

EFFECT OF PREGNANCY ON BODY TEMPERATURE REGULATION AND
HABITAT USE OF NORTHERN WATERSNAKES (*NERODIA SIPEDON*) IN
TENNESSEE

by

Alexis M. Hamous-Miller

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Biology

Middle Tennessee State University

December 2022

Thesis Committee:

Dr. Vincent Cobb, Chair

Dr. Matthew Klukowski

Dr. Donald Walker

ACKNOWLEDGMENTS

I would like to first thank Dr. Cobb for allowing me to join his lab and his support throughout my time at MTSU. Secondly, I would like to recognize my grandparents for their unending support and unconditional love. My husband for being my rock, helping us move cross country to live in a camper with three dogs and his willingness to learn how to catch snakes without fear. Dr. Walker and Alex Romer for their patience and guidance throughout the project and more specifically with statistics. Dr. Klukowski for his support throughout my project. My three URECA undergraduate students (Kayla Moores, Skylar Carson-Reynolds, and Molly Cooper) for all their help catching snakes and collecting data. Finally, all my fellow graduate students that made the last couple of years a little more fun and pushed me through to the finish line.

ABSTRACT

Snakes of several species select and maintain higher and less variable body temperatures (T_b s) during pregnancy by means of behavioral thermoregulation. The leading hypothesis for this behavior is that the rate of embryonic development is facilitated by warmer temperatures, shortening gestation time, and there are optimal temperature ranges for successful embryonic development which are typically warmer than baseline body temperatures. Therefore, maintaining a warm and relatively constant body temperature could be more beneficial than costly to a reproductive snake. Body temperature selection can be accomplished through behavioral actions such as modifying body postures, choosing different habitats and moving throughout the environment. To test this hypothesis, in 2021 and 2022 I implanted reproductive and non-reproductive female *Nerodia sipedon* from middle Tennessee with radio transmitters and temperature loggers to continuously record their body temperatures throughout the gestation season. With a sample size of 12 female snakes (7 reproductive, 5 non-reproductive) and a total of 7440 T_b s (from 7 July – 14 August for 2021 and 2022), daily (24-hour) mean T_b was 27.1 ± 0.03 °C. Non-reproductive snakes overall 24-hour mean T_b (27.0 ± 0.05 °C) was the same as that of reproductive snakes (27.2 ± 0.04 °C). After running a generalized additive mixed model with body temperature as the response variable and pregnancy status as the main predictor variable and accounting for time of day (in hours), air temperature, water temperature, month, snake ID (individual snakes) and site as random variables, pregnancy appeared to have no effect on body temperature selection. Because habitat use and snake movement can influence body temperature selection, I also collected data on movements and habitat use. Reproductive and non-reproductive females had similar daily

movements, generally moving less than 50 m between each telemetric location. However, snakes did show differences in habitat use with reproductive snakes predominately choosing rocks out of water and forbs while non-reproductive females chose rocks within the river or close to the water. Comparison of this study to others suggests the possibility that *N. sipedon* may exhibit thermal plasticity in body temperature selection based on geographic variation and that pregnant *N. sipedon* in warmer climates may not need to thermoregulate as strongly to maintain body temperatures at appropriate levels for embryonic development.

TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	ii
ABSTRACT.....	iii
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
Introduction.....	1
Methods.....	7
Study Site.....	8
Transmitter and Implantation.....	9
Environmental Measurements.....	10
Habitat Analysis.....	11
Data Analysis.....	11
Results.....	14
<i>Temperature Analysis</i>	14
<i>Environmental Temperatures</i>	15
<i>Habitat Analysis</i>	16
<i>Movement Analysis</i>	17
Discussion.....	18
Literature Cited.....	22

APPENDICES.....	30
APPENDIX A: TABLES.....	30
APPENDIX B: FIGURES.....	36
APPENDIX C: IACUC APPROVAL FORM.....	47

LIST OF TABLES

Table 1. <i>Nerodia sipedon</i> snout vent length (SVL), weight (g) and reproductive status..	31
Table 2. Body temperatures (mean $\pm$ SE) of reproductive ($n = 4344$ T_{bs}) and non-reproductive ($n = 3096$ T_{bs}) female <i>Nerodia sipedon</i> during July and August of 2021 and 2022 combined for 24 hr., daytime (07:00-20:00) and nighttime (21:00-06:00).....	32
Table 3. Habitat use of reproductive and non-reproductive female <i>Nerodia sipedon</i> based on daily relocations of snakes for both 2021 and 2022.....	33
Table 4. Compositional analysis of relocation sites (proportionally) for reproductive and non-reproductive female <i>Nerodia sipedon</i> for both 2021 and 2022.....	34
Table 5. Activity range size estimates using minimum convex polygons for reproductive and non-reproductive female <i>Nerodia sipedon</i> during 2021 and 2022.....	35

LIST OF FIGURES

Figure 1. Maps of study site locations for <i>Nerodia sipedon</i> in 2021 and 2022.....	37
Figure 2. Maps of study sites with relocation sites (indicated by red dots) with minimum convex polygons to estimate activity range size exhibited by <i>Nerodia sipedon</i> . A through D are along the Stones River (Rutherford Co.).....	38
Figure 3. Maps of study sites with relocation sites (indicated by red dots) with minimum convex polygons to estimate activity range size exhibited by <i>Nerodia sipedon</i> . E and F are along the Stones River (Rutherford Co.), G is Barton Creek (Wilson Co.).....	39
Figure 4. Distribution of reproductive ($n = 4344$) and non-reproductive ($n = 3096$) female <i>N. sipedon</i> body temperatures over the pregnancy period, not proportional.....	40
Figure 5. Reproductive and non-reproductive female <i>Nerodia sipedon</i> body temperatures over the pregnancy period smoothed with the GAMM function to better fit the body temperatures to a normalized curve.....	41
Figure 6. Air and water temperatures for 2021 and 2022 during the pregnancy period for <i>Nerodia sipedon</i> . 2021 temperatures are represented by grey solid line (air) and grey dotted line (water). 2022 temperatures are represented by black solid line (air) and black dotted line (water). The overall average air temperature for 2021 was 24.7°C and 2022 was 25.3 °C. The overall average water temperature for 2021 was 24.2°C and 2022 was 25.4°C.....	42
Figure 7. Daily mean body temperatures for reproductive (black solid line), non-reproductive (dark grey solid line) female <i>Nerodia sipedon</i> and operative model maximum and minimum temperatures (dotted light grey lines) on 4 August 2021 (A) and 4 August 2022 (B).....	43
Figure 8. Proportion of reproductive and non-reproductive female <i>Nerodia sipedon</i> daily movement over the pregnancy period for 2021 and 2022.....	44

Figure 9. Seasonal mean body temperatures over a 24-hour period for all *Nerodia sipedon* during the pregnancy period. Reproductive females are indicated by the black line and non-reproductive females are indicated by the grey line.....45

INTRODUCTION

Body temperature has a profound influence on the physiology and ecology of ectothermic animals (Huey 1982). Physiological processes ranging from neural responses of stimuli to the rate of embryonic development are temperature dependent in ectothermic animals and, ultimately, can alter their life history (Fox 1948, Huey 1982, Ingermann et al. 1991, Shine and Harlow 1993, Stevenson et al. 1985). Therefore, the majority of life history traits in ectothermic animals are strongly linked to maintenance of appropriate body temperatures (Huey 1982, Stevenson et al. 1985). Behavioral thermoregulation is a means for ectothermic animals to use the temperatures available in their environment to actively maintain a range of body temperatures needed for physiological process (Bogert 1948). Body postures, shuttling, moving up and down on structures (e.g., vegetation), finding warmer or cooler retreats and basking are all examples of behavioral thermoregulation (Bogert 1948, Peterson et al. 1993).

Snakes are one such taxa that have received considerable attention in the literature regarding behavioral thermoregulation. Such behavior may be particularly important for adult females that are viviparous and maintain their developing young throughout pregnancy. Viviparous snakes, unlike oviparous snakes, have an advantage of almost completely controlling the developmental environment for the young develop including the temperature of embryonic development (Shine and Bull 1979). In viviparous snakes, the mother's body temperature can influence the development of the offspring. For instance, there is increased delivery of oxygen to the embryos due to the mother's hemoglobin having a lower affinity of oxygen in viviparous garter snakes when mothers

thermoregulate at warmer temperatures (Ingermann et al. 1991). Developmental errors in vertebral number and scutellation were found to decrease as maternal body temperature increased (Fox 1948, Osgood 1978, Peterson et al. 1993). Additionally, as body temperature increases, gestation time decreases, indicating the importance of maintaining an increased body temperature (Peterson et al. 1993). Consequently, it is not surprising that prior studies of several viviparous snake species have revealed that reproductive and non-reproductive snakes often thermoregulate differently. Reproductive females thermoregulate differently and more precisely than non-reproductive females for several species: *Nerodia rhombifer* [Diamondback Watersnakes] (Tu and Hutchison 1995), *Crotalus horridus* [Timber Rattlesnakes] (Gardener-Santana and Beaupre 2009), *Crotalus viridis* [Western Rattlesnakes] (Charland and Gregory 1990), *Crotalus v. viridis* [Prairie Rattlesnakes] (Graves and Duvall 1993), *Thamnophis sirtalis* [Common Garter Snakes] (Charland 1995), *Thamnophis elegans* [Western Terrestrial Garter Snakes] (Charland 1995), *Natrix natrix* [Grass Snakes] (Isaac and Gregory 2006) and *Nerodia sipedon* [Northern Watersnakes] (Brown and Weatherhead 2000). This thermoregulatory difference is typically acquired by gravid snakes actively seeking environmental features that allow for warmer basking temperatures which may facilitate embryonic development (Charland and Gregory 1990, Harvey and Weatherhead 2010, Osgood 1978).

Maintaining higher body temperatures has been found to be common in reproductive female snakes and this may result from the selection of different habitats than non-reproductive females (Crane and Greene 2008, Dorcas and Peterson 1998, Graves and Duvall 1993, Roth and Greene 2006, Sprague and Bateman 2018). A leading hypothesis for reproductive female snakes to seek warmer habitats and maintain higher

body temperatures is that the rate of embryonic development is facilitated by warmer temperatures (Osgood 1978, Shine and Harlow 1993) and that there are a range of temperatures for successful embryonic development, thereby shortening the gestation period (Arnold and Peterson 2002). Therefore, maintaining a near-optimal and relatively constant body temperature is more beneficial than costly to a reproductive female (Charland and Gregory 1990, Shine and Harlow 1993). To thermoregulate in this manner, snakes must select for habitats and environmental features with suitable thermal conditions and low risk of predation (Huey et al. 1989). Thus, one would predict that the gestation sites would have adequate refuge to lessen the likelihood of being preyed upon and allow for active thermoregulation without basking, such as hot rocks (Huey 1982).

Suitable environmental conditions usually allow snakes to select a range of temperatures at which the animal can function most efficiently (Huey et al. 1989, Stevenson et al. 1985). Climate and latitude can potentially expose individuals to temperatures that can reduce their thermoregulatory opportunities in northern temperate zones, especially during pregnancy, unlike in tropical zones (Charland 1995, Charland and Gregory 1990, Gardener-Santana and Beaupre 2009, Hughes et al. 2019, Isaac and Gregory 2006, Shine and Madsen 1996). Active thermoregulation involves movement within the environment to actively seek warmer or cooler microhabitats (Huey 1982, Peterson 1987, Robertson and Weatherhead 1991).

Habitat choices, specifically microhabitat, can influence the temperatures available for thermoregulation (Blouin-Demers and Weatherhead 2002, Graves and Duvall 1993, Harvey and Weatherhead 2010, Huey et al. 1989). Microhabitat preference may include

substrate type, frequency of visitation, movement within an area, activity within the area, type of retreats available, canopy cover, surface temperatures and water temperatures (Blouin-Demers and Weatherhead 2002, Harvey and Weatherhead 2010). While these features are used to assess the habitat that the individuals are using, available environmental temperatures are also important to consider to determine if the snakes are thermally restricted due to environmental temperatures (Peterson et al. 1993, Tracy 1982). A common approach to evaluating the thermal conditions of the environment for snakes is the use of operative temperature models (Bakken and Gates 1975, Peterson et al. 1993). Operative temperature models, when painted a color with similar reflectiveness of the snake's scales, functionally represent the true environmental temperatures experienced by the snake (Peterson et al. 1993).

The goal of this study is to test the effect of pregnancy on thermoregulation and habitat selection by using *Nerodia sipedon* as a model. *Nerodia* have received considerable attention in the literature (Brown and Weatherhead 2000, Greene et al. 1999, Lutterschmidt and Reinert 1990, Osgood 1970, Pattishall and Cundall 2008, Roth and Greene 2006, Winne and Keck 2005) partly because they are usually abundant, several species have large geographic ranges, and they are usually top-level predators in the waterways they occupy (Gibbons and Dorcas 2004). They have been model organisms to understand things such as anatomy, morphology, behavior, disease, ecology and distribution, reproduction, and taxonomy (Walley et al. 2012).

Although *Nerodia sipedon* is a well-documented species, few studies have measured body temperatures (T_b) of reproductive females (Brown and Weatherhead 2000, Rowe et al 2022). Brown and Weatherhead (2000) investigated the thermal ecology of Northern

Watersnakes in a telemetry study of 38 individuals over three years in Ontario, Canada. Their findings support the hypothesis that reproductive females thermoregulate differently than non-reproductive females. Reproductive female *N. sipedon* were found to maintain warmer and more precise body temperatures than non-reproductive females. However, their study site was near the northern extreme of the *N. sipedon* geographic range with climatic conditions and shorter daytime hours that would suggest the need for strong thermoregulatory precision due to a shortened activity season. Conversely, a study by Rowe et al. (2022) in Central Michigan ($n =$) did not find differences in T_b or thermal accuracy for *N. sipedon* between reproductive classes.

Here, I hypothesized that there will be less variation in body temperatures between reproductive and non-reproductive females in Tennessee because of a warmer climate than populations in *N. sipedon*'s northern range. I also hypothesized that reproductive females will choose similar habitats and have a similar movement pattern to non-reproductive females based on previous findings in other studies (Pattishall and Cundall 2008, 2009; Roth and Greene 2006; Row and Blouin-Demers 2006). To test my hypothesis, I compared the thermal properties and habitat selection of gestation sites to non-gestation sites (areas used by non-reproductive snakes) and recorded body temperatures throughout the active season. These data allowed me to investigate thermoregulation of *N. sipedon* at a lower latitude and in a more temperate climate (Tennessee). The results of this study will provide an additional example of how pregnancy affects the efficiency of thermoregulation, habitat choices and daily movements.

METHODS

Study species:

Nerodia sipedon are semi-aquatic and have one of the largest ranges for watersnakes, ranging from the east coast to Texas and spanning from the gulf coast north to Canada (Gibbons and Dorcas 2004, Walley et al. 2012). Population sizes on average are large even in small streams and wetlands; up to 1200 individuals (91 snakes/km) have been estimated for 9 of the 10 islands in Lake Erie and there have been similar sized populations found elsewhere (Brown and Weatherhead 2000, Cecala et al. 2010, King 1986, Pattishall and Cundall 2009). *Nerodia sipedon* appears to be a habitat generalist as long as a water source with prey is near, and they occur in river systems, swamps and lakes where they will commonly position themselves in areas with overhanging trees, rocky outcrops and vegetation (Gibbons and Dorcas 2004). *Nerodia sipedon* are mainly piscivores but also consume amphibians, and but tend to be dietary generalists and will consume what is available (Gibbons and Dorcas 2004). *Nerodia sipedon* display sexual dimorphism with the females being bigger bodied and having longer snout vent length (SVL) on average: males SVL rarely exceeded 70 cm but females frequently had a SVL > 80 cm in a study by Weatherhead et al. (1995). Adult *N. sipedon* tend to reach sexual maturity around 3 years of age (or 55 cm SVL for females) and can exhibit multiple paternity (Barry et al. 1992, Gibbons and Dorcas 2004, Weatherhead et al. 1995). Most females reproduce annually (Brown and Weatherhead 1997, Gibbons and Dorcas 2004) and they have been reported to have litters ranging from 4 to 99 neonates, although the

average is approximately 22 (Gibbons and Dorcas 2004). Females are viviparous; breeding occurs after emergence from hibernation between late March and May and parturition occurs early July into September (Gibbons and Dorcas 2004; Weatherhead et al. 1995).

Study site:

In 2021, I conducted a telemetry study on *N. sipedon* at two sites. The first site was along 0.5 km of Barton Creek (36°17'N, 86°34'W) in Wilson County, TN. Barton Creek flows into Old Hickory Lake. The creek has a shallow rocky bottom and two distinct shorelines. The creek had ample amounts of canopy cover dominated by deciduous trees with thick understory, open canopy areas and occasional exposed limestone escarpments that are less than 5 meters above the water. Barton Creek runs south of the city of Lebanon, so there are sections adjacent to residential properties and agricultural lands. The site provided adequate habitat for snakes and contained retreats such as rocks, trees, crevices, and thick vegetation. Barton Creek receives moderate foot traffic from fishing and is used occasionally by cattle.

The second site used in 2021 and 2022 was a non-continuous 4.5 km section of the West Fork of the Stones River in Rutherford County, TN. A paved public greenway runs adjacent to the West Fork of the Stones River (35°52'N, 86°24'W). This river flows into the Percy Priest Reservoir and has adequate canopy cover of mainly deciduous trees with thick understory, open canopy areas and occasional exposed limestone escarpments that are no more than 5 meters above the water. The Stones River has a shallow rocky bottom with two distinct shorelines and runs through the city of Murfreesboro, so there

are sections adjacent to residential, commercial and agricultural properties. The river provided plentiful amounts of habitat for snakes and contained retreats such as rocks, trees, crevices, and thick vegetation. The greenway along the Stones River in Murfreesboro receives considerably more foot traffic because of the accessibility for recreational activities (Fig. 1).

Transmitters and Implantation:

In May 2021 and 2022, adult female *N. sipedon* ($n = 20$) were hand captured and transported to Middle Tennessee State University for surgical implantation of radio transmitters (SB-2 transmitters 5.0 g, Holohil Systems, Ottawa, Ontario, Canada) and iButton® data loggers. Snout vent length, weight and reproductive status for each snake can be found in Table 1. Prior to implantation, an iButton® was attached with cyanoacrylate glue to the rear of the transmitter. These transmitter/data logger packages were then dipped in a polymer coating (Plasti Dip® International, Minnesota, USA) and dried for a minimum of 24 hours. The polymer coating was used to prevent any water or moisture from potentially entering the button and to keep the transmitter and button together as one unit. For implantation, snakes were placed in an anesthesia induction chamber and administered an inhalant anesthesia (isoflurane) by means of soaked cotton balls placed in a glass bowl in the chamber. Snakes were visually monitored constantly until all muscle tone was lost. At approximately 65% of the snake's body length posteriorly, a two cm long incision was made on the left side between the first and second lateral scale rows. A transmitter was inserted inside the body cavity and a 38 cm long hollow metal cannula was used to thread the antenna subcutaneously towards the anterior

of the snake. The incision was sutured using 4.0 silk sutures. This procedure is similar to that of Reinert and Cundall (1982). The total mass of the radio transmitter and the temperature logger package never exceeded 5% of the snake's body weight ($n = 12$, mean % of snake body mass = $2.7\% \pm 0.02g$). The iButtons® were programmed to record a single temperature (T_b) at the resolution of $\pm 0.0625^\circ\text{C}$ every hour for the duration of the study. All postoperative snakes were maintained in the MTSU vivarium for 24 hours in vision racks equipped with heating strips to facilitate wound healing. The snakes were then released at their point of capture.

Once released, the snakes were located by radiotelemetry at least 3 times per week for the duration of the study using a 3-element Yagi antenna and a radio receiver (R-1000, Communications Specialist, Inc., CA), hand-held telemetry system. Geographic location was recorded for each snake using a hand-held GPS (GPSMAP 76CSx, Garmin International, Inc., KS). To ensure data collection during just the gestation period, I defined the sample period as 8-July and 14-August and removed days that were rainy or completely overcast.

Environmental Measurements:

To obtain operative snake temperatures, operative temperature models were placed throughout the study sites for 3 consecutive sunny days. Fifty-four operative temperature models were randomly placed either in full sun or in fully shaded areas around snake locations to measure the upper and lower operative temperatures available at the site (Peterson et al. 1993). The models were composed of copper pipe (1.9 cm diameter x 20 cm long), and an iButton® data logger was inserted into the pipe and was sealed with

rubber stoppers at each end. Following Shipman (2011), Krylon® Ruddy Brown was used for the model color to match the reflectance of *N. sipedon*.

To measure air and water temperature within each study site, two iButtons® were coated with Plasti Dip®. The temperature loggers were used to measure water temperature after being placed into 50 ml centrifuge tubes with six 7 mm diameter holes drilled into them, then fastened to a submerged tree root. The temperature loggers that measured air temperatures were placed in a mesh bag and secured to a tree in the shade approximately 1.5 m off of the ground.

Habitat Analysis:

Habitat was assessed by two different methods: daily habitat use and compositional analysis. Daily use habitat consisted of the type of habitat the snakes were using when relocated (e.g., under a rock, in a brush pile etc.). I used daily use locations to determine compositional habitat by surveying the area around the daily use locations (e.g., percentage of rock, water, forbs, etc.). Snake locations (GPS coordinates and habitat use) were taken at least 3 times per week. Daily habitat use was described by the following 6 categories: brush piles, instream cover (tree islands), rocks, rocks in the water, shrubs, and tree branches. Each compositional analysis location described for reproductive and non-reproductive snakes also had a random site surveyed which was determined by a random number generator and flip of a coin to be between 5 m and 20 m from the snake's site either up or down river. Using a point intercept method, I measured habitat structure using 3 vertical transect lines (3 m long) horizontally 2 m apart, placed with the snake location as the center point (the center point being the center of the middle line). I

measured the type of plant or structure at each 10 cm mark using the following 5 categories: soil, water, rock, forb, woody plant. This provided 90 point measurements at each location.

Data Analysis:

Body temperature data was analyzed using a GAMM (generalized additive mixed model) from the R package “*mgcv*” (Wood 2017) due to the nature of temperature data not following a linear pattern and the data having both fixed, and a random effects structure (Rij et al. 2015, Wood 2006; Yang et al. 2012). Previous studies used generalized linear models (GLM) or extensions of ANOVAs to evaluate the relationship between temperature and time. However, the snake body temperatures in this study did not follow a linear pattern and cannot be properly fitted to a linear model curve (much like GLM and ANOVA tests). Therefore, a GAMM (because of the fixed and random effects) was the appropriate modeling application to explain the relationship between body temperature and time while accounting for the non-linear pattern (Rij et al. 2015, Yang et al. 2012). Rainy days (11 days for 2021; 13 days for 2022) were excluded from the dataset because of the constraint it posed on the ability to “normally” thermoregulate due to temperatures, solar radiation and limited opportunity. The model included body temperature as the response variable, pregnancy status as the main predictor variable and accounted for time of day (in hours), air temperature, water temperature, month, snake ID (individual snakes) and site as random variables. In a separate analysis the data were subset to only daytime hours and nighttime hours based on sunset and sunrise to test for differences in body temperatures between the two times of day.

Both habitat methods were analyzed using a chi-square test of independence. For the compositional analysis I tested reproductive snake sites against random sites, non-reproductive snake sites against random sites and reproductive snake sites against non-reproductive sites. For daily habitat use, I compared non-reproductive snake habitat choices against reproductive snake choices.

Snake movement was analyzed using a two-sample *t*-test to compare daily movements. I then created minimum convex polygons for each individual (Fig 2 and Fig. 3) to estimate activity range size using ArcMap 10.7.1 (ESRI, Redlands, CA; Row and Blouin-Demers 2006).

All data analysis was performed in Program R (R Core Team 2020) and maps for analysis were taken from USGS Earth Explorer (2000).

RESULTS

Temperature Analysis

Twenty *N. sipedon* were captured and surgically implanted with transmitters in 2021 and 2022. Only 15 snakes survived due to predation and other unknown causes that could not be determined and 3 iButtons® did not work properly. I collected over 10,000 body temperatures (T_b) and 201 daily movements throughout the entire season. To ensure I was only collecting data during gestation, I defined the sample period as 8-July through 14-August. After rainy or completely overcast days were removed T_b s were reduced to 7440 and 12 snakes. This sample included 7 reproductive (4344 T_b s) and 5 non-reproductive (3096 T_b s) snakes (Table 2). Reproductive snake T_b s followed a similar pattern to non-reproductive snakes for both years (Fig. 4). Overall average T_b for all snakes was $27.1 \pm 0.03^\circ\text{C}$, reproductive snake's average T_b $27.2 \pm 0.04^\circ\text{C}$ and non-reproductive snake's average T_b $27.0 \pm 0.05^\circ\text{C}$. After running a generalized additive mixed model where body temperature was the response variable, pregnancy status was the main predictor variable and accounting for time of day (in hours), air temperature, water temperature, month, snake ID (individual snakes), year and site as random variables, I found that pregnancy had no significant effect (GAMM, t -value = 0.595, $P = 0.552$, $R^2 = 54.7\%$) on T_b (Fig. 5).

Environmental temperatures

Air and water temperatures were collected for the entirety of the pregnancy period (Fig. 6). The overall average air temperature for 2021 was 24.7°C with a minimum of 15°C and a maximum of 33.8°C and in 2022 was 25.3°C with a minimum of 15.5°C and

a maximum of 36°C. The overall average water temperature for 2021 was 24.2°C with a minimum of 21.3°C and a maximum of 27.3°C and 2022 was 25.4°C with a minimum of 22.5°C and a maximum of 31°C. Temperatures overall were warmer in 2022 and there were more rainy days in 2022.

The 54 operative snake models recorded 6 days ($n = 3,168$ temperatures) of environmental temperatures (T_e). The number of rainy/overcast days in both years and the necessity for models to collect temperatures over more than two consecutive days each time only allowed for 6 recorded days. Snake body temperatures occurred between the maximum and minimum T_e , inferring that snakes may not have been thermally constrained by T_e (Fig. 7).

Habitat Analysis

Daily locations ($n = 201$) were collected for daily habitat use analysis. Daily habitat use showed that reproductive snakes proportionally chose brush piles 13%, instream cover (tree islands) 3%, rocks 64%, rocks in the water 3%, shrubs 9% and tree branches 8% of the time. Non-reproductive snakes proportionally choose brush piles 0%, instream cover (tree islands) 7%, rocks 25%, rocks in the water 36%, shrubs 16% and tree branches 16% of the time (Table 3). Using non-reproductive snake relocation sites as my observed outcome and reproductive snake relocation sites as my expected outcome, habitat use was significantly different between reproductive classes (chi-squared test: $X^2 = 62.10$, $P < 0.05$).

Compositional habitat was conducted on 36 sites for this study. Many relocation sites were within 5-10 m of each other or other snake relocation sites, so I eliminated these points to avoid sampling overlap. Habitat composition showed that reproductive snakes proportionally chose habitats with forbes 28%, rock 29%, soil 6%, water 26% and woody plants 11% of the time. Non-reproductive snakes proportionally choose habitats with forbes 9%, rock 59%, soil 0%, water 31% and woody plants 1% of the time (Table 4). When tested to random sites separately, reproductive and non-reproductive snake sites both were significantly different than random sites (chi-squared test: $X^2 = 401.55$, $P < 0.05$ and $X^2 = 448.33$, $P < 0.05$, respectively). Reproductive and non-reproductive sites compared to each other also were significantly different (chi-square test: $X^2 = 918.35$, $P < 0.05$).

Movement Analysis

For 2021 and 2022 daily snake movements averaged 18.5 m per day. Reproductive snakes moved 14.3 m per day and non-reproductive snakes moved 22.8 m per day on average. The longest distance moved in one day for reproductive snakes was 244 m and the longest distance moved in one day for non-reproductive snakes was 245 m. A Wilcoxon signed-rank test indicated that pregnant snakes did not move differently than non-reproductive snakes ($W = 5094.5$, $P = 0.944$). Figure 8 displays meters moved (proportionally) per day by reproductive and non-reproductive snakes for both years. Activity range size estimates averaged 0.30 ha (0.37 ha for reproductive snakes and 0.23 ha for non-reproductive snakes) based on size of minimum convex polygons calculated for each individual snake (Table 5).

DISCUSSION

This study found no difference in average daily body temperature (T_b) between reproductive and non-reproductive female *N. sipedon*. Despite reproductive and non-reproductive female snakes thermoregulating similarly and having similar daily movements, they used different habitat types.

Although there is a common hypothesis that reproductive viviparous snakes thermoregulate differently than non-reproductive snakes, snakes in this study selected similar average daily body temperatures (T_b s) irrespective of reproductive condition (Fig. 9). Rowe et al. (2022) studied *N. sipedon* in Central Michigan and found no difference in T_b between reproductive and non-reproductive females. Gier et al. (1989) also found no difference in T_b between reproductive classes in *Crotalus viridis oreganus* (Pacific Rattlesnakes). Both my results and other studies suggest thermal plasticity occurring between snake species and/or populations at different latitudes.

Contrary to my study, *Nerodia sipedon* in Ontario, Canada (Brown and Weatherhead 2000) displayed a difference in T_b between reproductive classes. Body temperatures found in my study (reproductive female snake mean $T_b = 27.2^\circ\text{C}$ and non-reproductive female snake mean $T_b = 27.0^\circ\text{C}$) were higher than the body temperatures Brown and Weatherhead (2000) recorded (reproductive female snake mean $T_b = 25.6^\circ\text{C}$ and non-reproductive female snake mean $T_b = 24.4^\circ\text{C}$). Rowe (2022) recorded reproductive female snake mean $T_b = 26.9^\circ\text{C}$ and non-reproductive female snake mean $T_b = 27.7^\circ\text{C}$. This study's mean air was also higher (24.7°C for 2021 and 25.3°C for 2022) than Brown and Weatherhead's (2000) observed air temperature in Ontario (23.7°C). My findings as well as Rowe et al.'s (2022) findings, suggest the possibility that when given

warmer seasonal temperatures (compared to temperatures in Ontario), certain populations of *N. sipedon* will maintain higher body temperatures and may not thermoregulate differently based on reproductive status (Weatherhead et al. 2012).

Thermoregulatory set points (i.e., preferred body temperature) have been found to vary with latitude in other species of watersnakes (Winne and Keck 2004). Variation of thermoregulatory set points along latitudes opens the possibility that thermoregulatory behavior associated with pregnancy in *N. sipedon* may vary along latitude as well. Brown and Weatherhead (2000) mentioned that a replicate study needed to be performed in a more southern and temperate climate to investigate differences based on species distribution because of the known climatic differences between Ontario and the southern portion of the United States. Further evidence of latitudinal variation in body temperature selection in snakes occur with neonate *Nerodia rhombifer* for laboratory preferred body temperatures (Winnie and Keck 2004). Snakes from lower latitudes had higher preferred body temperatures. Similarly, laboratory preferred temperatures and field body temperatures of *Elaphe obsoleta* increased at lower latitudes (Weatherhead et al 2012). Therefore, as suggested by this study, there is potential for *N. sipedon* to exhibit warmer body temperatures at more southern latitudes.

A shortened active season and cooler temperatures in *N. sipedon*'s northern range could accentuate the differences in T_b between reproductive classes. Studies that describe a difference in body temperatures between reproductive classes (Gardener-Santana and Beaupre 2009, Charland 1995) displayed a clear difference even with relatively small sample sizes. Here I used GAMM models, which are a new approach to analyze variable

body temperatures in snakes. Future modeling efforts using GAMMs in more northern snake species may help elucidate the contradictory patterns suggesting thermal plasticity. To further strengthen my body temperature findings, I collected environmental temperatures to test if the snakes were thermally constrained, however this did not appear to be the case (Fig. 7).

Reproductive snakes specifically chose rocks away from the water most of the time while non-pregnant snakes were observed using rocks in the water, basking in trees and using shrubs. These results are in line with similar findings in other studies on *N. sipedon* (Pattishall and Cundall 2009). Difference in body size may account for differences in habitat choice because of the requirements for different body sizes (e.g., because of their greater thermal inertia, pregnant snakes “larger snakes” may be able to use conduction of hot rocks to thermoregulate and non-pregnant snakes “smaller snakes” which change T_b more quickly may instead use tree branches, shrubs and rocks in water). Reproductive females in this study were 32% heavier than non-reproductive snakes. Difference in habitat choice based on reproductive status illustrates the need for different habitats based on snake size, regardless of reproductive status (Weatherhead et al. 1995).

Habitat choice difference between reproductive and non-reproductive snakes has been previously documented in *N. sipedon* (Pattishall and Cundall 2009). Pattishall and Cundall (2009) found the reproductive snakes chose habitats twice as far away from water than non-reproductive snakes. Based on Pattishall and Cundall (2009) and this study’s finding, reproductive *N. sipedon* may choose habitats away from water because they do not forage often during pregnancy, unlike non-reproductive individuals who were found in or near water in both studies. Although Aldridge and Bufalino (2003) found

food present in 71% of pregnant *N. sipedon* during July and August, most of the mass increase throughout pregnancy was due to water retention in the embryos. This leads to the possibility that while snakes do eat during pregnancy, pregnant snakes do not feed as often as non-pregnant snakes based on Pattishall and Cundall's (2009) findings on the habitats *N. sipedon* chose and based on this study's finding of *N. sipedon*'s on average, short movements. To further support this idea, non-reproductive snakes were observed using habitat (rocks in water) that allowed for easier access for feeding since they feed more often than reproductive snakes during the active season. It also needs to be mentioned that reproductive snakes have a higher risk factor (i.e., protecting their young from predation) than non-reproductive snakes, so choosing habitats away from the open canopy river and near brush would be more beneficial than costly to reproductive snakes.

Pregnancy is expected to influence snake movement because of the constraints associated with pregnancy (Shine 1980). Previous studies found reduced daily movements in reproductive *Hoplocephalus bungaroides* [Broad-headed Snakes] (Webb and Shine 1997) and reduced locomotor ability in reproductive *Thamnophis marcianus* [Checkered Garter Snakes] (Seigel et al. 1987). Additionally, some viviparous snake species may exhibit anorexia (i.e., reduced foraging behavior) during pregnancy (Gregory et al. 1999). For this population, I found that reproductive female snakes had similar daily movements to non-reproductive female snakes. These findings are similar to previous studies on *N. sipedon* (Roth and Greene 2006, Pattishall and Cundall 2008). Both reproductive and non-reproductive snakes moved between 0-50 meters a majority of the time (92% and 78% respectively). *Nerodia sipedon*, unlike other species whose movements have been studied during pregnancy (Charland and Gregory 1995, Blazquez

1995, Webb and Shine 1997), are sometimes restricted to a linear system (i.e., a river), such as in this study. Movements *N. sipedon* should be expected to follow a different pattern than other “terrestrial” colubrids, possibly giving an explanation to why it would be unlikely for pregnancy to influence daily movements.

This study points to the possibility that *N. sipedon* displays thermal plasticity between populations at different latitudes. Unfortunately, sample size is a weakness to my study design and sampling along a latitudinal gradient would be needed to test for thermal plasticity. Sample size was restricted in my study due to deaths, iButton® failures and inability to find additional pregnant females in publicly accessible rivers. Measurement of the available thermal environment with operative models turned out to be more difficult than expected on a river system because models could not be placed directly in water. Additionally, water level varied with season, so temperatures recorded were not as consistent and representative of specific microhabitats as expected. Lastly, a laboratory study examining the effect of reproductive condition on thermal preference would strengthen my ability to draw conclusions from body temperatures acquired in the field. This study provides preliminary results for a replicate study based on Brown and Weatherhead’s study in Ontario and Rowe et al.’s study in Central Michigan, while also connecting habitat use and movement patterns during pregnancy. It also allowed insight into the differences in natural history traits along latitudinal lines for *N. sipedon* and introduces the possibility that this may occur in other snake species with large distributions.

LITERATURE CITED

- Arnold, S.J., and C.R. Peterson. 2002. A model for optimal reaction norms: The case of the pregnant garter snake and her temperature-sensitive embryos. *The American Naturalist* 160:306-316.
- Aldridge, R.D., and A.P. Bufalino. 2003. Reproductive female common watersnakes (*Nerodia sipedon sipedon*) are not anorexic in the wild. *Journal of Herpetology* 2:416-419.
- Bakken, G.S. and D.M. Gates. 1975. Heat transfer analysis of animals: Some implications for field ecology, physiology, and evolution Pp. 255-290, *In* G.S. Bakken, and D.M. Gates (Eds.). *Perspectives of Biophysical Ecology*. Vol. 12. Springer Science & Business Media, Berlin, Germany. 624pp.
- Barry, F.E., P.J. Weatherhead, and D.P. Philipp. 1992. Multiple paternity in a wild population of northern watersnakes, *Nerodia sipedon*. *Behavioral Ecology and Sociobiology* 30:193-199.
- Blazquez, M.C. 1995. Body temperature, activity patterns and movements by gravid and non-gravid females of *Malpolon monspessulanus*. *Journal of Herpetology* 29:264-266.
- Bogert, C.M. 1949. Thermoregulation in reptiles, a factor in evolution. *Evolution* 3:195-211.
- Brown, G.P., and P.J. Weatherhead. 1997. Effects of reproduction on survival and growth of female northern water snakes, *Nerodia sipedon*. *Canadian Journal of Zoology* 75:424-432.

- Brown, G.P., and P.J. Weatherhead. 2000. Thermal ecology and sexual dimorphism in northern water snakes, *Nerodia sipedon*. *Ecological Monographs* 70:311-330.
- Cecala, K., M. Dorcas, and S. Price. 2010. Ecology of juvenile northern watersnakes (*Nerodia sipedon*) inhabiting low-order streams. *Amphibia-Reptilia* 31:169-174.
- Chamberlain, J.D., and M.E. Gifford. 2016. Physical and physiological costs of reproduction in watersnakes. *Copeia* 104:722-727.
- Charland, M.B. 1995. Thermal consequences of reptilian viviparity: Thermoregulation in gravid and nongravid garter snakes (*Thamnophis*). *Journal of Herpetology* 29:383-390.
- Charland, M.B., and P.T. Gregory. 1990. The influence of female reproductive status on thermoregulation in a viviparous snake, *Crotalus viridis*. *Copeia* 1990:1089-1098.
- Charland, M.B., and P.T. Gregory. 1995. Movements and habitat use in gravid and nongravid female garter snakes (*Colubridae:Thamnophis*). *Journal of Zoology* 236:543-561.
- Dorcas, M.E., and C.R. Peterson. 1998. Daily body temperature variation in free-ranging rubber boas. *Herpetologica* 1998:88-103.
- Earth Explorer. 2000. FS; 083-00. Geological Survey (U.S.). Available online at <https://earthexplorer.usgs.gov/>. Accessed 10 December 2020.

- Fitch, H.S. 1970. Reproductive cycles in lizards and snakes. Special technical report. Natural History Museum at The University of Kansas, Lawrence, KS. 247 pp.
- Fitch, H.S. 2000. Population structure and biomass of some common snakes in Central North America. Special technical report. Natural History Museum at The University of Kansas, Lawrence, KS. 8 pp.
- Fox, W. 1948. Effect of temperature on development of scutellation in the garter snake, *Thamnophis elegans atratus*. *Copeia* 1948:252-262.
- Gardner-Santana, L.C., and S.J. Beaupre. 2009. Timber rattlesnake (*Crotalus horridus*) exhibited elevated and less variable body temperatures during pregnancy. *Copeia* 2009:363-368.
- Gibbons, J.W., and M.E. Dorcas. 2004. North American Watersnakes: A Natural History. University of Oklahoma Press, Norman, OK. 496 pp.
- Gier, P.J., R.L. Wallace, and R.L. Ingermann. 1989. Influence of pregnancy on behavioral thermoregulation in the Northern Pacific rattlesnake *Crotalus viridis oreganus*. *Journal of Experimental Biology* 145:465-469.
- Greene, B.D., J.R. Dixon, M.J. Whiting, and J.M. Mueller. 1999. Reproductive ecology of the Concho water snake. *Copeia* 1999:701-709.
- Gregory, P.T., L.H. Crampton, and K.M. Skebo. 1999. Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: Are gravid snake anorexic? *Journal of Zoology* 248:231-241.

- Graves, B.M., and D. Duvall. 1993. Reproduction, rookery use, and thermoregulation in free-ranging, pregnant *Crotalus v. viridis*. *Journal of Herpetology* 27:33-41.
- Harvey, D.S., and P.J. Weatherhead. 2010. Habitat selection as the mechanism for thermoregulation in a northern population of massasauga rattlesnakes (*Sistrurus catenatus*). *Ecoscience* 17:411-419.
- Huey, R.B. 1982. Temperature, physiology, and the ecology of reptiles. Pp 25-92, In C. Gans and F.H. Pough (Eds.). *Biology of the Reptilia*. Vol 12. Academic Press, London, UK. 536 pp.
- Huey, R.B., C.R. Peterson, S.J. Arnold, and W.P. Porter. 1989. Hot rocks and not-so-hot rocks: retreat-site selection by garter snakes and its thermal consequences. *Ecology* 70:931-944.
- Ingermann, R.L., N.J. Berner, and F.R. Ragsdale. 1991. Effect of pregnancy and temperature on red cell oxygen-affinity in the viviparous snake *Thamnophis elegans*. *Journal of Experimental Biology* 156:399-406.
- Issac, L.A., and P.T. Gregory. 2006. Thermoregulatory behaviour of gravid and non-gravid female grass snakes (*Natrix natrix*) in a thermally limiting high-latitude environment. *Journal of Zoology* 264:403-409.
- King, R.B. 1986. Population ecology of the Lake Erie water snake, *Nerodia sipedon insularum*. *Copeia* 1986:757-772.
- Lutterschmidt, W.I., and H.K. Reinert. 1990. The effect of ingested transmitters upon the temperature preference of the northern water snake, *Nerodia s. sipedon*. *Herpetologica* 46:39-42.

- Osgood, D.W. 1970. Thermoregulation in water snakes studied by telemetry. *Copeia* 1970:568-571.
- Osgood, D.W. 1978. Effects of temperature on the development of meristic characters in *Natrix fasciata*. *Copeia* 1978:33–37.
- Pattishall, A., and D. Cundall. 2008. Spatial biology of northern watersnakes (*Nerodia sipedon*) living along an urban stream. *Copeia* 2008:752-762.
- Pattishall, A., and D. Cundall. 2009. Habitat use by synurbic watersnakes (*Nerodia sipedon*). *Herpetologica* 65:183-198.
- Peterson, C.R., A.R. Gibson, and M.E. Dorcas. 1993. Snake thermal ecology: the causes and consequences of body-temperature variation. Pp. 241-314, *In* R.A. Seigel and J.T. Collins (Eds.). *Snakes: Ecology and Behavior*. Vol. 1. McGraw-Hill Inc, New York, NY. 414 pp.
- R Core Team. 2020. R: A language and environment for statistical computing, version 3.6.3. R Foundation for Statistical Computing, Vienna, Austria. Available online at <https://www.R-project.org/>. Accessed 1 November 2020.
- Reinert, R., and D. Cundall. 1982. An improved surgical implantation method for radio-tracking snakes. *Copeia* 1982:702-705.
- Rij, J.V., M. Wieling, R.H. Baayen, and D.H. Rijn. 2015. itsadug: interpreting time series and autocorrelated data using GAMMs. R Packag. Available online at <https://cran.r-project.org/web/packages/itsadug/itsadug.pdf>. Accessed 1 November 2020

- Roth, T.C., and B.D. Greene. 2006. Movement patterns and home range use of the northern watersnake (*Nerodia sipedon*). *Copeia* 2006: 544-551.
- Row, J.R., and G. Blouin-Demers. 2006. Kernels are not accurate estimators of home-range size for herpetofauna. *Copeia* 2006:797-802.
- Rowe, J.W., T.M. Goerge, C.E. Martin, and W.P. Mulligan. 2022. Thermal ecology of northern water snakes (*Nerodia sipedon*) in a northern wetland in central Michigan. *Journal of Thermal Biology* 105:103204.
- Seigel, R.A., M.M. Huggins, and N.B. Ford. 1987. Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia* 73:481-485.
- Shine, R. 1980. "Costs" of reproduction in reptiles. *Oecologia* 46:92-100.
- Shine, R., and J.J. Bull. 1979. The evolution of live-bearing in lizards and snakes. *The American Naturalist* 113:905-923.
- Shine, R., and P. Harlow. 1993. Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* 96:122-127.
- Shine, R., and T. Madsen. 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology* 69:252-269.
- Shipman, J.D. 2011. Thermal ecology of hibernation in the northern watersnake, *Nerodia sipedon* (Linnaeus). Master's thesis. Middle Tennessee State University, Murfreesboro, TN. 59 pp.

- Sprague, T.A., and H.L. Bateman. 2018. Influence of seasonality and gestation on habitat selection by northern Mexican garter snakes (*Thamnophis eques megalops*). PLOS ONE [Internet] 13(1)e0191829. Available online at doi.org/10.1371/journal.pone.0191829. Accessed 7 September 2022.
- Stevenson, R.D. 1985. The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. The American Naturalist 126:362-386.
- Stevenson, R.D., C.R. Peterson, and J.S. Tsuji. 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. Physiological Zoology 58:46-57.
- Tu, M.C., and V.H. Hutchison. 1995. Influence of pregnancy on thermoregulation of watersnakes (*Nerodia rhombifera*). Journal of Thermal Biology 19:255-259.
- Walley, H.D., R.B. King, J.M. Ray, and T.L. Wusterbarth. 2012. *Nerodia sipedon*. Catalogue of American Amphibians and Reptiles 899:1-58.
- Weatherhead, P.J., F.E. Barry, G.P. Brown, and M.R. Forbes. 1995. Sex ratios, mating behavior and sexual size dimorphism of the northern water snake, *Nerodia sipedon*. Behavioral Ecology and Sociobiology 36:301-311.
- Weatherhead, P.J., J.H. Sperry, G.L. Carfagno, and G. Blouin-Demers. 2012. Latitudinal variation in thermal ecology of North American ratsnakes and its implications for the effect of climate warming on snakes. Journal of Thermal Biology 37:273–281.
- Webb, J.K., and R. Shine. 1997. A field study of spatial ecology and movements of a threatened snake species, *Hoplocephalus bungaroides*. Biological Conservation 82:203-217.

- Winne, C.T., and M.B. Keck. 2004. Intraspecific differences in thermal tolerance of the diamondback watersnake (*Nerodia rhombifer*): Effects of ontogeny, latitude and sex. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 140:141-149.
- Wood, S.N. 2017. mgcv: mixed GAM computation vehicle with automatic smoothness. R Package. Available online at <https://cran.r-project.org/web/packages/mgcv/mgcv.pdf>. Accessed 1 November 2020.
- Wood, S.N. 2006. Generalized additive models: an introduction with R. Department of Mathematical Sciences, University of Bath, U.K. Available online at <https://www.academia.edu/download/6418651/7-gam.pdf>. Accessed 1 November 2020.
- Yang, L., G. Qin, N. Zhao, C. Wang, and G. Song. 2012. Using a generalized additive model with autoregressive terms to study the effects of daily temperature on mortality. *BMC Medical Research Methodology* 12:1-13.

APPENDIX A: TABLES

Table 1. *Nerodia sipedon* snout vent length (SVL), weight (g) and reproductive status.

Snake ID	SVL (cm)	Weight (g)	Reproductive Status
Snake 1	72.5	369.9	Reproductive
Snake 3	72.5	344	Reproductive
Snake 4	76.0	334	Reproductive
Snake 6	74.0	445	Reproductive
Snake 7	80.0	490	Reproductive
Snake 11	78.0	472.1	Reproductive
Snake 16	70.0	256.1	Reproductive
Mean	74.7	387.3	Reproductive
Snake 5	64.5	221	Non-reproductive
Snake 9	69.0	330.6	Non-reproductive
Snake 12	72.0	275	Non-reproductive
Snake 14	65.0	228.5	Non-reproductive
Snake 18	73.0	262.3	Non-reproductive
Mean	68.7	263.5	Non-reproductive

Table 2. Body temperatures (mean $\pm$ SE) of reproductive ($n = 4344 T_{bs}$) and non-reproductive ($n= 3096 T_{bs}$) female *Nerodia sipedon* during July and August of 2021 and 2022 combined for 24 hr., daytime (07:00-20:00) and nighttime (21:00-06:00).

Sex	Time Period	Month	Tb (°C)	Range (°C)
Reproductive (N=7)	24 Hr.	July	27.63 $\pm$ 0.05	20.8 - 34.8
		August	26.48 $\pm$ 0.06	20.5 - 33.6
	Daytime	July	28.22 $\pm$ 0.07	21.4 - 38.8
		August	26.83 $\pm$ 0.08	20.5 - 33.6
	Nighttime	July	26.75 $\pm$ 0.08	20.8 - 32.9
		August	26.00 $\pm$ 0.08	20.8 - 32.7
Non-Reproductive (N=5)	24 Hr.	July	27.10 $\pm$ 0.06	19.1 - 34.2
		August	26.46 $\pm$ 0.07	16.6 - 34.7
	Daytime	July	27.71 $\pm$ 0.08	19.4 - 34.2
		August	27.01 $\pm$ 0.09	16.6 - 34.7
	Nighttime	July	26.23 $\pm$ 0.09	19.1 - 40.0
		August	25.70 $\pm$ 0.10	16.9 - 31.9

Table 3. Habitat use of reproductive and non-reproductive female *Nerodia sipedon* based on daily relocations of snakes for both 2021 and 2022.

Habitat Use	Proportion of Reproductive	Proportion of Non-Reproductive
Rock	0.64	0.25
Rock in water	0.03	0.36
Shrub	0.09	0.16
Tree branch	0.08	0.16
Instream cover	0.03	0.07
Brush pile	0.13	0.00

Table 4. Compositional analysis of relocation sites (proportionally) for reproductive and non-reproductive female *Nerodia sipedon* for both 2021 and 2022.

Habitat Type	Proportion of Reproductive	Proportion of Non-Reproductive
Open soil	0.06	0.00
Water	0.26	0.31
Rock	0.29	0.59
Forbes	0.28	0.09
Woody plants	0.11	0.01

Table 5. Activity range size estimates using minimum convex polygons for reproductive and non-reproductive female *Nerodia sipedon* during 2021 and 2022.

Snake ID	MCP	Reproductive Status
Snake 1	3230	Reproductive
Snake 3	4031	Reproductive
Snake 4	6785	Reproductive
Snake 6	4411	Reproductive
Snake 7	5423	Reproductive
Snake 11	640	Reproductive
Snake 16	1600	Reproductive
Snake 9	3964	Non-Reproductive
Snake 10	102	Non-Reproductive
Snake 12	3536	Non-Reproductive
Snake 14	2550	Non-Reproductive
Snake 18	1190	Non-Reproductive

APPENDIX B: FIGURES

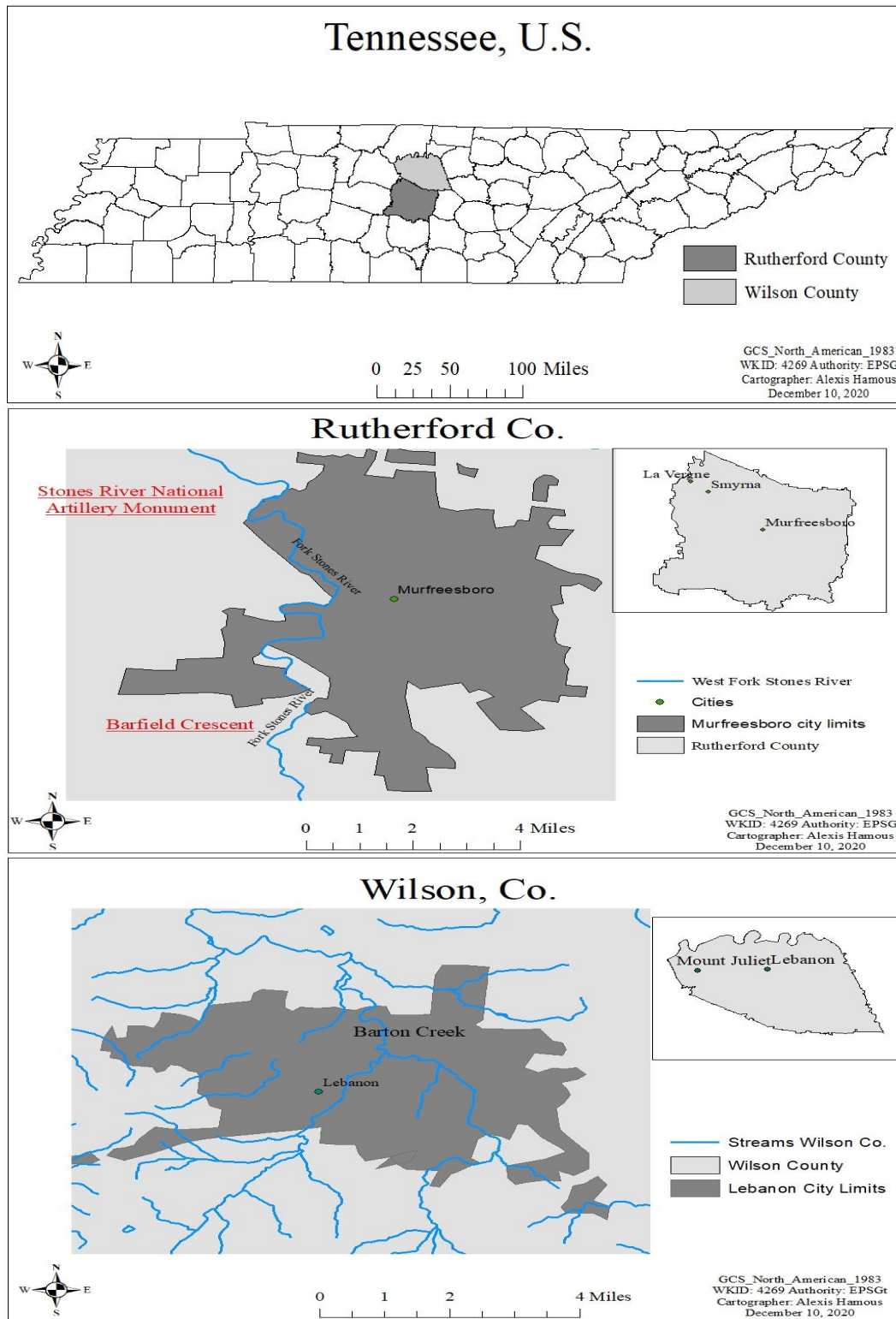


Figure 1. Maps of study site locations for *Nerodia sipedon* in 2021 and 2022.

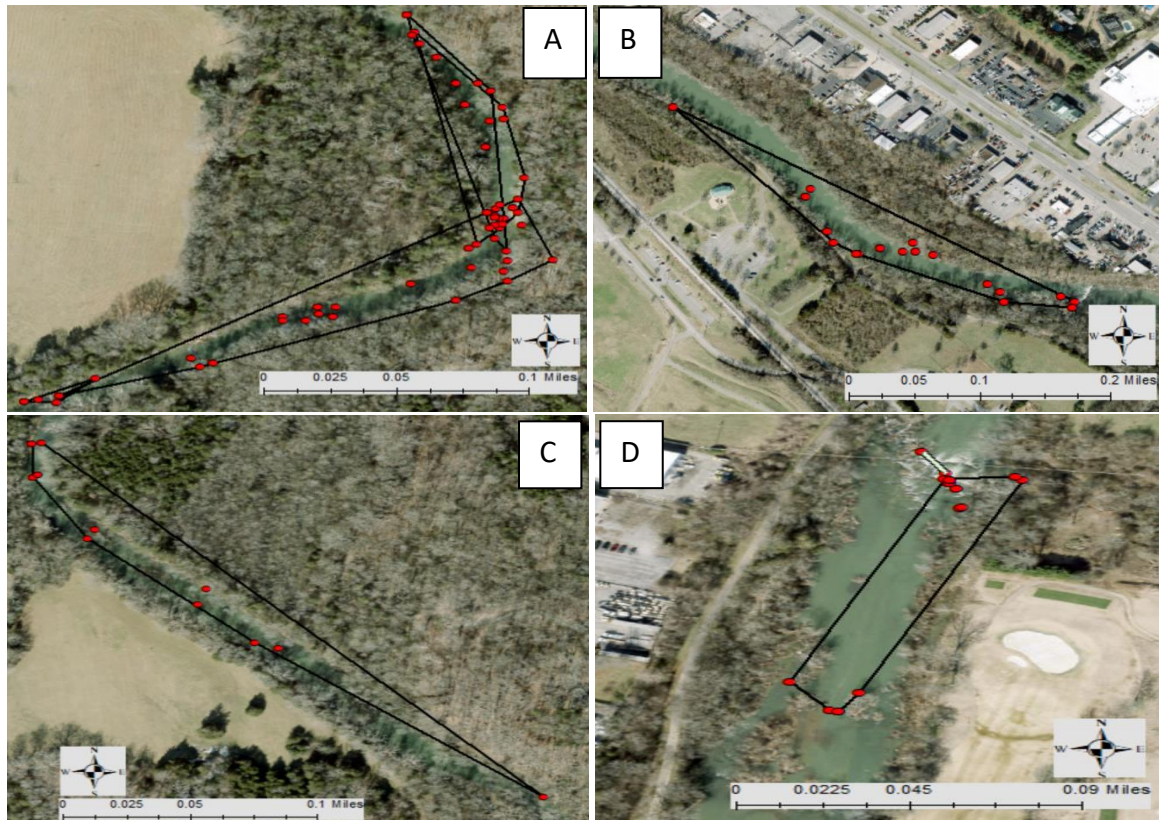


Figure 2. Maps of study sites with relocation sites (indicated by red dots) with minimum convex polygons to estimate activity range size exhibited by *Nerodia sipedon*. A through D are along the Stones River (Rutherford Co.).

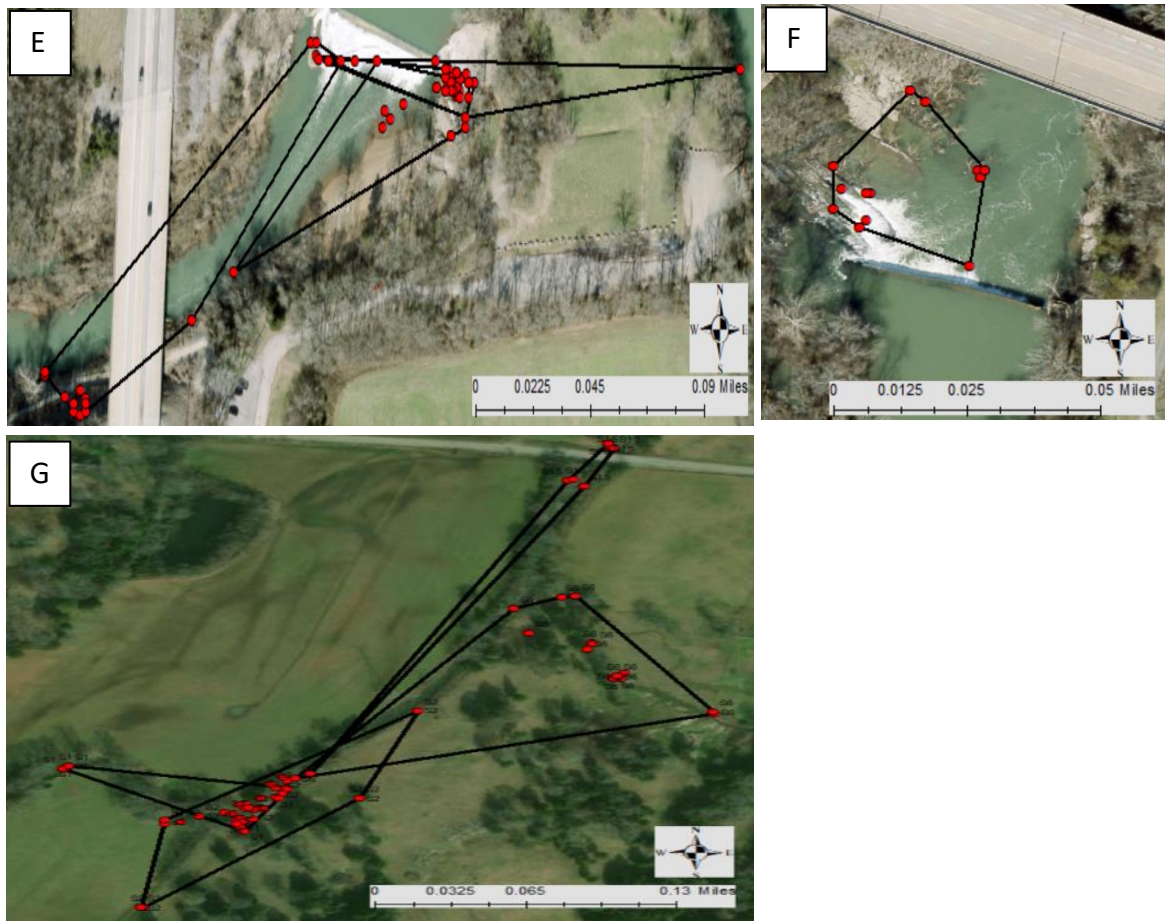


Figure 3. Maps of study sites with relocation sites (indicated by red dots) with minimum convex polygons to estimate activity range size exhibited by *Nerodia sipedon*. E and F are along the Stones River (Rutherford Co.), G is Barton Creek (Wilson Co.)

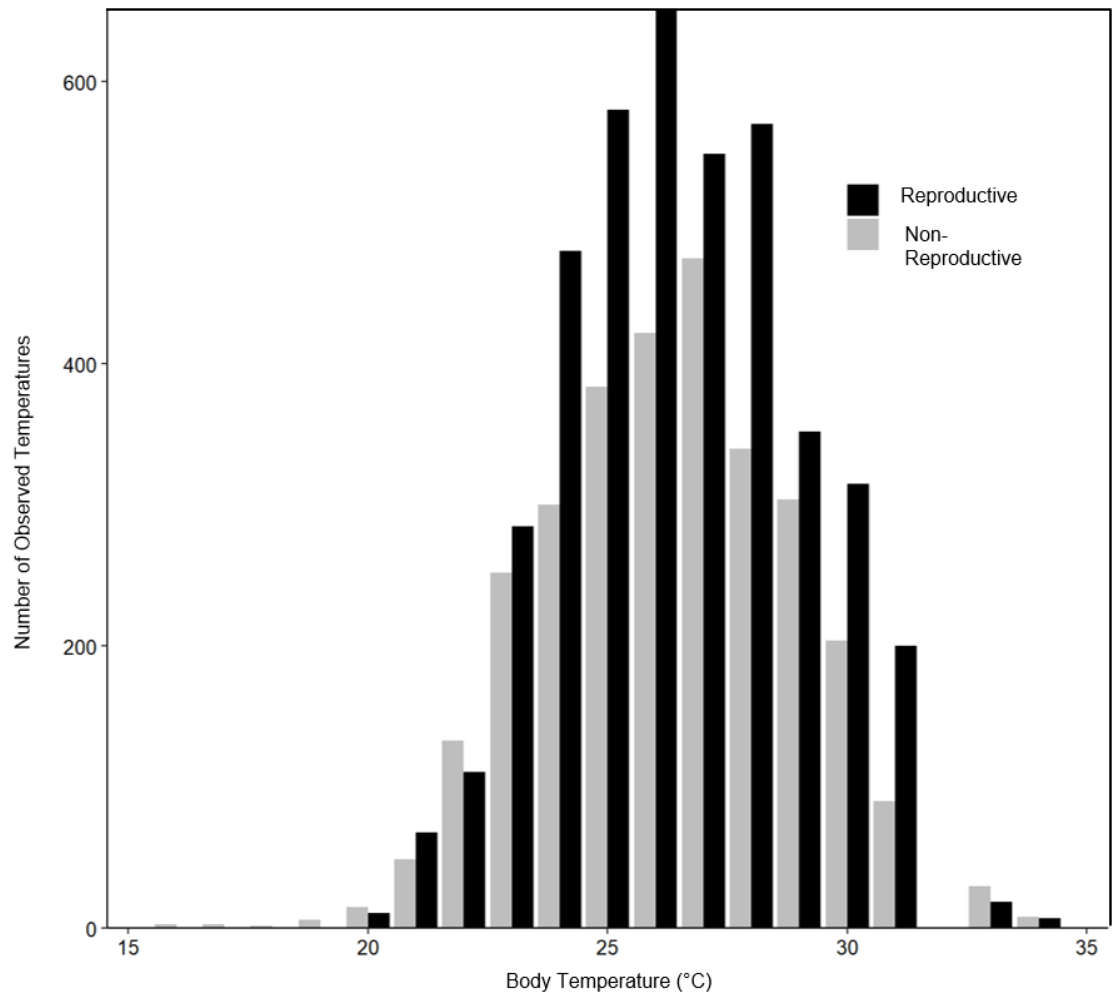


Figure 4. Distribution of reproductive ($n = 4344$) and non-reproductive ($n = 3096$) female *Nerodia sipedon* body temperatures over the pregnancy period, not proportional.

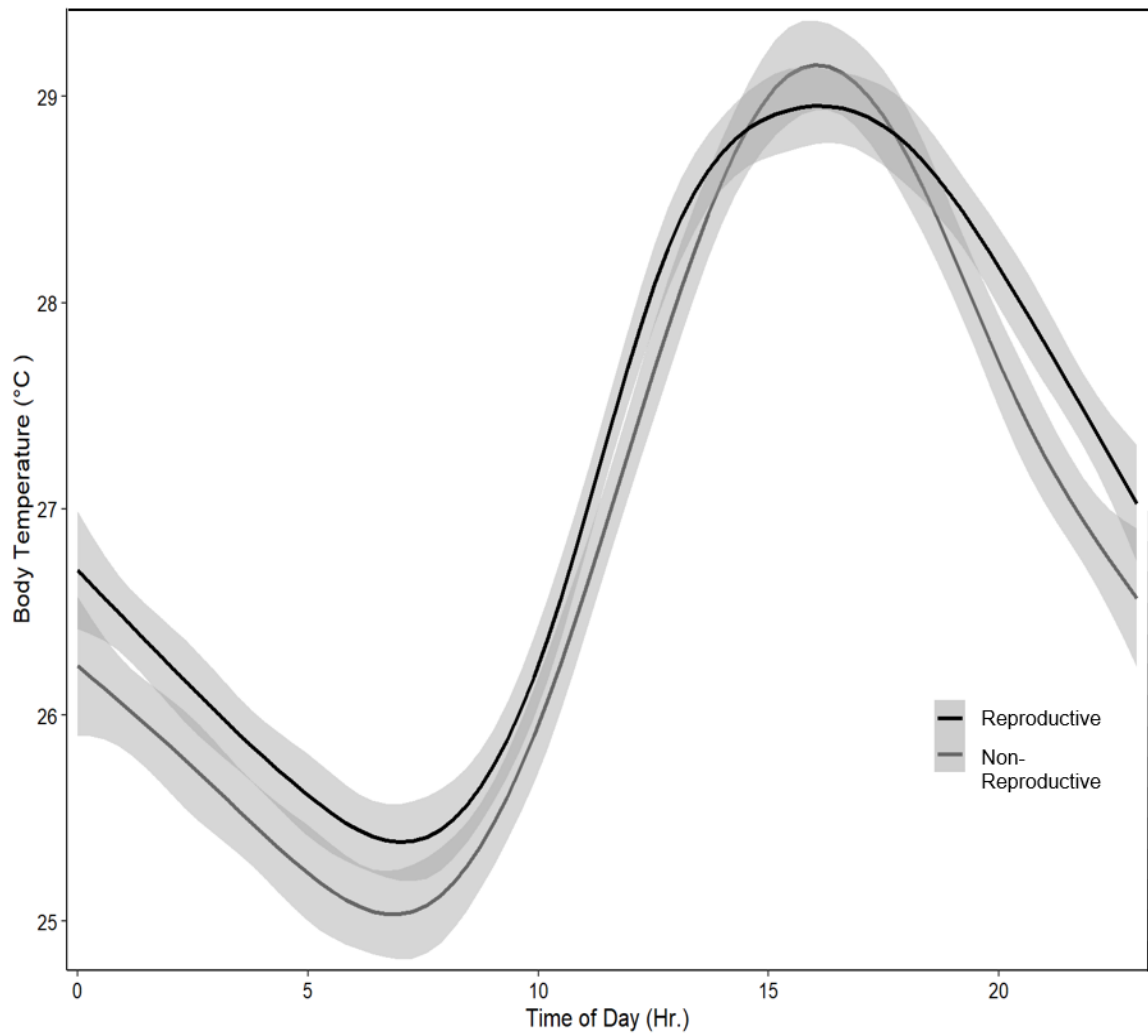


Figure 5. Reproductive and non-reproductive female *Nerodia sipedon* body temperatures over the pregnancy period smoothed with the GAMM function to better fit the body temperatures to a normalized curve.

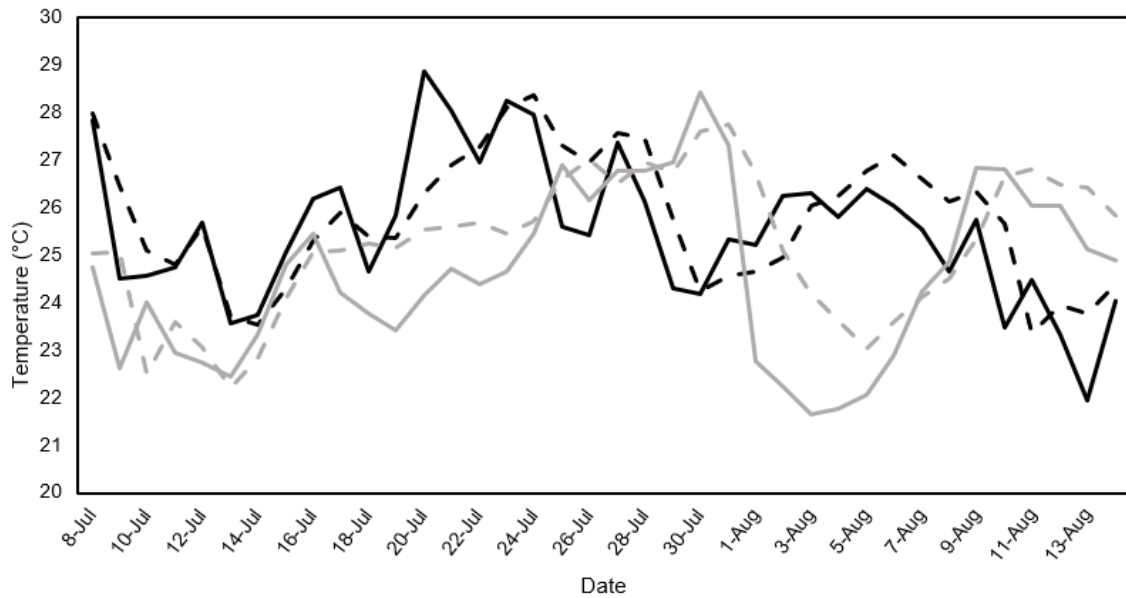


Figure 6. Air and water temperatures for 2021 and 2022 during the pregnancy period for *Nerodia sipedon*. 2021 temperatures are represented by grey solid line (air) and grey dotted line (water). 2022 temperatures are represented by black solid line (air) and black dotted line (water). The overall average air temperature for 2021 was 24.7°C and 2022 was 25.3 °C. The overall average water temperature for 2021 was 24.2°C and 2022 was 25.4°C.

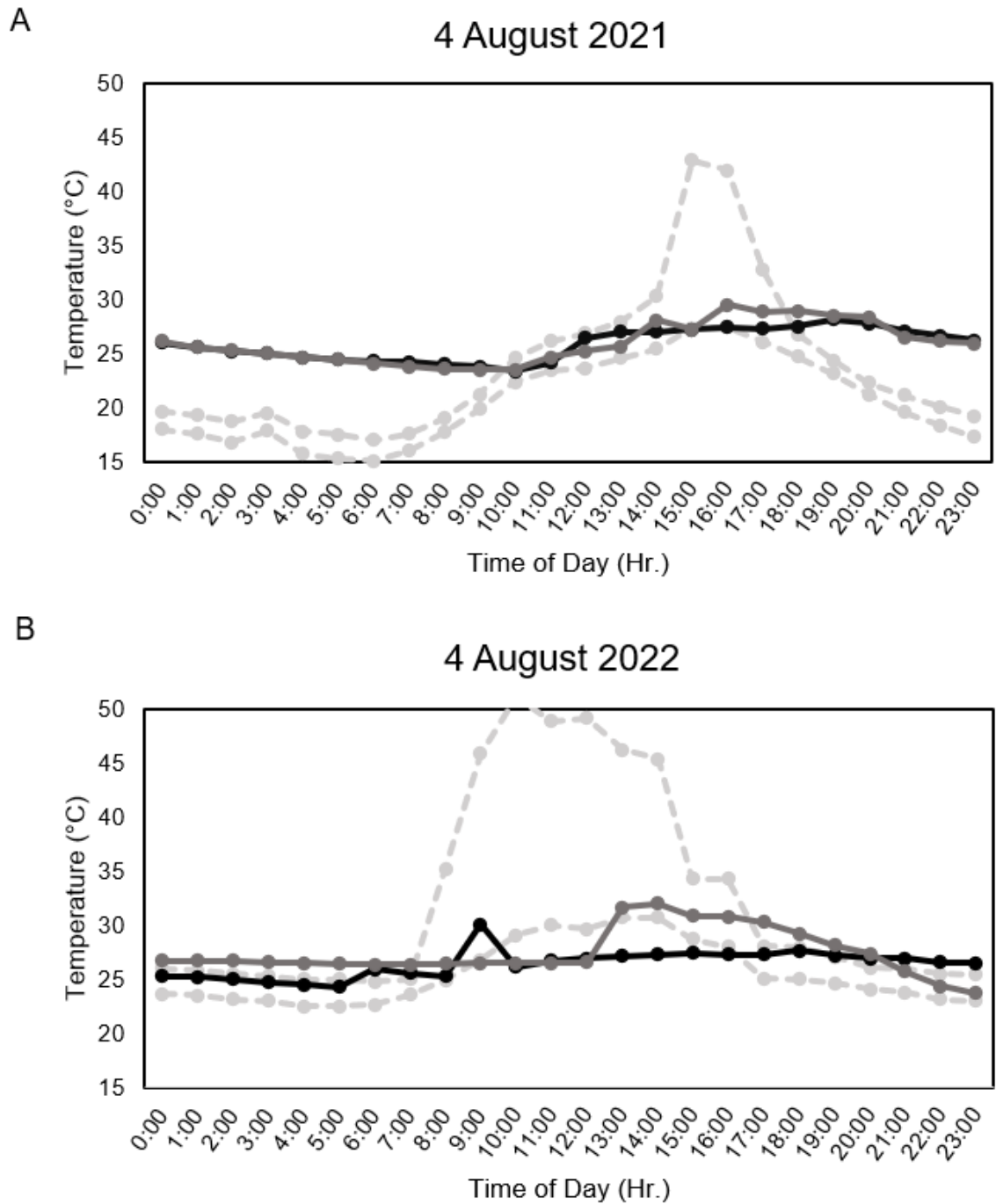


Figure 7. Daily mean body temperatures for reproductive (black solid line), non-reproductive (dark grey solid line) female *Nerodia sipedon* and operative model maximum and minimum temperatures (dotted light grey lines) on 4 August 2021 (A) and 4 August 2022 (B).

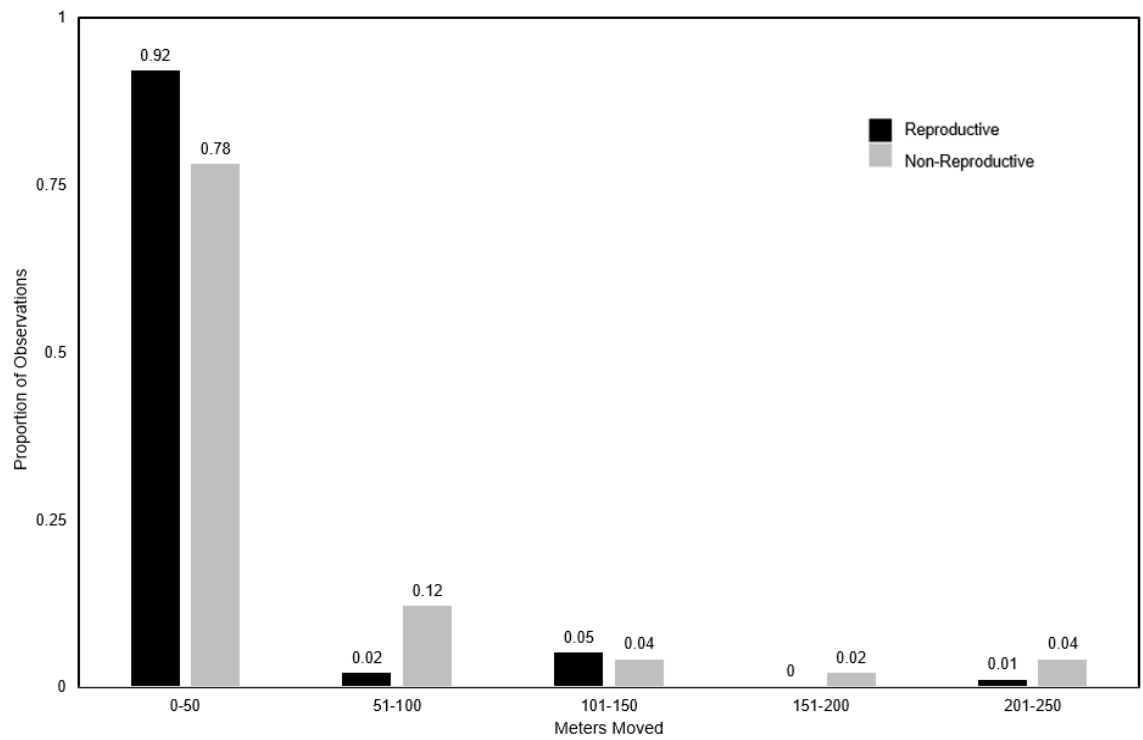


Figure 8. Proportion of reproductive and non-reproductive female *Nerodia sipedon* daily movement over the pregnancy period for 2021 and 2022.

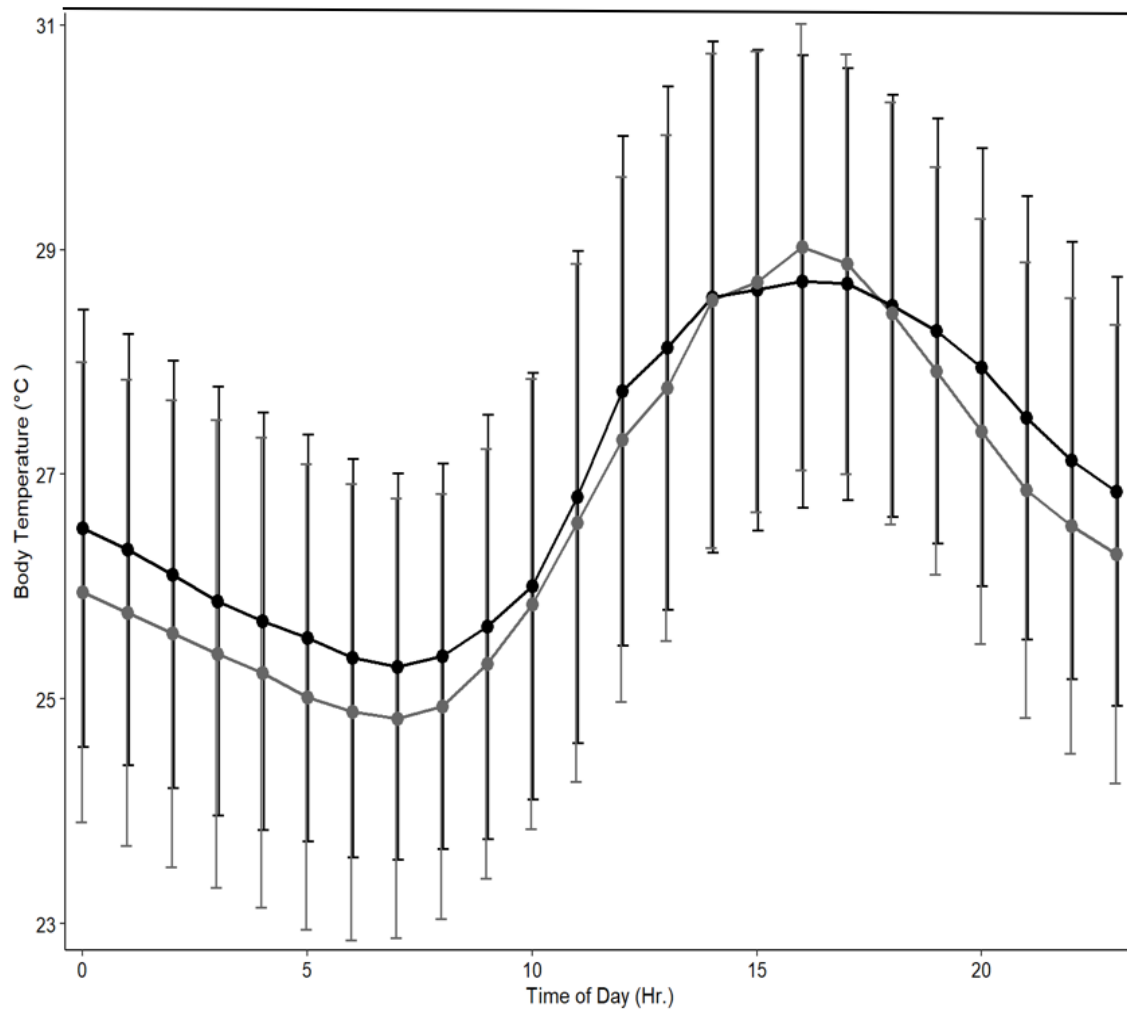


Figure 9. Seasonal mean body temperatures over a 24-hour period for all *Nerodia sipedon* during the pregnancy period. Reproductive females are indicated by the black line and non-reproductive females are indicated by the grey line.

APPENDIX C: IACUC APROVAL FORM

IACUC

INSTITUTIONAL ANIMAL CARE and USE COMMITTEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129



IACUCN006: FCR PROTOCOL APPROVAL NOTICE

Tuesday, April 20, 2021

Senior Investigator	Vincent Cobb (ROLE: Principal Investigator)
Co-Investigators	Alexis Hamous
Investigator Email(s)	<i>vincent.cobb@mtsu.edu; amh2gf@mtmail.mtsu.edu</i>
Department	Biology
Protocol Title	<i>Behavioral Ecology of Common Watersnakes</i>
Protocol ID	21-3008

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the REVISED animal use proposal identified above under the **Full Committee Review (FCR) mechanism**. The IACUC initially met on 4/9/2021 to determine if your proposal meets the requirements for approval. The IACUC recommended approval pending to your clarification on missing animal number statistics. Upon receipt of your clarification on 04/12/2021, the IACUC has determined through an unanimous vote that your animal use protocol meets the guidelines for approval in accordance with PHS policy. In view of the current COVID-19 crisis, the IACUC also introduced a few restrictions. A summary of the IACUC action(s) and other particulars of this protocol are tabulated below:

IACUC Action	APPROVED for one year	
Date of Expiration	4/30/2022	
Number of Animals	TOTAL TWENTY (20) animals (refer below for species-specific numbers)	
Approved Species	Nerodia sipedon (15 animals) and Regina septemvittata (5 animals)	
Category	☐ Teaching	☒ Research
Subclassifications	☐ Classroom	☐ Laboratory ☒ Field Research ☐ Field Study
	☐ Laboratory	☒ Handling/Manipulation ☐ Observation
	Comment: NONE	
Approved Site(s)	Field Research and MTSU Vivarium: Rooms (SCI 1170L for housing)	
Restrictions	1. Must comply with all FCR requirements; 2. Mandatory compliance with CDC guidelines during COVID-19; Social distancing guidelines are made by the Dean of CBAS. 3. The PI must make alternative plans to ensure proper animal care, including euthanasia if needed, in the event the research team is quarantined due to COVID19	
Comments	NONE	

IACUCN006

Version 1.3

Revision Date 05.03.2016

This approval is effective for three (3) years from the date of this notice **till 4/30/2024**. The investigator(s) MUST file a Progress Report annually updating the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be **approved** by the IACUC prior to **4/30/2021** for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	3/31/2022	NONE
Second year report	3/31/2023	NONE
Final report	3/31/2024	NONE

Post-approval Amendments:

Date	Amendment	IACUC Notes
04/19/2021	Student worker Alexis Hamous is added	Administrative Action

Post-approval Actions:

Date	Amendment	IACUC Notes
NONE	NONE	NONE

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. In addition, refer to MTSU Policy 129: Records retention & Disposal (<https://www.mtsu.edu/policies/general/129.php>) for Tennessee State requirements for data retention. Please be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

Compliance Office
(On behalf of IACUC)
Middle Tennessee State University
Tel: 615 494 8918
Email: iacuc_information@mtsu.edu (for questions) and
iacuc_submissions@mtsu.edu (for sending documents)