

2019

## Efforts in estimating habitat usage of neonatal garter snakes in central Iowa

Jeremy Andersen  
*Iowa State University*

Follow this and additional works at: <https://lib.dr.iastate.edu/etd>



Part of the [Developmental Biology Commons](#), and the [Evolution Commons](#)

---

### Recommended Citation

Andersen, Jeremy, "Efforts in estimating habitat usage of neonatal garter snakes in central Iowa" (2019). *Graduate Theses and Dissertations*. 17637.  
<https://lib.dr.iastate.edu/etd/17637>

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact [digirep@iastate.edu](mailto:digirep@iastate.edu).

**Efforts in estimating habitat usage of neonatal garter snakes in central Iowa**

by

**Jeremy Robert Andersen**

A thesis submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee:  
Anne Bronikowski, Co-major Professor  
Brent J. Danielson, Co-major Professor  
Robert Klaver

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the contents of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2019

Copyright © Jeremy Robert Andersen, 2019. All rights reserved.

## TABLE OF CONTENTS

	Page
LIST OF FIGURES .....	iii
LIST OF TABLES.....	iv
ACKNOWLEDGMENTS .....	v
ABSTRACT.....	vi
CHAPTER 1. GENERAL INTRODUCTION .....	1
Background of the Study .....	1
Thesis Organization .....	3
Literature Cited.....	3
CHAPTER 2. THE CONSPECIFIC TRAILING ABILITIES OF NEONATAL .....	
<i>THAMNOPHIS SIRTALIS</i> .....	
Abstract.....	5
Introduction.....	5
Methods .....	7
Results .....	14
Discussion .....	17
Literature Cited .....	19
CHAPTER 3. GENERAL CONCLUSIONS.....	23
Summary and Discussion.....	23
REFERENCES .....	30

**LIST OF FIGURES**

	Page
Figure. 1. Scent choice apparatus.....	10
Figure 2. Change in latency (s) with each subsequent exposure.....	16

**LIST OF TABLES**

	Page
Table 1. Three treatments of scent choice .....	11
Table 2. Results of choice experiment .....	14
Table 3. Analysis of variance for exposure 1, all exposures pooled, and latency to complete an exposure .....	15
Table 4. Preference results .....	17

## ACKNOWLEDGMENTS

First and foremost, I am grateful to my family, Lindsay and Addyson, whose amazing support and encouragement have enabled me to pursue my passion. I am also thankful to my mom who constantly exposed me in my youth to exotic pets and wildlife documentaries, and to my dad who taught me the value of a hard day of work.

Thank-you also to Drs. Anne Bronikowski and Brent Danielson for their patience, guidance, and support during this process. I could not have asked for a better pair of advisors.

I am also thankful to Professor Robert Klaver, for his patience while answering yet another of my many questions.

I am also grateful to Drs. Bill Clark and Mike Rentz, for their support which enabled me to complete my research.

Finally, I am especially grateful to the Bronikowski-Janzen lab coalition for their stimulating conversations, insightful feedback, and openness to differing opinions.

**ABSTRACT**

This study investigated *Thamnophis sirtalis* habitat usage, a species that is highly adaptive and has a wide range across North America. *Thamnophis sirtalis* have been found in various habitats ranging from meadows and prairies to woodland edges and swamps. They can survive the bitter cold winters of Northern Canada and the metabolically taxing high elevations of the Rockies and Cascade mountains. To survive in the northern regions of North America, adult *T. sirtalis* are known to seek underground refuge called Hibernaculums. However, it has not been observed previously where neonates choose to overwinter. The research presented in this study provides a possible method of how neonates locate overwintering sites in the first years.

The first study established that *T. sirtalis* used a pit in the study area with a combination of drift fencing and trail cameras. Over the course of the study, 90 events were photographed moving in and out of the pit. No neonates were photographed suggesting that either they were not present or did not utilize the same portions of the habitat. In the second study, thermal data were obtained for 3 habitat types: prairie, woodland edge, and woodlands for a 3-month period. The thermal data provided the monthly averages for each month and also showed the thermal variance between soil and elevated locations within each location. Thermal preference for *T. sirtalis* was not quantified due to a lack of captures. The thermal variance suggested that the cover objects most likely did not provide a large enough thermal gradient that snakes prefer. The third study was the continuation of a long-term survey to determine the population size, growth rate, and survival probabilities. The study was unsuccessful due to a lack of recaptures. The lack of recaptures might be remedied with the implementation of sampling methods such as radio telemetry, drift fencing, pit fall traps, and

funnel traps. The final study demonstrated that neonates show a propensity to follow adult scent tracks that is statistically significant from chance for naïve neonates ( $P=0.002$ ) and repeated exposures to the apparatus ( $P=0.0006$ ). Neonates also demonstrated a statistically significant result for latency ( $P=0.04$ ) to complete the apparatus. Scent trailing could be the method that neonates use to locate hibernaculums in the first years of life. The results of the latency analysis suggest that the neonates learned the apparatus and made their choice quicker with each subsequent exposure.



## CHAPTER 1. GENERAL INTRODUCTION

### Background of the Study

Investigating the functional significance of behaviors that animals display while interacting with the environment is a fundamental question in behavioral ecology. The use of reptiles in behavioral studies has been disproportionately lower than other vertebrate taxa (Bonnet et al. 2002). Conducting behavioral studies on snakes can pose a unique challenge due to the lack of limbs, visual displays, or vocal signals (Doody et al. 2013). Instead, snakes rely heavily on chemical cues to provide information about the habitat and other animals present in the area (Mason, 1992). *Thamnophis* species have been used as a model snake genre for close to 40 years for studying various reptile behaviors (Mason et al. 2010).

The ability to observe secretive species, such as snakes, in the field has been limited primarily to adult behaviors consisting primarily of mating behaviors, migration events, and thermoregulation models (Huey et al. 1989; Lemaster et al. 2001; Lutterschmidt et al. 2006). While this has been exceedingly insightful for understanding reptilian life histories, there are still many gaps in early life behaviors because of the small body size (Pike et al. 2008). In pairing camera traps with a method of individual recognition such as Passive Integrated Transponders (PIT tags) or Visible Implanted Elastomer (VIE) researchers can begin to build a working model for secretive, trap shy, or endangered species without the negative impacts of repeated invasive sampling.

With the recent availability of small digital video cameras that are both reliable and fiscally inexpensive, researchers are able to remotely observe species possibly reducing the bias that might otherwise be incurred by the presence of a person. This opportunity brings researchers another step closer to elucidating the natural reactions to a stimulus in a

laboratory setting. With respect to snakes, behavioral experiments focusing on the reaction when a specific chemical cue is encountered have been predominately focused on adults. The few studies that used juvenile garter snakes as a study subject, focused on the number of tongue flicks as a measurement of interest in a particular cue (Graves et al. 1988). While I am unaware of any studies that use juvenile garter snakes in a Y-Tube Maze, there have been several studies using adults in this apparatus (Ford et al. 1984; Heller et al. 1981;). The chemical cue is applied to a material that is then placed inside of an apparatus commonly referred to as a Y-tube olfactory maze. These mazes are often in the shape of a Y but can be constructed in other configurations to suit the needs of the research goals. Once the chemical cue is applied to the apparatus the test subject is released and the results are recorded. While this method is hardly unheard-of, a few details can lead to a bias in the results. The apparatus is often constructed with transparent materials that allow the researchers to visually watch the outcome, often with the researcher remaining in the room. Constructing the apparatus of transparent materials can cause an animal, who is predated upon from above, to react in response to the researcher's presence thereby creating a false response to the chemical cues.

This thesis research provides a method to reduce the bias that might be present in previous scent trailing research in the common garter snake, *Thamnophis sirtalis*. This species is found throughout eastern North America from Florida to Quebec and British Columbia. Like other snakes that live in northern areas of North America, *T. sirtalis* must seek winter refuge to survive. The species is also known to give birth to young during late July through early September, which only provides the young with a small window of opportunity to locate an appropriate winter refuge. This research provides the first steps in

understanding the effects that adult chemical cues have on juveniles while limiting potential induced bias.

### Thesis Organization

This thesis is organized into three main chapters. The first chapter introduces population sampling methods and to the original research covered in the second chapter. Chapter 2 focuses on the experiment that was conducted to elucidate the behaviors displayed by juvenile common garter snakes. Chapter 3 discusses the study methods utilized to conduct the research and the results.

### Literature Cited

- Bonnet X, Shine R, Lourdais O. 2002. Taxonomic chauvinism. *Trends Ecol Evol.*, 17(1):2000–2002.
- Doody JS, Burghardt GM, Dinets V. 2013. Breaking the social-non-social dichotomy: A role for reptiles in vertebrate social behavior research ethology. *Intl J Behav Biol.* 119(2):95–103.
- Ford NB, Schofield CW. 1984. Species specificity of sex pheromone trails in the plains garter snake, *Thamnophis radix*. *Herpetologica.* 40(1):51–55.
- Graves BM, Halpern M. 1988. Neonate plains garter snakes (*Thamnophis radix*) are attracted to conspecific skin extracts. *J Comp Physiol.* 102(3):251–253.
- Heller S, Halpern M. 1981. Laboratory observations on conspecific and congeneric scent trailing in garter snakes (*Thamnophis*). *Behav Neural Biol.* 33(3):372–377.
- Huey RB, Peterson CR, Arnold SJ, & Porter WP. 1989. Hot rocks and not-so-hot rocks : Retreat-site selection by garter snakes and its thermal consequences. *Ecology.* 70(4):31–944.
- Lemaster MP, Moore IT, Mason RT. 2001. Conspecific trailing behaviour of red-sided garter snakes, *Thamnophis sirtalis parietalis*, in the natural environment. *Anim Behav* 61(4):827–833.
- Lutterschmidt DI, LeMaster MP, Mason RT. (2006). Minimal overwintering temperatures of red-sided garter snakes (*Thamnophis sirtalis parietalis*): A possible cue for emergence? *Can J Zool.* 84(5):771–777.
- Mason RT 1992. Reptilian Pheromones. In: C Gans, D Crews: *Hormones, Brain, and Behavior* (pp. 114–228). Chicago, Il: The University of Chicago Press.

Mason RT, Parker MR. 2010. Social behavior and pheromonal communication in reptiles. *J Comp Physiol.* 196:729–749.

Pike DA, Pizzatto L, Pike BA, Shine R. 2008. Estimating survival rates of uncatchable animals : The myth of high juvenile mortality in reptiles. *Ecology.* 89(3):607–611.

## CHAPTER 2: CONSPECIFIC TRAILING BY NEONATAL GARTER SNAKES, AND IMPLICATIONS TO OVER-WINTERING SURVIVAL.

*A paper submitted to the Iowa Academy of Science*

Jeremy Anderson, Anne Bronikowski, and Brent Danielson

### ABSTRACT

Snakes rely heavily on chemical cues to navigate the world around them, so it is important that we understand what behaviors cues elicit. While the behaviors are well studied in adult reproduction and prey preference, the roles that chemical cues play in immature snake behaviors is still being established. With the use of a modified Y-tube apparatus, we tested 27 neonatal *Thamnophis sirtalis* predilection for following adult scent tracks. We found that in an unfamiliar environment a large percentage of neonates followed adult scent tracks and, with repeated exposures, the neonates still preferred the adult scent track regardless of the sex of either neonate or adult. The propensity for neonates to follow adult scent tracks suggests a biological and ecological dependence on scent tracks.

KEY WORDS: juvenile, neonate, chemical cues, scent trailing, behavior, *Thamnophis sirtali*

### Introduction

A critical component of an ectothermic vertebrate's life is surviving when environmental conditions are outside a desirable range. The effects of the environment on metabolic function and behavioral adaptations at thermal extremes, has been studied in various ectotherms, on their ability to withstand wide variation in their body temperatures (Deutsch et al. 2008; Gangloff et al. 2016; Huey et al. 1989; Telemeco et al. 2017). North American snake species provide examples of metabolic/behavior functions during thermal extremes. The life cycle of many North American snake species includes a period of winter

dormancy, emergence during the spring when breeding occurs, followed by birthing events in mid to late summer (Shine 2005; Shine et al. 2001). Some period of foraging presumably ensues, followed by movement to a suitable over-wintering site. Unfortunately, the method in which snakes locate appropriate overwintering sites has been less studied than other behaviors, particularly for neonates and young juveniles. This paucity of information exists even for widely distributed and common species, such as *Thamnophis sirtalis*.

The importance of chemical cues to snakes has been well established in numerous studies summarized in Chapter 4 of, *Methods in Chemical Ecology* (BN Ford et al. 1993; also see Mason et al. 1998). Two families, Viperidae and Colubridae, have served as model taxa for research in this area. In Viperidae, scent tracks of conspecifics are used by both adults and juveniles to locate communal dens for reproduction and overwintering (Brown et al. 1983, 2007; Cobb et al. 2005; Diller et al. 2002; Gomez et al. 2002). The scent-tracking abilities of various juvenile *Crotalus* and *Sistrurus* species have been investigated in depth, and they are known to use female scent tracks to locate overwintering sites (Clark et al. 2012; Hileman et al. 2015). In Colubridae, scent cues are involved in prey selection, predator avoidance, and reproduction (Placyk et al. 2011; Shine 2005;). In *Thamnophis*, males will track conspecific females more reliably than heterospecific females, and adults will track conspecifics and congeneric individuals when given the choice between a scented track or a non-scented track (Heller and Halpern 1981; Lemaster et al. 2001; Shine et al. 2001).

At the far northern limits of the range of *T. sirtalis*, in Manitoba, Canada, researchers examined the social and reproductive behaviors of adults during the spring emergence from large communal overwintering dens (Aleksiuk 1976; Larsen 1987). Young *T. sirtalis* were not observed aggregating with adults at these overwintering sites (Shine et al. 2001); hence,

several hypotheses have been proposed to explain their absence, including the risk of cannibalism and injury (Seigel and Collins 1993). The information concerning the trailing abilities of *T. sirtalis* has been limited to adult mating events and prey detection. Few studies in either laboratory or field settings have focused on conspecific trailing abilities of neonates (also see Halpin 1990; Mason and Parker 2010).

To discern whether chemical cues are used by neonates to locate overwintering sites, we sought to answer two questions: First, do neonate *T. sirtalis* use chemical cues from adults to determine movement routes in general? Secondly, if so, do neonate *T. sirtalis* distinguish between closely related and distantly related conspecifics? If neonate *T. sirtalis* follow chemical trails from adults, and furthermore preferentially aggregate with relatives, then this suggests the possibility of cryptic parental care. We tested the propensity of neonate *T. sirtalis* to follow adult scent tracks in a lab experiment. We exposed naïve newborn garter snakes to the scents of related and unrelated adults in a choice experiment to test whether they chose to follow the scented or unscented route through an experimental apparatus and to test whether they more reliably follow their mother relative to non-related adults. If neonatal garter snakes preferentially used olfactory cues to navigate a novel environment, neonatal snakes should choose the path with the scent trail of adults significantly more often than a non-scented path.

## **Methods**

### *Study Population*

The field site was in Story County in central Iowa, approximately 2 km south of Story City (42°09'50.4"N 93°34'49.1"W). The site was on private land and consisted of restored oak savannah prairie, woodland edge, woodland, and river edge habitats spread across

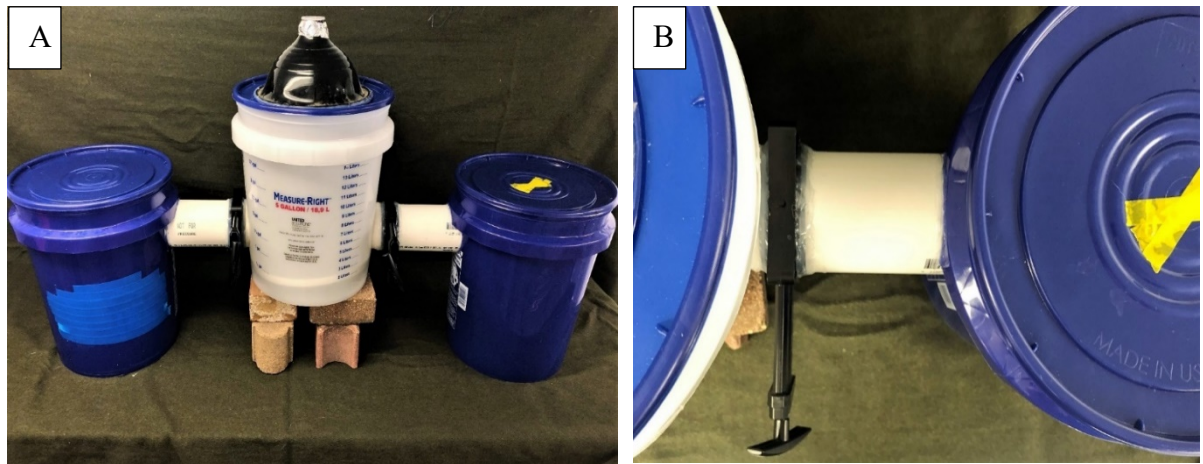
approximately 12 ha, as well as some cultivated land. Surveys of amphibians and reptiles conducted annually at this site since 2012, found *T. sirtalis*, *T. radix*, *Pantherophis ramspotti*, *Lithobates pipiens*, *Anaxyrus americanus*, and *Hyla versicolor*.

From 15 May 2018 – 15 July 2018, snakes were hand-captured under cover objects and moving freely within the vegetation. I caught only a single pregnant *T. sirtalis* in 2018. This female, along with a non-pregnant female and an adult male, were brought into captivity for acclimation (male and female), and gestation (gravid female). Each adult was housed separately in a 40-l aquarium lined with paper substrate, maintained in a room with an ambient temperature of 23 C, and a thermal gradient with supplemental heat of 25-38 C. Animals were provided with a water dish that also served as a retreat site and water was provided *ad libitum*. The three adults were fed weekly: the pregnant female was offered four small pinkie mice twice per week until parturition; the two additional adults, and the post-parturition female were offered two small pinkie mice once per week. The gravid female gave birth to a litter of 30 offspring, of which 27 survived to be used in the experiment. Neonates were removed from the mother's enclosure and placed into individual plastic shoe boxes lined with brown paper towels. Each shoe box was equipped with a cover item and a water bowl. The neonates were fed a diet of appropriately sized nightcrawler (*Lumbricus terostris*) pieces twice per week. Feeding for adults and neonates was voluntary and we recorded propensity to eat. Animals were fasted for four days prior to the start of the scent choice experiment detailed below. All animals were obtained in accordance with an Iowa Department of Natural Resources collection permit (SC 1234) and maintained in the laboratory under an approved IACUC protocol (1-12-7285-J).



### *Materials*

The general method used to elucidate trailing behaviors of snakes is with an apparatus known as a Y-maze (hereafter “maze”) (Brown and Maclean 1983; Graves and Halpern 1988; Hileman et al. 2015; Lemaster et. al. 2001; Mason and Parker 2010). The bottom of the maze is often covered with a paper product to allow easy transference of scents and to reduce contamination between exposures. The animal is introduced to the maze through a predetermined leg where it is presented with a choice between two avenues to proceed. Typically, only one avenue is scented, but is dependent on the experimental design. My modified Y-maze the, Scent Choice apparatus (hereafter “SC”), was constructed from one translucent and two opaque 5-gallon buckets, two 8-inch sections of 3-inch I.D. PVC tunnels, and two 3-inch I.D. plastic gate valves (Figure 1A & 1B). The translucent bucket was at the center of the SC apparatus with the remaining opaque buckets attached at 180 degrees from the central bucket. PVC tubes served to connect the central bucket to the two opaque side buckets (hereafter “tunnels”). Polyethylene freezer paper lined the bottom of the opaque buckets, and scented freezer paper was used to line the tunnel that would serve as the scented tunnel in each exposure. Scent was applied to the freezer paper by allowing an adult to crawl freely across it for 24 hours in an aquarium. All experiments were video recorded using the following components sourced from adafruit.com: a Linux OS control board, pi camera module version 2, and PFC8523 real-time clock module situated above the center bucket. The video recording software was PiKrellCam (Wilson 2015) and used a web-based vector system to track movement and trigger a video recording or still images.



**Figure 1. Scent choice apparatus.** (A) Neonates begin each experiment in the translucent center bucket. On either side of the center bucket are the scent track choice buckets. Leading into the choice buckets are manually operated three-inch plastic gates. (B) Close-up of a manually operated gate in the closed position. To open the gate and allow access to the choice buckets the handle at the bottom left of the photo is pulled.

### *Experiment*

Three treatments of the scent-choice experiment were conducted using the neonates. Each treatment was composed of four males and five females. The difference among treatments was the order of adult scents to which the neonates were exposed (Treatment 1 (n=9): Mother-Male-Female; Treatment 2 (n=9): Female-Mother-Male; Treatment 3 (n=9): Male-Female-Mother, where “Female” is the non-mother adult female). Because the time required to prepare and then clean the apparatus was significant, and the time from start-to-finish for a single neonate in the apparatus was up to 1 hour, only one exposure (n=9) could be run in a single day. The three treatments were conducted such that each neonate’s first exposure was run on three consecutive days, each neonate’s second exposure was run on the next three consecutive experiment days, etc. Each set of three consecutive exposures was separated by one week to feed the animals and provide a rest (total of 9 experimental days, in three sets of three consecutive days, separated by one week; see Table 1 for details). Each

**Table 1. Three treatments of scent-choice.** Each treatment differed in the order of exposure to the adult scent. The shaded row corresponds to Exposure 1 and is highlighted to emphasize that this was the first exposure of neonates to the apparatus. All subsequent exposures were no longer in a novel environment.

	<b>TREATMENT 1</b> (N=9) <b><u>Day 1</u></b>	<b>TREATMENT 2</b> (N=9) <b><u>Day 2</u></b>	<b>TREATMENT 3</b> (N=9) <b><u>Day 3</u></b>
<b>EXPOSURE 1</b>	Mother	Male	Female
<b>EXPOSURE 2</b>	Female	Mother	Male
<b>EXPOSURE 3</b>	Male	Female	Mother

group was naïve to both the apparatus and the male and female adult they were presented with the first time they were tested in the apparatus. The specific tunnel that was left unscented or that received the scented paper trail was randomized for the first exposure of the apparatus. For the subsequent exposures, the scented tube was placed opposite of the previous exposure.

The adult was placed in an enclosure lined with pre-scored wax paper the evening before the experimental treatment was left to saturate the paper with scent. The pre-scored paper was separated into 1-inch strips and used to line the tunnel for each neonate exposure for the treatment day. Moreover, the bucket at the end of the tunnel selected to receive the scented paper was further scented with that adult's scent by placing the adult in the bucket lined with wax paper for 10 minutes before the start of each experimental trial. The entire apparatus was designed with translucent and opaque materials to reduce stress caused by the animal's ability to visually detect the researcher. The adult was removed after the 10-minute scenting period, and the neonate from the testing group was released into the central bucket, allowing for a 10 minutes acclimation period before manually opening the sliding gates. After the neonate made its choice, it was removed, and the apparatus was sterilized with a 10% bleach solution and wiped with fresh paper towels before commencing the next

exposure. Thus, each neonate was given a fresh scent track from a single adult. Each neonate was allotted a time limit of 30 minutes to choose between tunnels before removal. If an individual stayed within the central bucket, data were recorded as a non-movement. We recorded the choice made and total time for completion per neonate.

### *Analysis*

For the dependent variable, “choice”, the outcomes were: traverse the tube lined with adult-scented paper, traverse the tube with no scent (hereafter “blank tube”), and remain in place (N=4). Because most individuals traversed one of the tubes as opposed to remaining in place (see Table 1), the third choice was not included in the final model. Because the data from the first exposure represents the truly naïve neonate experience, the data for Exposure 1 only were analyzed first, and then all data simultaneously for all exposures.

For the analysis of Exposure 1, the question of interest was whether neonates chose the tube with the scented paper or the blank tube. Secondary to this was whether neonates varied in their behavior as a function of their sex (M or F) and the identity of the adult. To address the first question, if tube choice was random, the probability of choosing the scented tube would be 0.5. The appropriate probability is calculated with the summation of exact binomial probabilities:

$$P(k \geq n) = \sum_k^n \frac{n!}{k!(n-k)!} (p^k)(q^{n-k}) \quad (1)$$

where k is the number of individuals that followed the scent track, n is number of individuals that moved, p and q equal 0.5 under  $H_0$ .

The second question was addressed using logistic regression in R (R Core Team, 2014), where the dependent variable was choice (chose scent versus chose blank, coded with

a 1 and 0), and the main effects were sex of the neonate (“sex”: M or F) and identity of the adult (“adult”: mom, male, female). The model for Exposure 1 data was:

$$\text{Choice} = \text{Sex} + \text{Adult} + (\text{Sex} \times \text{Adult}) \quad (2)$$

For the analysis of all data (all three exposures pooled), the question of interest was whether neonates choose the scented or blank tube. Here again, this was tested by calculating the probability that the observed data were significantly different than random given that each neonate had a choice between two tubes. The same formula was used as in equation (2). To analyze additional effects on neonate choice, not only were adult identity and sex of neonate important, but also exposure number (“Exposure”: 1, 2, 3), order of exposure (Treatment: 1, 2, 3), and repeated subjects (ID). These data were analyzed using a generalized linear mixed effects model using ID as a random effect in R (R Core Team, 2014) with the lme4 (Bates et al. 2015) and car (Fox et.al. 2019) packages. Because “Exposure” is a blocking variable, interactions with “Exposure” were not tested. The final model for all data on neonate choice was:

$$\text{Choice} = \text{Sex} + \text{Adult} + (\text{Sex} \times \text{Adult}) + \text{Exposure} + \text{Treatment} + \text{RepeatedID} \quad (3)$$

The other dependent variable analyzed was latency to complete the exposure (time from the end of the 10 minutes of acclimation to completion of movement through a tunnel, defined as the time the tail could no longer be seen in the live-streaming camera). These data were analyzed using a linear mixed effect model with the added effect of “tube choice” (1, 0).

$$\begin{aligned} \text{Latency} = & \text{Sex} + \text{Adult} + (\text{Sex} \times \text{Adult}) + \text{Exposure} \\ & + \text{Treatment} + \text{RepeatedID} + \text{Tube Choice} \end{aligned} \quad (4)$$

## Results

### *Choice: First exposure*

In week 1, all 27 of the neonates were tested with 20 that chose the scented tube, 5 that chose the unscented tube and 2 that reached the experimental time cutoff (Table 2). The probability of this occurring by chance was  $P = 0.002$  (using equation (1) with  $k = 20$ ,  $n = 25$ ,  $p$  and  $q = 0.5$ ). The remaining variables of sex ( $P = 0.845$ ), adult ( $P = 0.498$ ), and interaction of sex\* adult ( $P = 0.352$ ) were not found to be statistically significant (Table 3).

**Table 2. Results of choice experiment.** Choices of each treatment are shown and totaled in the format of [number followed, number did not follow, remain in place]. Treatment refers the adult scent rack used. Exposure refers to the week of the experiment. Shaded rows correspond to Exposure 1 movement numbers and the p-values associated with Exposure 1 and “all-Exposures”.

	TREATMENT 1 (N=9)	TREATMENT 2 (N=9)	TREATMENT 3 (N=9)		
	<u>Day 1</u>	<u>Day 2</u>	<u>Day 3</u>	Total	
EXPOSURE 1	[6,3,0]	[6,1,2]	[8,1,0]	[20,5,2]	<b>P = 0.002</b>
EXPOSURE 2	[4,4,1]	[6,2,1]	[5,4,0]	[15,10,]	
EXPOSURE 3	[7,2,0]	[7,2,0]	[4,5,0]	[18,9,0]	
<b>TOTAL</b>	[17,9,1]	[19,5,3]	[17,10,0]	[53,24,]	<b>P = 0.0006</b>

### *Choice: All Exposures*

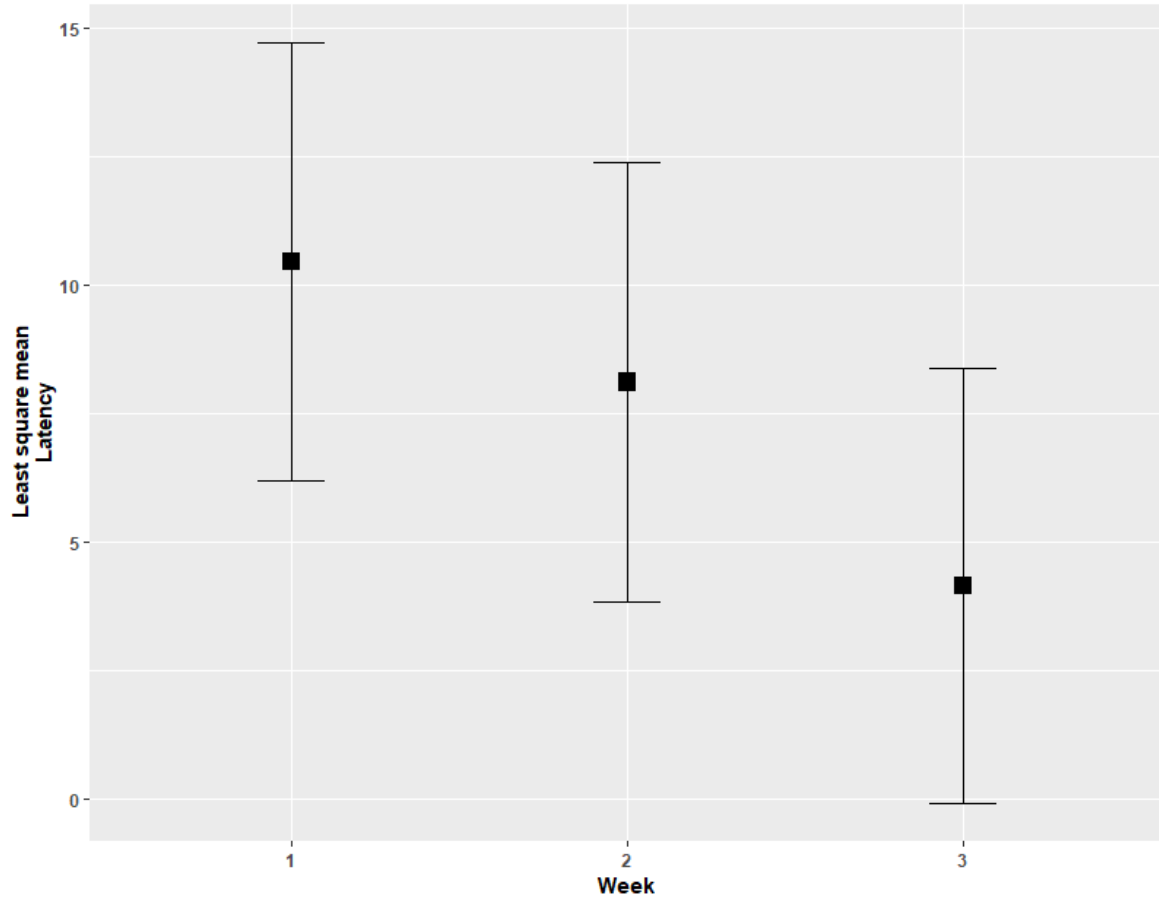
For the pooled data for all exposures, 81 total exposures with 53 that chose the scented tube, 24 that chose the unscented tube and 4 that reach the experimental cutoff. I again used equation (1), with  $k = 53$ ,  $n = 77$ ,  $p$  and  $q = 0.5$ . Across all exposures, similar to exposure 1, 53 of 77 neonates followed the scented tube. The probability of this occurring by chance was  $P = 0.0006$ . The remaining variables of sex ( $P = 0.623$ ), adult ( $P = 0.127$ ), exposure ( $P = 0.326$ ), treatment ( $P = 0.292$ ), and interaction of sex \* adult ( $P = 0.572$ ) were not found to be statistically significant (Table 3).

**Table 3. Analysis of variance for exposure 1, all exposures pooled, and latency to complete an exposure.** Choice is the decision that the neonate made when exposed to a scent track. Latency is the time between exposure and completion. The results of each variable are listed as the F-values, degrees of freedom, and P-values. Variables with  $p < 0.05$  in bold.

Source of Variation	Exposure 1 Choice	All Exposures Choice	All Exposures Latency
<b>Sex</b>			
<i>F (df<sub>n</sub>, df<sub>d</sub>)</i>	0.039(1,19)	0.247(1,24)	0.533(1,24)
Pr > F	0.845	0.623	0.472
<b>Adult</b>			
<i>F (df<sub>n</sub>, df<sub>d</sub>)</i>	0.721(2,19)	2.161(2,45)	0.507(2,44)
Pr > F	0.498	0.127	0.605
<b>Sex × Adult</b>			
<i>F (df<sub>n</sub>, df<sub>d</sub>)</i>	1.103(2,19)	0.564(2,45)	0.073(2,44)
Pr > F	0.352	0.572	0.929
<b>Exposure</b>			
	---		
<i>F (df<sub>n</sub>, df<sub>d</sub>)</i>		0.984(1,45)	<b>4.322(1,44)</b>
Pr > F		0.326	<b>0.043</b>
<b>Treatment</b>			
	---		
<i>F (df<sub>n</sub>, df<sub>d</sub>)</i>		1.160(2,45)	2.858(1,24)
Pr > F		0.292	0.103
<b>Choice</b>			
	---	---	0.088(1,44)
<i>F (df<sub>n</sub>, df<sub>d</sub>)</i>			0.767
Pr > F			

*Latency: All Exposures*

For the latency data all 81 exposures were used. Latency to complete the tube choice was affected only by “Exposure” ( $P = 0.043$ ); the remaining variables of choice ( $P = 0.76$ , treatment ( $P = 0.103$ ), sex ( $P = 0.472$ ), adult ( $P = 0.605$ ), and interaction of sex\* adult ( $P = 0.929$ ) were not found to be statistically significant (Table 3). The directionality of the LSmeans (Figure 2) over the three weeks suggests a decrease in time to completion with each exposure to the apparatus.



**Figure 2. Change in latency (s) with each subsequent exposure.** The graph shows the average in competition times for neonates for weeks 1-3. Results for Week 1 were 10.45 min. with a S.E.  $\pm$  1.73, Week 2 were 8.10 min. with a S.E.  $\pm$  1.74, and Week 3 were 4.14 min. with a S.E.  $\pm$  1.72. The average completion time decreased each week suggesting the neonates are learning with each exposure to the apparatus. Boxes indicate the LS mean. Error bars indicate the 95% confidence interval of the LS means. (Tukey-adjusted comparisons).

### *Preferential Following*

The tabulated number of individuals that followed the adult out of the number individuals that were exposed to the adult scent did not show a preference for the sex of the adult or the assumed relatedness (Table 4). All 27 neonates were exposed to each adult for a total of 81 exposures. For the sex of adult preference, the pooled number of neonates that followed the female adults was 35 chose the scented tube, 17 chose the unscented tube, and 2 reached the experimental cutoff. For the males, 19 chose the scented tube, 6 chose the



**Table 4. Preference results.** The table shows the number of neonates that followed each adult based on sex of the neonates. There is no strong preference for a specific adult or sex of the adult. The total row is the total number of neonates that followed of the total number of neonates exposed to the adult scent.

	<b>MOTHER</b>	<b>FEMALE</b>	<b>MALE</b>
<b>MALES</b>	6	10	7
<b>FEMALES</b>	12	12	12
<b>TOTAL</b>	14/26	21/26	19/25

unscented tube, and 2 reached the experimental cutoff. For the preference for related adults, among the pooled females and males 40 chose the scented tube, 11 chose the unscented tube, and 3 reached the experimental cutoff. For the preference for a related adult, the females and males were pooled resulting in 40 chose the scented tube, 11 chose the unscented tube, and 3 reached the experimental cutoff. And the results of the exposures to a related adult were 14 chose the scented tube, 12 chose the unscented tube, and 1 reached the experimental cutoff.

## **Discussion**

An individual's scent tracking behaviors could mean the difference between life and death during a time period assumed to have low survival rates (Pike et al. 2008). *Thamnophis* species can differentiate between conspecific and congeneric individual scents (Costanzo 1989; Ford et al. 1984, 1993; Graves et.al. 1988; Heller et.al 1981; Lemaster et al. 2001), recognize predator scent tracks (Placyk et.al. 2011; Herzog Jr. et.al. 1989), differentiate and track prey items et.al. 1991), and males track females for reproductive activities et.al. 2001; Shine et al. 2004; Mason et.al. 2010). The trailing behaviors tested in this research were hypothesized as a method for neonate *T. sirtalis* to use the scent tracks passively left by conspecific adults to navigate their habitat. The major findings indicate that neonates do follow adults regardless of the sex of the neonate, or the identity of the adult, and with repeated exposures the neonate's completion time decreased (Table 3).

The results from “first choice only” and “all exposures pooled” supports our hypothesis that neonatal snakes have a propensity to follow adult scent tracks. During the first week of the experiment, neonates displayed a higher following rate that may indicate a dependency on conspecific tracks in novel environments. The propensity of neonates to follow a previous conspecific in our experiment provides further support of previous investigations by Ford et al. 1984, and Halpin 1990. The results of my study and previous studies (Costanzo 1989; Ford et al. 1984; Halpin 1990; LeMaster et al. 2001) suggest a strong ecological and biological dependency of on conspecific tracking abilities.

Additionally, we did not find any evidence to support a preference for a specific adult. Similar trailing experiments have been conducted on adults and concluded that any scent preference is only present in males during the breeding season (Lemaster et al. 2001). The lack of a preference was not overly surprising as the subjects were not sexually mature, and the presence of reproductive pheromones were not likely present in the female tracks. In addition, in the analysis of the latency data, we found that the completion time decreased with each exposure to the apparatus. This result could simply be a learned behavior from previous exposures, or the choice of between a known and unknown outcome, or a combination of both explanations.

Our study was limited by small sample size, with only a single litter of 27 individuals and a single unrelated adult female and adult male due to reduced encounters during field sampling periods. We are unsure of the reasons for such a low encounter rate, but one possibility could be that the large amount of snow melt and rains in late winter 2017 could have led to increased winter mortality. Further studies need to be conducted to estimate both the snake and prey populations to determine the natural ebb and flow of the population.

While the results of our research suggest neonates trail adults, future studies are needed with larger sample sizes of neonates from multiple mothers and the determined degree of relatedness among all adults. An increased sample size would provide more naïve neonates eliminating the possibility of preferential tube choice caused by repeated exposures. An understanding of the degrees of relatedness of the individuals would elucidate any preference or avoidance of closely related individuals. Another limitation imposed by small sample size was the inability to run control experiments with naïve neonates to investigate any effect of waxed paper on trailing behaviors. If future studies reproduce the findings of our data with a larger sample size, the next step from laboratory experimentation should be to perform field surveys of neonates and adults. With current tracking device size and battery limitations, the tracking of neonate garter snakes is difficult. However, tracking migrations of adults with the aid of camera traps and drift fences could support the existence of this behavior in nature.

### **Literature Cited**

- Aleksiuk M. 1976. Reptilian Hibernation: Evidence of adaptive strategies in *Thamnophis sirtalis parietalis*. *Copeia*. 1:170.
- Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 1(67):1–48.
- Brown WS, Kéry M, Hines JE, Brown WS, Ke M, Hines JE. 2007. Survival of timber rattlesnakes (*Crotalus horridus*) estimated by capture-recapture models in relation to age, sex, color morph, time, and birthplace. *Copeia*. 3:656–671.
- Brown WS, Maclean FM. 1983. Conspecific scent-trailing by newborn timber rattlesnakes, *Crotalus horridus*. *Herpetologica*. 39(4):430–436.
- Clark RW, Brown WS, Stechert R, Greene HW. 2012. Cryptic sociality in rattlesnakes (*Crotalus horridus*) detected by kinship analysis. *Biol Lett*. 8(4):523–525.
- Cobb VA, Jeffrey Green J, Worrall T and Pruitt J. 2005. Initial den location behavior in a litter of neonate *Crotalus horridus* (Timber Rattlesnakes). *SE Nat*. 4(4):723–730.

- Costanzo JP. 1989. Conspecific scent trailing by garter snakes (*Thamnophis sirtalis*) during autumn further evidence for use of pheromones in den location. *J Chemical Ecol.* 15(11):2531–2538.
- Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude thermal safety margin. *PNAS.* 105(18):6688–6672.
- Diller L V, Wallace RL. 2002. Growth, reproduction, and survival in a population of *Crotalus viridis oregonus* in North Central Idaho. *Herpetol Monogr.* 16:26–45.
- Ford BN, Burghardt MG. 1993. Perceptual mechanisms and the behavioral ecology of snakes. In: RA Seigel, JT Collins, *Snakes: Ecology and Behavior.* pp. 117–164.
- Ford NB, Schofield CW. 1984. Species specificity of sex pheromone trails in the plains garter snake, *Thamnophis radix*. *Herpetologica.* 40(1):51–55.
- Fox J, Weisberg S. 2019. *An R companion to applied regression.* Third edition. Thousand Oaks, CA: Sage.
- Gangloff EJ, Holden KG, Telemeco RS, Baumgard LH, Bronikowski AM. 2016. Hormonal and metabolic responses to upper temperature extremes in divergent life-history ecotypes of a garter snake. *J Exp Biol.* 219(18):2944–2954.
- Gomez ML, Gregory PT, Larsen KW. 2002. Habitat use and movement patterns of the northern pacific rattlesnake (*Crotalus o. oregonus*) in British Columbia. Master of Science Thesis. British Columbia, Can. University of Victoria.
- Graves BM, Halpern M. 1988. Neonate plains garter snakes (*Thamnophis radix*) are attracted to conspecific skin extracts. *J Comp. Psychol.* 102(3):251–253.
- Halpin ZT. 1990. Responses of juvenile eastern garter snakes (*Thamnophis sirtalis sirtalis*) to own, conspecific and clean odors. *Copeia.* 4:1157–1160.
- Heller S, Halpern M. 1981. Laboratory observations on conspecific and congeneric scent trailing in garter snakes (*Thamnophis*). *Behav Neural Biol.* 33(3):372–377.
- Herzog Jr HA, Burghardt GM. 1989. Stimulus control of antipredator behavior in newborn and juvenile garter snakes (*Thamnophis*). *J Comp Psychol.* 103(3):233–242.
- Hileman ET, Bradke DR, Delaney DM, King RB. 2015. Protection by association: Implications of scent trailing in neonate eastern massasauga (*Sistrurus catenatus*). *Herpetol Conserv Biol.* 10(2):654–660.
- Huey RB, Peterson CR, Arnold SJ, Porter WP. 1989. Hot rocks and not-so-hot rocks: Retreat-site selection by garter snakes and its thermal consequences. *Ecology.* 70(4):931–944.

- Larsen KW. 1987. Movements and behavior of migratory garter snakes, *Thamnophis sirtalis*. Can J Zool. 65(Brown 1960):2241–2247.
- Lebreton AJ, Burnham KP, Clobert J, David R. 1992. Modeling survival and testing biological hypotheses using marked animals: A unified approach with case studies. Ecol Monogr. 62(1):67–118.
- LeMaster MP, Mason RT. 2001. Evidence for a female sex pheromone mediating male trailing behavior in the red-sided garter snake, *Thamnophis sirtalis parietalis*. Chemoecology. 11(3):149–152.
- Lemaster MP, Moore IT, Mason RT. 2001. Conspecific trailing behaviour of red-sided garter snakes, *Thamnophis sirtalis parietalis*, in the natural environment. Anim Behav. 61(4):827–833. doi:10.
- Mason RT, Chivers DP, Mathis A, Blaustein AR. 1998. Bioassay methods for amphibians and reptiles. In: Myers, KF, Millar, JG, Methods in Chemical Ecology, Vol. 2 (pp. 271–325): Bioassay Methods. Boston, MA: Springer.
- Mason RT, Parker MR. 2010. Social behavior and pheromonal communication in reptiles. J Comp Physiol. 196:729–749.
- Pike DA, Pizzatto L, Pike BA, Shine R. 2008. Estimating survival rates of uncatchable animals: The myth of high juvenile mortality in reptiles. Ecology. 89(3):607–611.
- Placyk JS, Burghardt GM. 2011. Evolutionary persistence of chemically elicited ophiophagous antipredator responses in garter snakes (*Thamnophis sirtalis*). J Comp Psychol. 125(2):134–142.
- R Core Team. 2014. R: A Language and Environment for Statistical Computing. Vienna, At: R Foundation for Statistical Computing.
- Seigel RA, Collins JT. 1993. Snakes : Ecology and Behavior. New York: McGraw-Hill.
- Seigel RA, Collins JT, Novak SS. 1993. Snakes : Ecology and Evolutionary Biology. New York, NY: McGraw-Hill.
- Shine R. 2005. Life-history evolution in reptiles. Annu Rev Ecol Evol Syst. 36(1):23–46.
- Shine R, Elphick MJ, Harlow PS, Moore IT, LeMaster MP, Mason RT, Maso. 2001. Movements, mating, and dispersal of red-sided garter snakes (*Thamnophis sirtalis parietalis*) from a communal den in Manitoba. Copeia. 1(1):82–91.
- Shine R, Lemaster M, Wall M, Langkilde T, Mason R. 2004. Why did the snake cross the road? Effects of roads on movement and location of mates by garter snakes (*Thamnophis sirtalis parietalis*). Ecol Soc. 9(1):9.

- Telemeco RS, Gangloff EJ, Cordero GA, Polich RL, Bronikowski AM, Janzen FJ. 2017. Physiology at near-critical temperatures, but not critical limits, varies between two lizard species that partition the thermal environment. *J Anim Ecol.* 86(6):1510–1522.
- Wilson B. 2015. PiKrellCam. <https://billw2.github.io/pikrellcam/pikrellcam.html>.
- Yeager CP, Burghardt GM. 1991. Effect of food competition on aggregation: Evidence for social recognition in the plains garter snake (*Thamnophis radix*). *J Comp Psychol*, 105(4):380–386.

## CHAPTER 3. GENERAL CONCLUSIONS

### Summary and Discussion

I started this research with the goal of learning more about the neonatal/juvenile population at a long-time study site in central Iowa. I attempted to achieve this goal through four different studies to determine various aspects of the population. In the first study the goal was to establish if the target species, *Thamnophis sirtalis*, was using a pit as an overwintering site. The pit is approximately 3-4 meters deep and 15 meters in diameter and is the remnant of construction on the property that has been filled in with dead and decaying wood debris. I hypothesized that *T. sirtalis* utilized the pit as a hibernaculum to survive the winter months. I used a combination of drift fencing and camera traps to observe the animals entering and exiting the pit.

The goal of the second study was to observe the thermal properties of the microhabitat beneath cover objects spanning three habitat types: prairie, woodland edge, and woodland. I expected to identify a range of preferable thermal conditions from which I could make a thermal spatial map that would allow for targeted sampling efforts. I used a combination of cover objects, thermal data loggers, copper thermal models, and infrared temperature readings.

The third study was a continuation of a long-term data set involving opportunistic Capture-Mark-Recapture (CMR) studies from 2012-2018. The goal was to build a database of encounters for individuals in the *T. sirtalis* population to estimate the population size, growth rate, and adult population survival. The hypothesis was this site was an example of a stable population despite the presence of human development. The CMR study design was

simply the capture and marking each individual encountered with a unique identifier and recording the subsequent encounters with previously identified individuals.

The fourth and final study was a behavioral study conducted in the laboratory at Iowa State University. The goal was to investigate the propensity of neonatal *T. sirtalis* to follow adult scent trails. The expected outcomes were that when presented with a conspecific scent trail, the neonates would choose to follow an adult, or they would be repelled by the scent.

In the first study, the drift fence was constructed from 46 cm tall aluminum flashing encircled the 15 m pit. During the installation of the drift fence, I identified several trails where 8 camera traps were also installed. These camera traps were constructed from inverted 5-gallon buckets with trail cameras pointed directly at the ground. Each bucket had openings cut in opposite sides to allow animals to pass under the camera lens. The design relied on detecting body heat to trigger the shutter in the camera. The buckets were intended to create an environment which would amplify the thermal difference between ambient temperatures inside and outside the bucket (presumably the entering snake would have a body temperature near this ambient outside temperature). This method was successful in recording 90 sighting events but lacked the ability to successfully identify individuals. I believe the absence of the neonates moving through my camera traps may be for multiple reasons. The simplest explanation to be that neonates are not present at this hibernaculum site, meaning that they do not overwinter in the same location as adults. However, I believe that neonates may need a much smaller range to forage for prey than adults. Given the size of the pit they would have ample foraging opportunities and thus would not need to move outside the hibernaculum at this site. Without the ability to identify specific individuals, it was impossible to identify any information about sex ratio or population size. Sex ratio in the population would allow an



estimation of sex-specific population growth that would depend on number of mature females present given the knowledge of alternating breeding years and known clutch size averages. Estimates of the population size when analyzed with sex ratio, snout-to-ventral-length, and population growth would have allowed me to determine the size of the juvenile population present at the site. Pairing the camera trap with another method of individual recognition such as a visible identifier or subcutaneous radio tag along with a recorder would allow for a complete population analysis. Without an accurate analysis, I could not develop or recommend any habitat or population management plans.

My second goal of profiling microhabitat preferences for these snakes began with creating a thermal database for all captures during 2018 sampling periods with covariates of beginning and ending temperatures, humidity, and times. In addition to the site-specific thermal data, each cover object was broken down into 8 thermal quadrants. 4 of the quadrants were the surface of the cover object exposed to the environment and 4 of the quadrants were below the cover object in direct contact with the soil. I also placed 6 dual-channel and 4 single-channel thermal data loggers at various points of interest across the study site. The 6 dual-channel data loggers were attached to a 91.5 cm x 0.95 cm fiberglass rod that was vertically staked in the ground, with one thermal probe placed 2.5 cm and the other probe placed at the 91.5 cm above ground level. The goals were to record the variance in temperatures between full sun exposure and ground level with vegetative cover and to record the thermal differences between prairie, woodland edge, and woodland habitats. I could then use the thermal reading from each encounter to locate areas that have a higher probability of encounters. The 4 single-channel loggers were used to model the thermal variance above and below a cover board in a tallgrass prairie and a cover board in a wooded environment. Each

of the thermal probes was inserted into a small copper tube and painted to match the thermal reflectiveness of my target species, *T. sirtalis* (Seigel et al. 1993). While I was successful in gaining thermal data, ultimately the low capture rate under a cover object did not allow me to quantify a thermal preference. The thermal data provided average temperature differences (Table 1) between the months of June, July, and August for each of the 3 habitat types (Figures 1-3). Increasing the number of thermal loggers, increasing the sampling area, and adding radio telemetry with body temperature logging capabilities could provide enough data for identifying targeted sampling periods. These targeted sampling periods could then be employed by other researchers to maximize field efforts and capture rates.

My third goal, estimating population size and growth utilized the long-term Capture-Mark-Recapture data dating back to 2012 (CMR). Doing this effectively requires estimating survival and capture probabilities using Program MARK (White and Burnham 1999). MARK provides adult survival probabilities from the capture history and the capture probabilities. The overall annual adult survival probability estimates the surviving members of the population for a given year. The population growth rate is based on the estimated number of reproductively active females in a given year combined with the estimated number of offspring per female.

After adding the capture history into MARK, I began by building sex, encounter year, and sex by time interaction models using open population Cormack-Jolly-Seber (CJS) parameters to estimate survival and capture probabilities. The open population type of analysis assumes that a population is changing over time through events such as births, deaths, immigrations, and emigrations. Although studies may often violate an assumption to a small degree, when studies severely deviate from the assumptions the model does not

accurately reflect the population. This is due to the lack of subsequent encounters compounding over frequent sampling periods. MARK's ability to provide accurate, biologically significant estimates relies on the probability of encountering an individual for the first time, the probability that individuals survive until the next sampling event, and the compounded probabilities of the individual surviving between sampling events and the probability of encountering during a sampling event.

The relationship between recapture rates and an accurate estimate of population analysis is often overlooked. For example, if the capture history of an individual is only a single encounter (initial) but then never captured again, the survival probability is not estimable because the individual cannot be ruled out as either dead or alive or present or absent from the sampling area. Each unsuccessful recapture event of a single individual decreases the estimate of survival of the entire population. Thus, until the individual is recaptured the probabilities remain undefined due to the presences of only a zero in the denominator.

MARK uses the recaptures to calculate the survival probability and the capture probabilities between 3-time intervals. This means that without at least a two captures for an individual, the survival probability cannot be estimated reliably without other covariates (Lebreton et al. 1992). In the entire data set (2012 to 2018), only 11% of the individuals were captured for a second time and only a single individual with a capture history greater than 3 encounters. The inability to accurately estimate the survival probabilities of this study is due to lack of recaptures for individuals and the haphazard encounters.

The results of the MARK survival probabilities are further evidence that CMR studies on secretive or cryptic species will most likely require more than simply transects, cover-

board surveys and/or multiple sites. Combining the CMR with other sampling techniques like transects and drift fences could increase the rate of recapture allowing for a more accurate estimation of survival and capture probabilities and population size estimates.

An ideal sampling design would be the use of combining systematic transect sampling with PIT tags and reader/recorders surrounding the sampling area. The transects would provide a method of increasing the number of identifiable individuals and the PIT tags would provide a constant accounting for known individuals within the transect by monitoring those whom leave or enter the transect. Therefore, as animals are implanted with the PIT tags are added to the database with the transects, the PIT tag reader/recorders will be capturing the individuals that are moving into and out of the transect. This could increase the overall sample size and improve the recapture rates, thus improving the survival, population size, and population growth estimates.

Finally, my fourth set of hypotheses involved determining whether juvenile snakes may utilize the scent trails of adults in making movement decisions, especially related to hibernation. Snakes rely on acquiring and processing tactile and chemical signals to make decisions that impact individual survival. Since snakes live in close proximity to the ground, they must rely on senses other than sight and sound alone to navigate their habitat. Snakes rely on acquiring and processing tactile and chemical signals to make decisions that affect individual survival.

My thesis research provided strong evidence for the importance of chemical cues in juvenile decision making. The results of my experiment revealed that juveniles have a propensity to follow adult scent trails in a novel test environment and make faster decisions

after repeated exposures to the test environment. My experiment did not elucidate a sex preference among the test subjects, perhaps due to the small sample size.

This research has provided a foundation for future endeavors concerning juvenile scent trailing behaviors and their real-world implications. More research is needed on the scent trailing preferences of neonates and juveniles with regards to seasonality. Perhaps there is a seasonal effect on the preference to follow a conspecific before maturation. Could the immature snakes be repulsed in the Spring to increase dispersal, limit resource competition, or provide safety from physical harm during Spring emergence mating activities? Could the immature snakes be attracted in the Fall to conspecifics in order to locate suitable winter survival locations?

**REFERENCES**

- Lebreton AJ, Burnham KP, Clobert J, David R. 1992. Modeling survival and testing biological hypotheses using marked animals: A unified approach with case studies. *Ecol Monogr.* 62(1):67–118.
- Seigel RA, Collins JT, Novak SS. 1993. *Snakes: Ecology and Evolutionary Biology*. New York: McGraw-Hill.
- White GC, Burnham KP. 1999. Program mark: Survival estimation from populations of marked animals. *Bird Study.* 46:S120–S139.