2010). This raises the question as to whether or not EMRs may exhibit some road avoidance. However, there are observations of EMRs crossing natural park roads in Ontario (Colley et al., 2017). A western relative of the EMR, the Prairie Massasauga Rattlesnake (*Sistrurus t. tergeminus*) has also been observed crossing refuge roads in northwestern Missouri (Seigal and Pilgrim, 2002). Perhaps type of road and traffic intensity are factors, but these questions are unresolved for the EMR. Interestingly, Maag and Greene (2017) concluded that road surveys are effective for another relative of the EMR, the Pygmy Rattlesnake (*Sistrurus miliarius*). The authors found other traditional survey methods for Pygmy Rattlesnakes to be ineffective at their sites in southwestern Missouri.

OTHER SURVEY METHODS

Environmental DNA (eDNA)

Environmental DNA (eDNA) has surged in popularity in recent years as a tool to monitor biodiversity (Bohmann et al., 2014; Thomsen and Willerslev, 2015). The method entails taking environmental water or soil samples and filtering them for an assemblage of DNA. Researchers then use PCR primers designed for target organism(s) to determine presence in an area. This non-invasive method is lauded for its applications to cryptic and elusive species (Thomsen and Willerslev, 2015), particularly in aquatic environments (Jerde et al., 2011).

Advocacy for eDNA extends into herpetology (Dejean et al., 2012; Lacoursière-Roussel et al., 2016). Hunter et al. (2015) determined the presence of invasive Burmese Pythons (*Python bivittatus*) in the Everglades with a detection probability of 0.91. While this figure is very promising, it is important to note that Burmese Pythons are particularly ideal for eDNA detection. This is due to their large body size, ubiquitous population and semi-aquatic ecology (Goldberg et al., 2016).

Indeed, eDNA has some significant limitations that can lead to frequent false-positive and false-negative results (Goldberg et al., 2016; Wilson et al., 2016). Environmental factors such as temperature and pH affect how well and how long DNA is preserved in the environment. If eDNA degrades before collection, researchers may obtain a false-negative of species occupancy. Environmental factors also influence the efficiency of filtration and PCR that can lead to false results (Strickler et al., 2015). Erroneous data can create sampling biases that draw incorrect conclusions on occurrence estimates. Statistical approaches are being developed to help identify and account for false positives in eDNA data (Lahoz-Monfort et al., 2016). However, use of such statistical approaches appears to be limited in the current literature.

Application of eDNA to the EMR would face additional challenges. Presumably, eDNA would need to be collected from crayfish burrow hibernacula. Burrows can be difficult to find in vegetated fields, and extracting adequate samples without damaging the hibernacula may be challenging. Further, detection would relate to when the EMR was last in the burrow. Finally, each burrow may have differing rates of eDNA degradation due to localized environmental conditions (Strickler et al., 2015).

Nonetheless, Baker et al. (2018) undertook an eDNA study of EMR in Illinois. They collected samples from crayfish burrows in an effort to detect EMR and the snake fungal disease bacterium (*Ophidiomyces ophiodiicola*); both of which were known to occur in high density in the study area. The results did not detect any *Ophiodiomyces* and only gave two possible detections of EMR. As of this writing, eDNA has not yet been a successful EMR survey tool, nor do any protocols advocate for its use (**Figure 2**).

Detector Dogs

Training dogs for use in wildlife research is not a new idea (Zwickel, 1980). Indeed, Klauber (1956) claimed to have 'used a hound' in Florida to find 500 Eastern Diamond-backed Rattlesnakes (*Crotalus adamanteus*) in just two years! Today, detector dogs are used in a variety of conservation efforts (Woollett et al., 2013). They are frequently used to detect the fur or scat of large mammals (Wasser et al., 2004). Dogs have also been used for taxa as diverse as foxes (Smith et al., 2003), game birds (Gutzwiller, 1990), tortoises (Cablk and Heaton, 2006) and even termites (Brooks and Koehler, 2003).

There are few applications of detector dogs in modern snake studies, but we highlight a couple here. Recalling the invasive Brown Tree Snake (*Boiga irregularis*) in Guam, dogs have been used to detect snakes hidden aboard outbound cargo. In their first attempts, the dogs found snakes with 61% accuracy (Engeman et al., 1998a), and later marginally improved to 64% accuracy (Engeman et al., 2002).

Dogs have also been used to detect snake occurrence *in-situ*. Stevenson et al. (2010) trained a dog to detect live Eastern Indigo Snakes (*Drymarchon couperi*) and their sheds. The dog was trained to detect snakes and sheds above ground and within their Gopher Tortoise (*Gopherus polyphemus*) burrow refugia. The dog was 100% accurate with sheds, but had more difficulty with above and below-ground snakes (88%, 75% accuracy respectively). Overall, the dog successfully used sheds and burrows to indicate snake presence at six of the seven sites.

However, the authors could not confirm if the dog's 'presence' signal was false-positive for below-ground snakes. The authors used a camera to check 17 of the 18 burrows determined by the dog to be or have been inhabited by a snake. They could only visually verify three below-ground snakes. Still, the non-predicted burrows did not capture any snakes by trapping for 2–10 days.

Overall, Stevenson et al. (2010) found the dog to be effective as a survey tool. However, the dog was not perfect and struggled in temperatures above 23°C and in thorny habitat. Therefore, the authors recommend to also conduct VES. As an interesting note, the authors also mention that in 2007 the New Jersey Division of Fish and Game – Endangered Species Program successfully used a trained dog to find Northern Pinesnake (*Pituophis m. melanoleucus*) adults and eggs.

To our knowledge, dogs have not been used to survey for the EMR. However, the ability of the dog in Stevenson et al. (2010) to predict presence of snakes from sheds and burrows raises the possibility of its application. However, researchers should be cautious about using a dog to detect a venomous species, especially as EMRs appear to more readily strike at dogs than human surveyors (Lipps, pers. obs.). Further, dogs may have difficulty working in scrubby early-successional habitat.

TRADITIONAL CAMERA TRAPS

Overview

Camera traps are a widely-accepted tool in conservation research and monitoring (Cutler et al., 1999; Swann et al., 2005; O'Connell et al., 2011). They are frequently used to observe cryptic or elusive species (Carbone et al., 2001; Rovero et al., 2013). However, camera trap success varies by species, site, study design and equipment, causing limitations on species detectability (Cutler et al., 1999; Hamel et al., 2013; Meek et al., 2015).

The debate on how to address these issues has largely focused on the type of camera. Typically, commercial camera traps use Passive Infrared (PIR) triggers. Infrared radiation is emitted from the surface of objects, which is detected by the PIR sensor. Thermal change in the detection zone due to a passing animal activates the PIR sensor (Hughson et al., 2010).

We want to emphasize that PIR sensors do not detect "body temperature", "core temperature" or "ambient background temperature". Further, PIR sensors are not limited to "heat-in-motion". Rather, they detect infrared discrepancy from an object surface that is sufficiently hotter or colder than the detection zone surfaces (Welbourne et al., 2016).

The PIR detection system becomes less effective when background objects in the detection zone do not have uniform surface temperatures. If the background surfaces are thermally heterogeneous, the PIR sensor requires a larger infrared discrepancy to trigger the camera. This can lead to the sensor not detecting the animal or triggering without the presence of an animal (Welbourne, 2013). Critiques highlight this limitation to promote the use of active triggers, particularly for application to rare or elusive species. Active triggers record when an infrared beam is interrupted (Rovero et al., 2013; Hobbs and Brehme, 2017) or a pressure-sensitive switch is activated (Guyer et al., 1997).

Analysis of camera trap images or video can be a time-consuming challenge for researchers (Hamel et al., 2013). Recent progress has been made in computerized image processing (Swinnen et al., 2014), including a developed open-access R package ('camtrapR'; Niedballa et al., 2017). Still, variation of study design poses a challenge when comparing research. To address this, Harris et al. (2010) proposed a standardized design for camera trap studies using a three-step system. However, camera trap placement and study design still largely remain at the discretion of the researcher.

Application to reptiles and small mammals

Camera traps are frequently used in studies of reptiles and small mammals (Guyer et al., 1997; McGrath et al., 2012; Merchant et al., 2013; Ballouard et al., 2016). However, typical PIR camera traps face additional limitations when applied to these taxa. PIR sensors do not emit any radiation and only detect infrared discrepancy between the animal's surface and the background surfaces (Welbourne et al., 2016). Ectothermic animals often have surface temperatures similar to background surface temperatures. Therefore, they may not create a great enough differential in infrared emission to trigger PIR sensors (Welbourne et al., 2013).

Further, many reptiles and mammals have small body sizes or narrow profiles. This reduces the chance that these animals will cross the detection zone in a manner that triggers the PIR sensor. Still, PIR have been used in Thailand to image King Cobras (*Ophiophagus hannah*) after they emerge from overnight refugia (Strine, unpublished data; Amber, pers. obs.).

One way that researchers have addressed PIR issues is simply to switch to active trigger cameras. Sadighi et al. (1995) used active trigger cameras to study Timber Rattlesnakes (*Crotalus horridus*) at three Massachusetts sites. Their best results came where the infrared beam projected for one meter in partial shade along a rock outcrop and the ground. This site resulted in 35 Timber Rattlesnake images in one week.

Guyer et al. (1997) used a pressure switch placed in front of a Gopher Tortoise (*Gopherus polyphemus*) burrow to trigger a timer box wired to a camera. However, the greater power consumption of active triggers compared to PIR meant that the timer box required recharging every 1-2 weeks. The authors also found that active trigger cameras also more easily false-trigger compared to PIR. Guyer et al. (1997) noted that this was a particular issue when the camera is close to the ground or when natural disturbances (such as rainstorms) cause false- triggering.

Hobbs and Brehme (2017) have attempted to alleviate this issue by developing the Hobbs Active Light Trigger (HALT). The system is designed to capture small animals moving in relatively confined spaces, such as along narrow trails or drift fences. The custom HALT pre-aligned three-millimeter near-infrared beam is mounted on an elevated surface and was coupled with a PIR camera for comparison. The elevated threshold and extended beam termination points are designed to reduce false triggers from the environment and small invertebrates. In controlled tests, the authors found that the HALT system obtained higher detection probabilities than their PIR cameras. Indeed, in these tests the HALT had perfect detection of target objects moving at slow and medium speeds no matter their tested temperature differential. However, in field trials, 38% of HALT images were blank, mostly due to mud splashes during rainstorms, small invertebrates and mice. As of this writing, the HALT system does not appear to be commonly-used.

A second strategy is to use time-lapse cameras (Pagnucco et al., 2011; Adams et al., 2017). This method has been particularly applied to aquatic turtles whose wet surfaces and slow movements often don't trigger PIR or motion sensors. Such studies also used the idea of concentrating turtles on "basking rafts" (Geller, 2012b; Bluett and Consentino, 2013; Lipps and Smeenk, unpublished data). A similar method was used by McGrath et al. (2012), who concentrated Grassland Earless Dragons (*Tympanocryptis pinguicolla*) in artificial burrows. In an interesting adaptation, Merchant et al. (2013) attached a light emitting diode on a five-minute timer to a PIR camera. This forced the PIR sensor to act as a time-lapse to monitor American Alligators (*Alligator mississippiensis*) during nesting. One drawback of time-lapse is that the constant or frequent recording means that battery life is much shorter than PIR. Time-lapse also greatly increases the number of images that are captured, increasing the time required of researchers to examine and curate the photographs.

Time-lapse cameras have also been used in snake research, though to our knowledge has never been attempted for the EMR. Sisson and Roosenburg (2017) investigated Timber Rattlesnake (*Crotalus horridus*) use of a newly constructed road right-of-way in Ohio. The authors used 30 time-lapse cameras to collect more than six-million images. Their work detected three new snakes and one new area of use that was not identified by their radio-telemetry study. Alexy et al. (2003) recorded Eastern Indigo Snakes (*Drymarchon couperi*) by continuous time-lapse video of burrow entrances.

Even with these solutions, camera trapping for reptiles and small mammals is a challenge. Researchers must compromise between animal detection and the ability to identify species from the imagery. A smaller field-of-view is better for acquiring photos capable of identifying species than a wide field-of-view. However, a smaller field-of-view will miss more animals due to small detection zone. Further, cameras set on timers will necessitate more frequent photos to ensure animal detection. This is because an animal can pass through a small field-of-view quicker than a wide one, and may exit the field-of-view before the time-lapse camera records. Finally, the large number of images and video to store and analyze can be logistically difficult and costly in person-hours (Harris et al., 2010). This challenge may be alleviated with proper study design and researcher experience (Hamel et al., 2013). For example, Adams et al. (2017) assert that they could process 10,000 images in 1.5 hours.

RECENT CAMERA TRAP APPROACHES FOR REPTILES AND SMALL MAMMALS

Camera Overhead Augmented Temperature (COAT)

Traditional drift fence arrays are effective for intercepting a wide array of amphibian and reptile species and a large number of individuals (Ryan et al., 2002). Welbourne (2013) applied this idea to develop the Camera Overhead Augmented Temperature (COAT) method to capture small mammals and reptiles encountering drift fences. The COAT method uses a drift fence to concentrate animals to an opening. A cork board is placed in the opening underneath a professional PIR Reconyx HC600 camera. The cork board reduces some of the background thermal heterogeneity in the PIR detection zone to improve trigger accuracy. Since the system does not trap animals, it does not need to be checked frequently and does not risk harm to the animals.

The COAT method has been largely successful in field tests. In 300 trap days, Welbourne (2013) imaged 2,900 mammals, 118 reptiles and 57 birds, with only 73 false-trigger events. Welbourne (2014) used COAT to capture images of eight reptile species in a month. He noted that VES and trapping of 40 sites in the 2014 study area produced 11 reptile species. However, he does not report over what time period the reptile surveys took place, number of captures or person-hours, only referring to the efforts as ‘recent’. Welbourne et al. (2015) compared COAT to traditional survey methods, and found COAT to be more effective in detecting small mammals and reptiles and more cost efficient in the long run.

The Hunt Trap

Researchers face the issue of PIR sensors not detecting or not taking clear enough images of reptiles and small mammals. Some studies addressed these problems by using battery intensive active or time-lapse cameras on an artificial concentration of the animals (Geller, 2012b; Bluett and Consentino, 2013; McGrath et al., 2012). However, these concentrations were fairly specific to the target species and were not designed to capture biodiversity. McCleery et al. (2014) developed the Hunt Trap to concentrate small mammals into a narrow detection zone of a PIR camera. The small field-of-view has high image quality and the trap ensures that animals aren’t missed by concentrating them into the detection zone.

The Hunt Trap is essentially an inverted bucket with the base removed. A camera, attached to an acrylic base, rests on top aiming down towards the lid. The bucket has entry and exit openings cut for small mammals, and is baited on the inside. McCleery et al. (2014) made their design for a tidal environment in Florida. Their original Hunt Trap can float with the tides on poles embedded into the substrate. However, the design could also be applied to non-tidal environments.

The Hunt Trap performed well for McCleery et al. (2014). Over 1,344 trap nights, the system detected three small mammal target species. The images had enough clarity to identify animals to the species-level in over 95% of images. The system did not flood or malfunction. Of significant importance, it was able to exclude Common Raccoons (*Procyon lotor*), which can cause problems with baited traps. It should be noted that the authors used a professional-grade custom focal-length Reconyx camera. Recreational camera traps do not have the sensitivity or clarity to take consistent quality images of small-bodied animals at a close focal distance (Newey et al., 2015). However, customized professional cameras are expensive ($400–$600 each).

Adapted-Hunt Drift Fence Technique (AHDriFT)

The COAT method was successful in concentrating a diversity of animals through a fence opening. Yet the open-air system had limited applicability during certain hours due to thermal heterogeneity of the cork board. It also still could not capture quality images of very small animals, and only incorporated one camera trap per array (Welbourne, 2013; 2014). Meanwhile, the Hunt Trap was successful in increasing species-level image quality and image capture of target species. However, it excludes animals not attracted to the bait and captures a large number of images of the same individual (McCleery et al., 2014).

Martin et al. (2017) developed a combination of the Hunt Trap and COAT methods, the Adapted-Hunt Drift Fence Technique (AHDriFT). Like the COAT method, the system uses a drift fence to force animals to move along the fence. Instead of an opening with an overhead camera, at either end of the fence are adapted Hunt Traps. The bucket lids provide a homogenous thermal background surface to improve PIR efficiency (Welbourne et al., 2016). Further, concentrating animals in a small detection zone allows for PIR efficiency and to obtain clear images of small animals (McCleery et al., 2014).

Rather than using bait, the openings in the AHDriFT inverted bucket are affixed with wooden guide boards. These naturally coax animals moving along the fence to enter the trap. Smaller guide boards inside the trap force animals to move directly under the PIR sensor. As with the Hunt Trap, the overhead camera platform is easily removable to change batteries and to download images. Martin et al. (2017) tested four different cameras using a Common Gartersnake (*Thamnophis sirtalis*; snout-vent-length 30cm) and a juvenile Northern Watersnake (*Nerodia s. sipedon*; snout-vent-length 12cm). They found that only custom fixed focal-length cameras detected both test snakes. Although it has not been attempted, AHDriFT's adaptation of the Hunt Trap method may make it applicable to aquatic and tidal habitats (McCleery et al., 2014).

Martin et al. (2017) constructed nine AHDriFT arrays in Florida coastal sand dunes. In one year, 16 cameras (4,502 trap nights) captured 2,523 distinct images. These included 21 reptile, three amphibian, eight mammal and one bird species. Only 51 (2%) of images could not identify the species, and there was no trap mortality. Unlike the COAT method, AHDriFT operated both day and night. The low PIR power-use meant that the batteries only needed to be replaced every four to eight-weeks. Further, the images only needed to be downloaded monthly. Overall, the authors report that field time was reduced by 95% compared to using traditional drift fences with traps.

The initial AHDriFT deployment by Martin et al. (2017) was effective in capturing snakes. They captured 355 images of nine snake species, though 227 captures were of a single species, the North American Racer (*Coluber constrictor*). Still, AHDriFT also captured one image of an Eastern Diamond-backed Rattlesnake (*Crotalus adamanteus*) and three images of Pygmy Rattlesnakes (*Sistrurus miliarius*). The Pygmy Rattlesnake is closely related to the EMR. This raises the possibility that AHDriFT can be an effective and cost-efficient tool to survey for EMR.

EMR SURVEY METHODS ACROSS MANAGEMENT AGENCIES

The widely-used traditional snake survey methods all have their strengths and weaknesses (Dorcas and Willson, 2009; **Table 1**), including different biases (McKnight et al., 2015). The USFWS recommends intensive long-term VES supplemented by drift fence with funnel trap arrays to survey for the EMR (Casper et al., 2001). Although many state agencies across the EMR range follow this recommendation, each state varies in their survey protocol (**Table 2**). We defined Ohio's and Wisconsin's survey protocols by what is described in their specific EMR conservation plans. For other states, we examined the EMR species profile webpages on the state agency websites, internal EMR research reports and their 2015 State Wildlife Action Plans (SWAP). We did not include Ontario in our comparison.

The most commonly-used method by state agencies across the EMR range is VES (**Figure 2**). While ACO is not part of the USFWS recommendations, it is commonly combined with VES for snake surveys (Olson and Warner, 2003; Sewell et al., 2012). The two states with formal EMR-specific conservation strategies, Ohio (Lipps and Smeenk, 2017) and Wisconsin (WIDNR, 2017) both employ VES and ACO. Ohio also uses mark-recapture, while Wisconsin emphasizes radio-telemetry. Drift fences are also widely-used, typically following the USFWS recommendation for funnel traps. However, Iowa and Missouri both use pitfall traps.

Non-traditional methods have not been widely-adopted for the EMR. While there has been road mortality work in Illinois (Shepard et al., 2008), road surveys have not been incorporated into any state's EMR strategies. Also absent is eDNA, which is unlikely to gain traction due to its poor performance in an attempted EMR study in Illinois (Baker et al., 2018). No states indicated the use of detector dogs as a viable survey method for EMR.

The Michigan Department of Natural Resources used element occurrences population modeling (Royle et al., 2012) and 'expert opinion' to delineate and rank EMR habitat. A 2015 Phase I study using aerial images to examine sites of element occurrence delineated 187 possible EMR "populations", of which 42 (22%) are suspected to be extirpated. The majority of the populations were in the lower southern peninsula, and 110 (57%) of these were ranked as "excellent". The Phase I plan also identified 43 "priority core populations" and 38 "back-up populations". However, these delineations did not take human dimensions into account (Lee and Enander, 2015). Current Phase II work is underway to assess populations and connectivity through genetic sampling and VES (Lee, pers. comm., 2019).

To our knowledge, camera traps have not been applied to EMR surveys. However, AHDriFT had success in capturing snake images, including a cousin of the EMR (Martin et al., 2017). AHDriFT had much better effort-efficiency compared to other methods of EMR and general herpetofauna surveys (**Table 3**). Therefore, AHDriFT may be a viable tool for future EMR surveys. Current research is underway in Ohio through The Ohio State University, funded by the Ohio Department of Transportation.

FIGURES AND TABLES

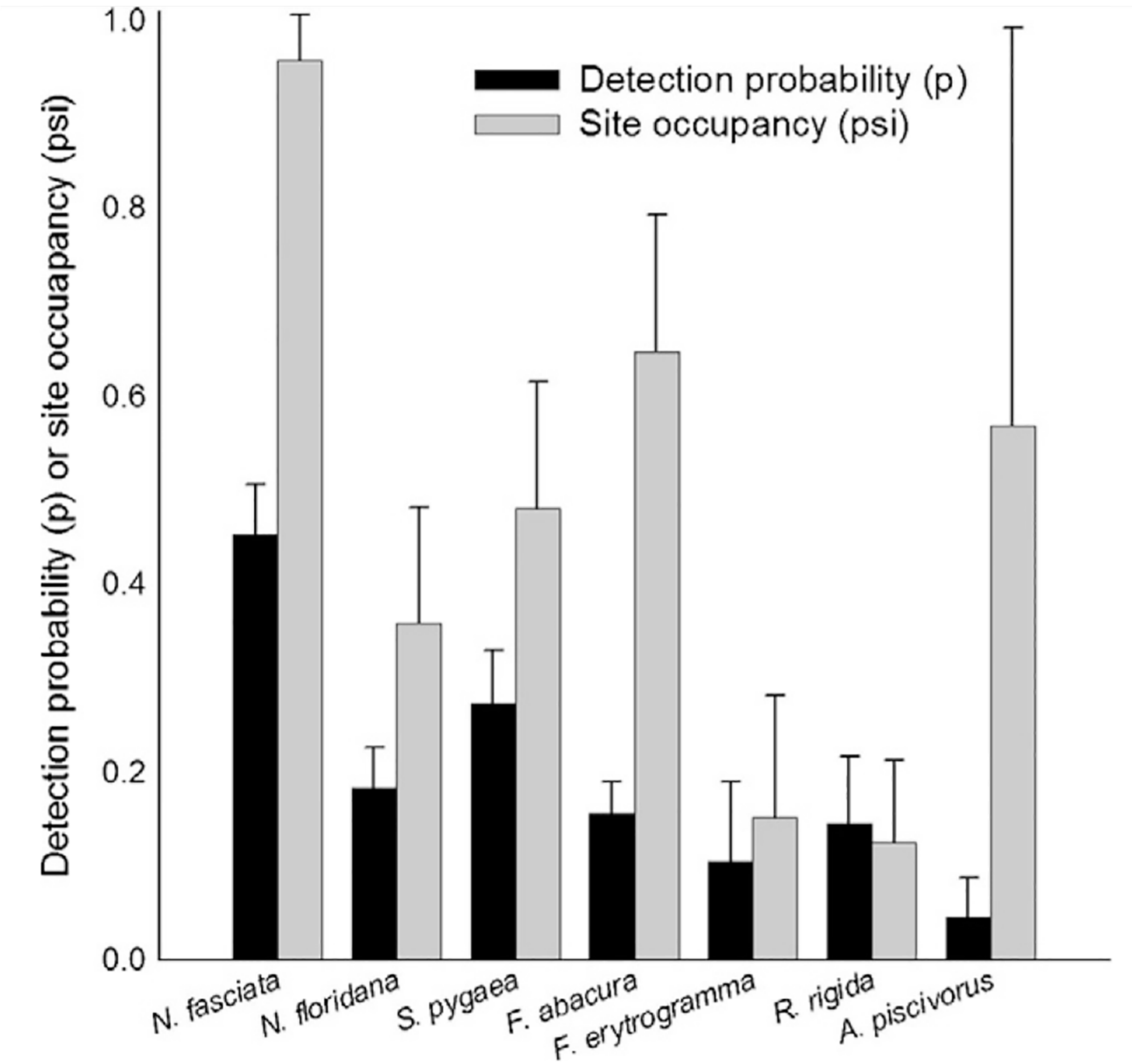


Figure 1. From Durso et al. (2011). Detection probability and site occupancy for seven aquatic snakes. Surveyed using drift fences with funnel traps.

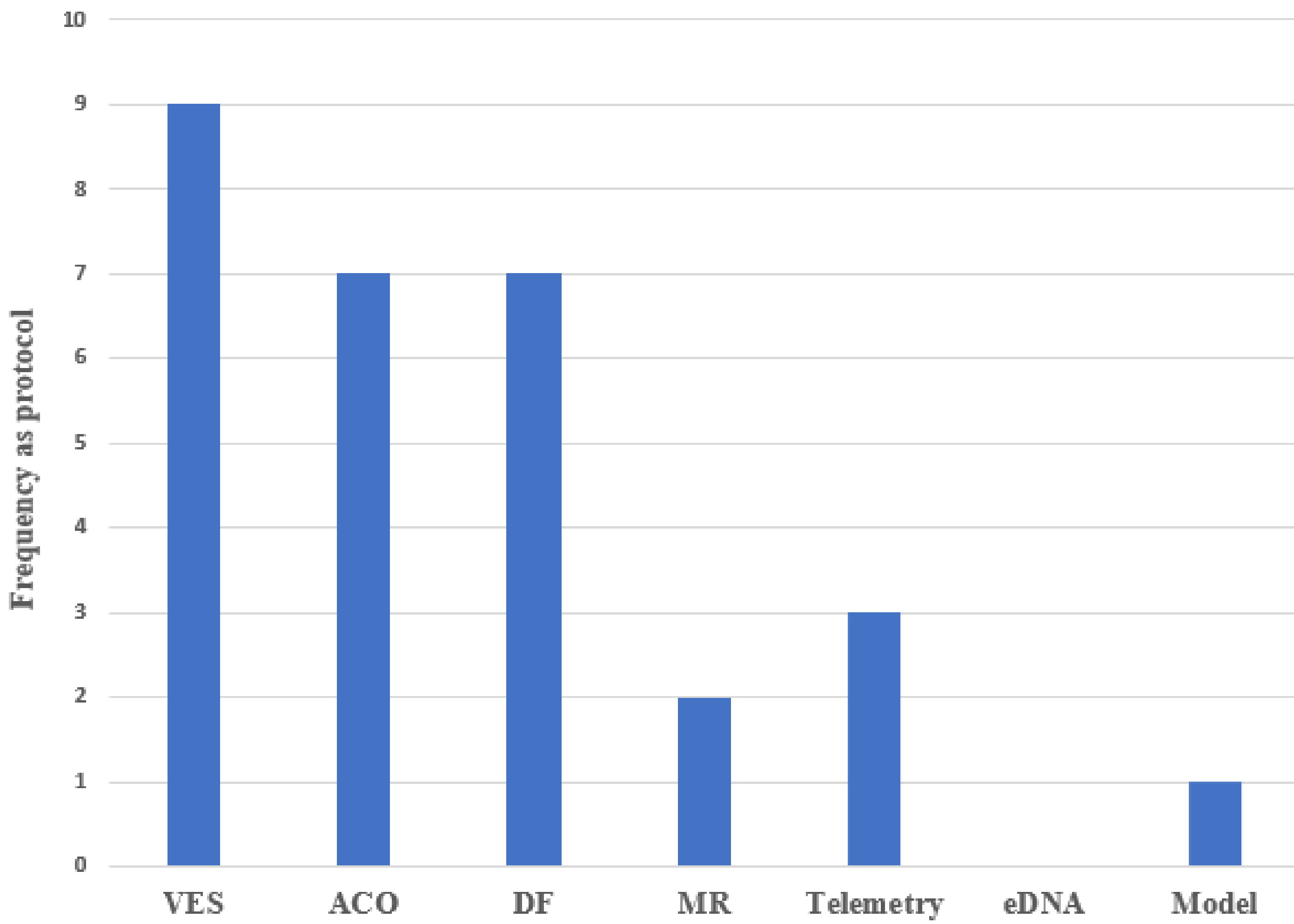


Figure 2. Frequency of different survey protocols for the EMR across states and the USFWS. Included eDNA since it is a commonly asserted method in herpetology. DF is Drift Fences with funnel and/or pitfall traps, MR is mark-recapture, and Model is element occurrence delineation.

Table 1. Qualitative comparison of the traditional survey method traits.

Method	Effort	Capture Efficiency (number of individuals and diversity)	Researcher Bias
VES	Medium	Medium	High
ACO	Medium	High	Low
Drift Fence/Traps	High	High	Low
Road Survey	Low	Medium	Medium

Table 2. State and USFWS survey methods for the EMR across their range.

Region	EMR Survey Method
Federal	VES supplemented by drift fences with funnel traps
Illinois	VES, ACO, drift fences with funnel traps
Indiana	VES, ACO, drift fences with funnel traps
Iowa	VES, ACO, drift fences with pitfall traps
Michigan	Models (element occurrence) with expert opinion. Plan to use VES in genetics study.
Minnesota	VES, drift fences with funnel traps
Missouri	VES, ACO, drift fences with pitfall traps* *Methods are not for the EMR, but for the prairie Massasauga (*Sistrurus t. tergeminus*)
New York	Telemetry, mark-recapture, drift fences with funnel traps
Ohio	VES, ACO, mark-recapture
Pennsylvania	VES, ACO, telemetry
Wisconsin	VES, ACO, telemetry

Table 3. Comparison of examples of detection effort-efficiency for different survey methods.

Method	Detection Effort-Efficiency (observations/person-hour)	Species	Reference
VES	0.41	*Sistrurus catenatus*	Bartman et al., 2016; Dreslik et al., 2016
ACO	1.11	*Sistrurus catenatus*	Bartman et al., 2016
Drift-Fence, Funnel Trap	2.64	*Sistrurus catenatus*	Bartman et al., 2016
eDNA	Near 0 (2 possible detections in full study)	*Sistrurus catenatus*	Baker et al., 2018
Camera trap (COAT)	3	*Pseudechis porphyriacus*	Welbourne, 2013
Road Survey	0.94	General reptiles	Coleman et al., 2008
AHDriFT	13	General snakes, mostly *Coluber constrictor*	Martin et al., 2017