

THE OVERWINTERING ECOLOGY OF A COLD-ADAPTED AND REFUGIA-
DEPENDENT RATTLESNAKE

BY

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DISSERTATION

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ABSTRACT

My dissertation examines several behavioral strategies used by refugia-dependent reptiles, focusing on imperiled Timber Rattlesnakes (*Crotalus horridus*), to successfully navigate and survive harsh temperate winters. My findings expand on previous work on the overwintering strategies of *C. horridus* and provide insights into the ecology of refugia-dependent snakes valuable for conservation, management, and further study.

In the first data chapter (chapter two), I identified the overwintering habitat characteristics of *C. horridus* in Illinois and determined the distribution of likely hibernacula habitats throughout the state. Using habitat suitability modelling, I identified slope angle, topographical position index, forest patch area, and aspect as important drivers of hibernacula habitat. Together, the variables and site surveys suggest suitable overwintering habitat for *C. horridus* in Illinois is distributed primarily in southern Illinois and throughout the Mississippi and Illinois River border counties. My findings add to the current understanding of the species' overwintering requirements and provides a foundation for future ecological studies, management, and survey efforts to find new hibernacula throughout Illinois.

In the second data chapter (chapter three), I identified environmental cues of spring emergence for *C. horridus* to determine the species' emergence phenology and examined the applicability of identified cues in predicting emergence across the species' range. The most parsimonious model for predicting spring emergence included the additive effects of maximum daily temperature, accumulated degree days, and latitude. With notable exceptions in the southeastern U.S., the model accurately predicted the average emergence day for eight other populations range-wide, emphasizing the importance of temperature in influencing the phenological plasticity observed across the species' range. The apparent broad applicability of

the model to other populations suggests it can be a valuable tool in predicting spring emergence phenology.

In the third data chapter (chapter four), I demonstrated the existence, phenology, and likely purpose of the fall and spring transient periods— “transitional” periods between summer and overwintering periods— for *C. horridus* in west-central Illinois. My results suggest the transient periods can last upwards of a month, are phenologically flexible in response to variable annual temperatures, and serve as necessary buffers to ensure snakes have reliable and swift access to refugia when temperatures surpass lower thermal tolerances. Spring transient periods result from post-emergent snakes waiting at overwintering sites for a second thermal trigger— alongside the initial cue for egress—which signals the arrival of consistently warm temperatures appropriate for summer activity. Fall transient periods result from snakes intentionally arriving at refugia at higher temperatures to avoid being caught in summer habitats when temperatures fall below thermal limits. My findings complement and expand previous work on the overwintering strategies of *C. horridus* and provide further insights into an understudied, but important, aspect of the annual life cycle of refugia-dependent and migratory snakes.

For my wife, Alexandra, my father, Peter, and my Auntie, Susan.

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CHAPTER ONE: INTRODUCTION

1.1 GENERAL BACKGROUND

Ectotherms are capable of minimal thermogenesis and rely on external convective, conductive, and radiative heat sources to maintain optimum body temperatures (Bartholomew 1982). Key variables involved in the thermal ecology of ectotherms include an organism's behavior (Stevenson 1985), physiological capacities (Angilletta et al. 2002, Huey and Stevenson 1979), and environmental conditions (Schulte et al. 2015, Wieser 1973). All three variables synergistically aid thermoregulation (Huey 1982), but the physical environment sets fundamental limits on an individual's capacity to affect body temperature, exemplified by the marked decrease in species abundance in higher latitudes where exposure to lower average and extreme temperatures prevents effective thermoregulation (Lowe and Vance 1955). Despite such thermal impediments, ectotherms persist—and even thrive—in temperate regions where winter temperatures frequently fall well below physiological tolerances (Sunday et al. 2011, 2014).

Reptiles employ two generalized cold-hardiness strategies to survive winter: freeze tolerance and freeze avoidance (Storey 1990). Freeze tolerance strategies involve ice formation in extracellular fluid spaces, characterized by mechanisms such as the production of ice nucleators and cryoprotectant substances, which control ice propagation within the body (Costanzo et al. 1995, Storey and Storey 1992). Conversely, freeze avoidance strategies involve evasion of extracellular freezing altogether. Some temperate reptiles can depress body temperatures below freezing point using anti-freeze mechanisms for supercooling (Storey 2006), allowing temporary exposure to sub-zero temperatures. Supercooling is particularly beneficial for temperate reptiles subject to short-term temperature fluctuations (e.g., low night temperatures) but is not practical for surviving prolonged cold exposure (Costanzo and Claussen

1990, Voituron 2004). Instead, long-term survival at ambient temperatures below thermal tolerances is best achieved by seeking thermally insulated subterranean refugia (Sunday et al. 2014; used interchangeably with “hibernacula” throughout). Though buffered from external conditions, occupants within refugia are still subject to surface temperatures (Brown et al. 1982, Claussen et al. 1991, Firth 1998). Thus, as the ambient temperature falls, refugial temperatures and body temperatures of reptiles within refugia also fall, suppressing metabolic rates (Ultsch 1989) and allowing individuals to survive for prolonged periods in a state of cold-induced dormancy called brumation (Gregory 1982).

The dependency on overwintering refugia for extended periods presents terrestrial reptiles with the challenge of locating thermally adequate refugia each winter cycle. Availability of such overwintering refugia can limit a species' distribution (Harvey and Weatherhead 2006), particularly for taxa that cannot create their refugia. Such refugia often include those created by other animals (e.g., Harvey and Weatherhead 2006, Hyslop et al. 2009) or natural refugia such as root holes, natural caves, or the interstitial spaces left by rockfalls and erosion. Refugia with adequate thermal protection are often geographically limited (Browning et al. 2005), particularly in more northern latitudes where sub-zero temperatures increase the depth of frost lines. Consequently, several high-latitude species have adapted to communal brumation (Ultsch 1989), resulting in large multi-species congregations in a refugium.

Such limitations often mean brumating reptiles exhibit high fidelity to refugia (Gregory 1982). Some taxa—particularly snakes—often travel several km from seasonal ranges to return to the same refugium each winter cycle (Brown 1993, Zappalorti et al. 2015). Evidence of high fidelity and multi-species residency indicates such refugia have desirable characteristics required for overwinter survival (Browning et al. 2005, Keller and Heske 2000, Prior and Weatherhead

1996). Defining such characteristics is crucial for understanding the overwintering requirements and distribution of hibernacula and can assist in identifying critical habitats warranting special conservation concern. However, the distribution and drivers of overwintering habitats for many refugia-dependent reptiles are unknown.

In addition to finding suitable refugia, long brumation periods restrict vital active-season pursuits, including physiological maintenance, foraging, and reproduction. For example, high winter juvenile mortality in several species is associated with insufficient fat reserves due to inadequate foraging before brumation (Smits and Yorke 1980). Furthermore, parturition of several northern pit vipers can be delayed during seasons with unusually cool summers, forcing females to brumate with embryos that are then presumably aborted (Martin 1993, Viitanen 1968). In fact, compared to the continuous breeding cycle of most tropical species, winter interrupts the reproduction of most temperate pit vipers, resulting in late summer or fall mating which is temporally dissociated from the time of fertilization during the following spring (Aldridge and Duvall 2002). It is, therefore, advantageous for reptiles to limit the duration of brumation. Determining when to emerge from refugia relies on the ability to interpret when above-ground conditions are survivable. Such a decision is particularly complicated in temperate climates because of their stochastic environmental conditions.

While studies have proposed several cues for emergence, including physiological thresholds (Angilletta 2009), endogenous rhythms (Lutterschmidt et al. 2006, Weatherhead 1989), rainfall/humidity (Viitanen 1967), and photoperiod (Rismiller and Heldmaier 1982), the most prevalent for temperate reptiles is temperature. Several studies propose ectotherms retreat into refugia when specific temperature thresholds are surpassed. Reported thresholds often range from 8–15 °C (e.g., DeGregorio et al. 2017, Weatherhead 1989), perhaps indicative of voluntary

thermal minima of certain species. Some studies propose egress occurs as reptiles follow warm thermal clines to the surface (Macartney et al. 1989, Sexton and Marion 1981), while others suggest a temperature trigger within refugia (Jacob and Painter 1980) resulting from rising external temperatures or air currents transporting warm surface air into subterranean passages. If spring emergence in temperate reptiles is prompted by temperature, emergence phenologies should be predictable using local climatic data. However, specific emergence predictors for many temperate reptiles remain unclear. Furthermore, few studies examine the applicability of their results across populations, particularly for wide-ranging species exhibiting a high degree of phenological plasticity (Blouin-Demers et al. 2000, DeGregorio et al. 2017, Gregory 1982, Martin 1992). The lack of phenological schedules for such species limits our understanding of overwintering ecology and restricts effective conservation and land management (e.g., prescribed burns, tree thinning, invasive species removal).

Alongside deciding when to use overwintering refugia, some taxa—particularly snakes—may also conduct active-season activities (e.g., foraging and mating) away from their refugia and thus must decide when to undergo annual fall and spring migrations to and from summer habitats and overwintering sites (Brown 1993, Shine and Mason 2004, Zappalorti et al. 2015). Migration poses a complex phenological challenge to refugia-dependent snakes as they must time their ingress and egress into and from refugia and their inbound and outbound migrations to maximize restricted active seasons and avoid lethal external conditions. Several snake species exhibit asynchronous timing between their arrival to hibernacula and ingress in the fall and egress from hibernacula and departure to summer habitats in the spring (Brown 1992, Gregory 1982). During the resulting intermediary "transient" periods, snakes reportedly occupy areas near hibernacula bounding the overwintering period and may even shuttle between the surface and refugia during

warmer periods throughout the winter (Jacob and Painter 1980, Nordberg et al. 2016, Plummer 2002).

Several hypotheses have been proposed as to the purpose and duration of transient periods, including stimulating ecdysis (Shine 2001), facilitating mating (Shine et al. 2000), enhancing physiological processes (Angilletta 2009, Gregory 1982), or purging the gut of food before ingress (Sealy 2002). However, most studies suggest transient periods represent "waiting periods" to address temperature stochasticity (Martin 1992, Viitanen 1967). Specifically, snakes may arrive at refugia early in the fall to avoid being caught too far from the hibernacula when temperatures drop and may depart from the vicinity of the hibernacula later in spring for similar reasons. In short, transient periods might be coincidental to a survival strategy used by refugia-dependent and migratory snakes to navigate the stochastic temperate fall and spring periods. However, while plausible, such a hypothesis is rarely investigated, and whether transient periods are common is poorly documented.

1.2 STUDY SPECIES AND CHAPTER OBJECTIVES

A species illustrating the challenges faced by refugia-dependent ectotherms is the Timber Rattlesnake (*Crotalus horridus*), a forest-dwelling pitviper endemic to the eastern United States (Figure 1.1). Persisting historically as far north as southeastern Canada and as far south as the Gulf of Mexico, *C. horridus* occupies the largest geographic range of any rattlesnake (Figure 1.2), although populations have experienced drastic declines due to habitat loss and human persecution (Brown 1993, Clark et al. 2008). Consequently, the species is considered extirpated in Maine, Rhode Island, and Ontario and is currently threatened or endangered in 14 of the 30 U.S. states within its range (Martin et al. 2008). Southern *C. horridus* typically brumate singularly or in small groups in a wider diversity of overwintering structures such as animal

burrows, root holes, tree stumps, and logs (Andrews and Waldron 2017). Individuals are also subject to shorter overwintering periods, such as those in South Carolina (Andrews and Waldron 2017), where spring egress and fall ingress occur in March and November/December, respectively.

Conversely, northern *C. horridus* populations are subject to more prolonged and harsher winters and thus brumate for up to 8 months (September–May; Brown 1993), leaving only ~ 4 months for vital activities, and are limited to overwintering refugia which provide greater thermal protection. Dependency on refugia throughout its higher latitude locations has dictated the species' life history. For example, *C. horridus* centers its activity around communal refugia (Brown 1993, Martin 1992), with seasonal movements limited to 1–3 km between den sites and foraging/breeding grounds in the fall and spring. Furthermore, northern populations have adapted to communal brumation, resulting in large congregations of up to 200 philopatric individuals (Brown 1993) in a single refugium.

Objective One (Chapter Two). — Information on the overwinter habitat requirements and distribution of northern *C. horridus* is predominantly clustered in the north-east of the species range (Brown 1992, Martin 1992, Sealy 2002, Reinert 1988; but see Browning 2005) and is particularly sparse throughout the upper Midwest (e.g., Bielema 2022). Northern Refugia typically occur on south or west-facing outcrops, talus slopes, and bluffs (Brown 1993, Reinert 1984; 1.3), consisting of one or more entrances (holes/crevices), which form a network of underground conduits and chambers. Refugia occur in both open and heavily shaded locations but are almost always near exposed habitats such as prairies, ridgelines, and gaps in the tree canopy (Martin 1993), which are often used temporarily by individuals during the fall and spring transient periods and by gravid females during summer gestation (Brown 1991, Gardner-Santana

and Beaupre 2009). Despite its status as a threatened species in Illinois, the critical hibernacula habitat for *C. horridus* is unknown, severely limiting our understanding of the species' overwintering requirements and restricting effective conservation and management across the state.

In chapter two, I identified the overwintering hibernacula characteristics of *C. horridus* in Illinois and constructed a predictive model to determine the distribution of likely hibernacula throughout the state. The results of this chapter further our understanding of the species' overwintering requirements, identify sites for conservation, management, and survey efforts throughout Illinois, and lay the foundation for long-term monitoring regimes. The chapter was published in the open-access journal PeerJ in October 2023 (Jesper et al. 2023).

Objective Two (Chapter Three). — Northern populations of *C. horridus* must also contend with restricted activity periods of 4–6 months (Brown 1992, Martin 1992). The limited reproductive potential of females exemplifies the impact of a short active season, with delayed maturity at 6.2 years old and with low mean lifetime reproductive events of ~1.7 efforts per female (Brown 2016). Although the example relates to extreme northern *C. horridus*, it reflects the selective pressures central and northern populations face. Across its northeastern range, the species shows an extraordinary degree of phenological and behavioral variation (Brown 1993, Martin 1992, Reinert and Zappalorti 1988). Further, research into the phenological cues, often anecdotal, remains critically important because they have large congregations of philopatric individuals (Brown 1993). Consequently, entire denning communities are susceptible to threats during such congregations, particularly during spring emergence, including human persecution, poaching (Galligan and Dunson 1979), and management activities (e.g., Beaupre and Douglas 2012).

In chapter three, I identified environmental cues of spring emergence for *C. horridus* to determine the species emergence phenology and then examined the applicability of the cues in predicting emergence phenology across the species' wide range. I also used my results to generate predictions of surface presence across the latitudinal gradient of Illinois, allowing examination of the intra- and inter-annual differences in predicted (untested) surface presence across a finer latitudinal scale to aid in effective management and conservation strategies. The chapter was published in the open-access journal *Endangered Species Research* in March 2024 (Jesper et al. 2024).

Objective Three (Chapter Four). — Dependency on overwintering refugia dictates the movement ecology of *C. horridus*, characterized by migrations of typically 1–3km between summer foraging and mating habitats and communal overwintering sites to which individuals show high fidelity (Brown 1992, Eckert and Jesper 2024, Petersen et al. 2019). Migration is necessary for northern populations of *C. horridus* to access often restricted resources while reducing conspecific competition of individuals using geographically limited communal refugia (Eckert and Jesper 2024, Harvey and Larson 2020). However, to survive, snakes must navigate stochastic spring and fall temperatures by determining when to conduct seasonal migrations and access refugia without overly restricting already limited active seasons.

To overcome this phenological challenge, *C. horridus* has reportedly adapted to using fall and spring transient periods, which may serve as necessary buffers which ensure snakes have reliable and swift access to refugia when temperatures surpass lower thermal tolerances. While plausible, information regarding the duration and phenology of the species' transient periods is sparse and varies dramatically from over a month to not at all (Brown 1992, Martin 1992). Nevertheless, the apparent existence of the fall and spring transient periods, which would further

restrict limited active seasons, suggests they are ecologically necessary and warrant further study.

In chapter four, I demonstrate more clearly the existence, phenology, and likely purpose of the fall and spring transient periods for *C. horridus* in west-central Illinois. I used automated passive integrated transponder tag (PIT) readers and VHF telemetry to describe the duration, associated movements, and behavior of individuals during both the spring and fall transient periods. I then examined whether transient periods are coincidental to a survival strategy used by *C. horridus* to navigate stochastic fall and spring temperatures. My results complement and expand previous work on overwintering strategies and provide further insights into an understudied aspect of the annual life cycle of refugia-dependent and migratory snakes.

1.3 FIGURES



Figure 1.1 A male Timber Rattlesnake (*Crotalus horridus*). Principia College, Illinois, USA. 2022.

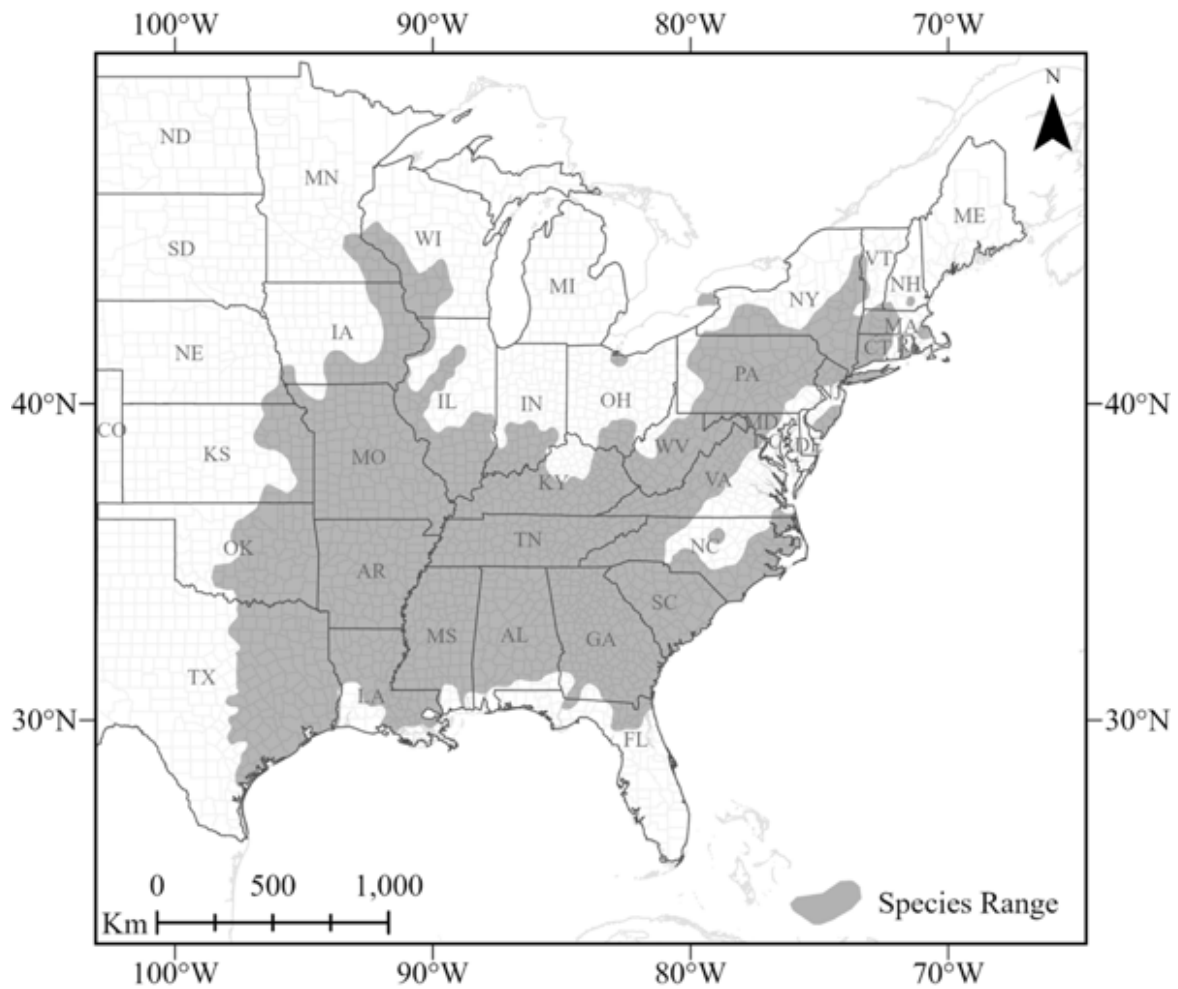


Figure 1.2 The geographic range of Timber Rattlesnakes (*Crotalus horridus*) throughout the east-central USA (Hammerson 2007).

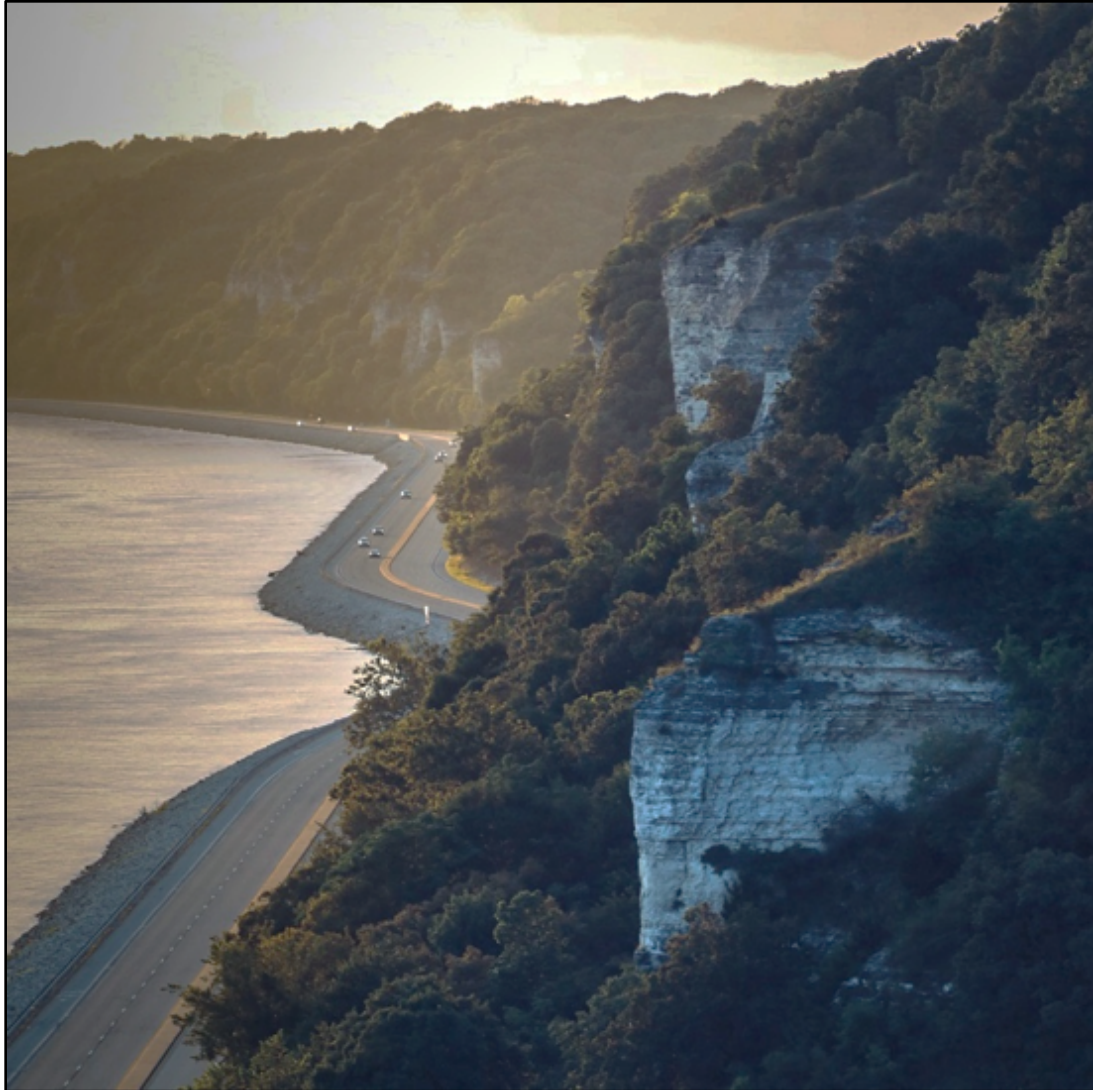


Figure 1.3 Characteristic Timber Rattlesnake (*Crotalus horridus*) overwintering habitat in Illinois: bluffs and talus overlooking the Mississippi River in Jersey County, Illinois, USA. 2022.

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CHAPTER TWO: DISTRIBUTION AND DRIVERS OF CRITICAL HIBERNACULA FOR THE TIMBER RATTLESNAKE (*CROTALUS HORRIDUS*) IN ILLINOIS

2.1 ABSTRACT

The dependency on hibernacula for extended periods presents terrestrial reptiles with the challenge of locating thermally adequate hibernacula each winter. Defining the habitat characteristics of hibernacula is crucial for understanding the overwintering requirements and distributions of hibernacula-dependent reptiles, alongside identifying habitats which warrant special conservation concern. My objectives were to identify the overwintering habitat characteristics of the imperiled *Crotalus horridus* (Timber Rattlesnake) in Illinois and to determine the distribution of likely hibernacula habitats throughout the state. Due to the initial sparsity of hibernacula records in Illinois, I adopted an iterative habitat suitability modeling process consisting of three distinct rounds of Maxent construction and revision. Each round was informed with updated information from model-guided surveys or building rapport with in-state naturalists and researchers who knew of additional hibernacula locations. I created the final model using 36 hibernacula and identified slope angle (indicative of rock outcrops and shallow soils), topographical position index, forest patch area, and aspect (decomposed into two linearized variables: *Southness* and *Eastness*) as important drivers of *C. horridus* hibernacula habitat in Illinois. Together, the five variables and site surveys suggest suitable overwintering habitat for *C. horridus* is located on south to southwest facing outcrops on upper slopes and ridges of larger forest patches. Such habitat is distributed primarily in southern Illinois and throughout the Mississippi and Illinois River border counties. My study adds to the current understanding of the species' overwintering requirements and provides a foundation for future ecological studies, management, and survey efforts throughout the state.

2.2 INTRODUCTION

The dependency on overwintering hibernacula for extended periods presents terrestrial reptiles with the challenge of locating thermally adequate hibernacula each winter. Species unable to create hibernacula often rely on uncommon or patchy landscape features, such as animal burrows or rock crevices, which limit species' departure capabilities and subsequent geographic distributions (Gregory 1982), often leading to high fidelity to specific hibernacula. Some taxa – particularly snakes – often travel several kilometers from activity season ranges to return to the same hibernaculum each winter (Brown 1993, Zappalorti et al. 2015). Several high-latitude species also overwinter communally (Ultsch 1989), resulting in large multi-species congregations in a hibernaculum. High fidelity, habitual use, and multi-species residency indicate such hibernacula have specific habitat characteristics required for overwinter survival (Browning et al. 2005, Keller and Heske 2000, Prior and Weatherhead 1996). Defining such characteristics is crucial for understanding the overwintering requirements and distribution of hibernacula-dependent reptiles and can assist in identifying critical habitats which warrant special conservation concern.

The imperiled Timber Rattlesnake (*Crotalus horridus*) is a wide-ranging pit viper dependent on hibernacula to survive critically low winter temperatures (Brown 1993, Martin 2002). Despite having the largest geographic range of any rattlesnake, spanning the eastern continental U.S. from Canada to Florida, information on the overwintering habitat requirements and distribution of *C. horridus* is sparse and predominantly clustered in the northeast of the species range (Brown 1993, Martin 1992, Sealy 2002; but see Browning 2005). Populations throughout such northerly regions use communal hibernacula which typically occur on south to west facing rock outcrops, talus slopes, and bluffs throughout upland forests (Brown 1993,

Martin 1992). Such hibernacula can occur in both open and shaded locations but are often near exposed basking areas such as prairies, ridgelines, and gaps in the tree canopy (Martin 1993). Basking areas (*sensu* Brown 1993; "transient habitat") often share similar habitat characteristics with hibernacula and are used temporarily by individuals, presumably to bask or shelter, before entering the hibernacula in fall (ingress) and immediately after emerging in the spring (egress). Gravid females also use transient habitats during summer gestation (Brown 1991, Gardner-Santana and Beaupre 2009). However, besides anecdotal reports (e.g., Bielema 2022, Breisch et al. 2021), we currently lack information regarding the overwintering characteristics and distribution of hibernacula habitat for *C. horridus* across the rest of the species' northern range.

In the past several decades, *C. horridus* has experienced drastic population declines throughout its range due to habitat loss and human persecution (Clark et al. 2008, Brown 1993). Consequently, the species is considered extirpated in Maine, Rhode Island, and Ontario and is currently threatened or endangered in 14 of the 30 U.S. states within its range (Martin et al. 2008). Despite its status as a threatened species in Illinois, critical hibernacula habitat for *C. horridus* is unknown, severely limiting our understanding of the species' overwintering requirements and restricting effective conservation and management across the state. The species' historical distribution in Illinois spanned the lower southern half of the state and throughout the western Mississippi River border counties (Smith 1961, Phillips et al. 2022). However, indiscriminate killing, hibernacula destruction (Warwick et al. 1992, Weir 1992), and landscape conversion (Yadav and Malanson 2008) has led to local extirpations (Foster et al. 2006) and range contraction (Phillips et al. 2022). Anecdotal reports on the distribution of *C. horridus* hibernacula throughout Illinois (Bielema 2022, Smith 1961, Foster et al. 2006) suggest hibernacula are limited by the extent of topographically rugged uplands where outcrops and

ridgelines intersect with most of the states' remaining forests. Due to the state's relatively flat topography, such habitat may only exist along the larger river valleys and unglaciated regions in northwestern and southern Illinois.

The objectives of my study were to: (1) identify the overwintering hibernacula characteristics of *C. horridus* in Illinois; and (2) construct a predictive model to determine the distribution of likely hibernacula throughout the state. Such information is vital to further our understanding of the species' overwintering requirements, identify sites for conservation, management, and survey efforts throughout Illinois, and lay the foundation for long-term monitoring regimes.

2.3 MATERIALS AND METHODS

I assessed the habitat requirements and distribution of *Crotalus horridus* overwinter hibernacula in Illinois using Habitat Suitability Models (HSMs). HSMs use occurrence records and environmental variables to predict the geographic distribution of a species' habitat based on its known distribution in environmental space. While there are two categories of HSMs based on the type of species occurrence data used (i.e., analyses requiring both presence and absence data versus those using presence-only), absence data for cryptic and imperiled species such as *C. horridus* is sparse due to difficulties in obtaining reliable detection and occupancy estimates. Therefore, I used Maxent (Phillips et al. 2006, Phillips et al. 2008) based on the maximum-entropy approach to modeling species distribution, which requires presence-only data. I chose Maxent due to its competitive performance, the ability to handle complex relationships between response and predictor variables, and robustness to small sample sizes (Elith et al. 2011).

Hibernacula occurrences and model iteration overview. — Determining hibernacula locations for enigmatic reptiles is difficult and is particularly challenging for *C. horridus* because

of difficult surveying conditions in topographically rugged terrain, the limited window of opportunity to locate snakes before their departure to summer habitats (spring egress) or ingress into hibernacula (Fall ingress), and low detection rates of snakes who frequently use inconspicuous locations (e.g., under rocks). Additionally, as a threatened and highly persecuted species, people are secretive in revealing known hibernacula locations to prevent potential poaching or harassment of snakes, an unfortunately common and encouraged practice across the species' range (Breisch et al. 2021, Brown 1993). Consequently, at the beginning of the study, I knew of only 13 hibernacula locations in Illinois. To bolster the sample size, I adopted an iterative HSM approach consisting of three rounds of Maxent modeling, revising the model as I determined additional hibernacula via model-guided surveys or as I built rapport with in-state naturalists and researchers who knew of or found additional hibernacula throughout the study period. I also refined the modeling procedures throughout the iterative process as I increased both the sample size and understanding of the species' overwintering requirements.

I briefly explain the data collection and modeling procedures of the first two modeling iterations below, but this chapter focuses primarily on the final (third) model iteration. Appendix A provides further details regarding modeling and field-survey methods for the first two iterations. In all cases, surveyors identified hibernacula via sightings of one or more *C. horridus* entering, or emerging from, a hole or a crevice during the species' overwintering period (September–May). Most hibernacula were vetted by follow-up visits or further study (e.g., Bielema 2022, Jesper et al. 2022). Hibernacula which were visited only once were all found during the first warm spring days, and consequently I am confident such locations were indeed hibernacula, as snakes would have had limited opportunity to emerge and depart to transient habitats prior to such dates. Surveyors recorded hibernacula locations using GPS receivers with a

horizontal accuracy of ≤ 10 m or marked them on U.S. Geological Survey topographic maps (7.5-minute series) before digitizing the locations.

In early 2019 (model round one), I created an initial Maxent model using 13 hibernacula locations (Appendix A), 10 of which were discovered during ongoing research of *C. horridus* in Jersey County between 2010–2018 (Scott Eckert, unpubl. data) and three were found via visual encounter surveys in Jo Daviess County in 1992 (Bielema 2022). To bolster sample sizes and field-validate the initial model, I conducted model-guided surveys in Spring 2019 at 24 sites in Jersey County (Figure A.1). To increase the chances of finding hibernacula, I constrained survey efforts to areas with a probability $\geq 50\%$ (as determined by a Maxent-derived C-loglog probability raster) and searched during the species' spring egress period when snakes were likely to be surface active at hibernacula. Overall, between 6–25 April, three surveyors spent 135.34 person-hours across 10 days searching for *C. horridus* (three sites per day with repeat site visits) and identified two new hibernacula during the surveys.

In 2022 (model round two), I created a revised Maxent model using 34 hibernacula locations (21 new hibernacula). Alongside the two hibernacula discovered in the first round, I included eight hibernacula located by Illinois Department of Natural Resources (IDNR) staff and in-state naturalists during (non-model guided) surveys in southern and west-central Illinois in 2020. I also included 11 hibernacula determined via opportunistic information from landowners and in-state naturalists who had located hibernacula in the past 20 years. As before, to bolster the sample size and to field-validate the revised model, I conducted model-guided surveys in northern Pope County (figure A.4) in April 2023. Learning from the previous survey efforts in Jersey County, I conducted surveys only on warmer days (daytime temps > 20 °C) during the spring egress period and made repeat visits to habitat deemed highly suitable ($P \geq 75\%$) by the

HSM to increase the chances of snake detection. Overall, I searched for 30.22 hr over 5 days (mean = 6.04 hrs/day) and found two new hibernacula.

Thus, I obtained 36 hibernacula occurrences throughout the study period distributed throughout southern, west central, and northwestern Illinois (Figure 2.1). I used all locations to create a final Maxent model (model round three), the primary focus for the remainder of the chapter.

Predictor variables. — For the final model, I created ten predictor variables based on *a priori* knowledge of the determinants of hibernacula distribution (Table 2.1), several of which I used in previous model iterations (Appendix A). Unless specified otherwise, all predictor variables were prepared as ASCII rasters using ArcGIS Pro 3.0 (Esri 2021) with a geographic extent of Illinois and a cell resolution of 10 m. I specifically used a higher resolution (30 m is commonly used, e.g., Browning et al. 2005) to capture finer topographical detail, particularly in the slope raster (see below). To avoid raster 'edge-effects,' or biased values at the edge of a raster due to N/A data values from neighborhood analyses, I created all rasters with the extent of a 5 km-buffered Illinois boundary before clipping them to the state boundary.

I prepared several LIDAR-derived predictors using a 10 m resolution Digital Elevation Model (DEM) obtained from the 3D Elevation Program ("3DEP"; USGS 2021). I calculated Slope angle (*slope*; degrees) as the maximum elevation change rate over each DEM cell and its eight neighbors using the "Surface Parameters" tool. Higher *slope* values represent steeper terrain, which studies frequently use as a proxy for rock outcrops and shallow soils (Browning et al. 2005, Garst 2007, Kakavas & Nikolakopoulos 2021). The Topographic Position Index (*TPI*; Weiss 2001) measures topographic position with positive TPI values representing upper slopes and ridges and negative TPI values representing lower slopes and ravines. I calculated TPI using

a neighborhood window size of 25 m to capture small-scale ridges and valleys throughout Illinois' dendritic drainage system.

I calculated aspect using the "Surface Parameters" tool before transforming it into two continuous (i.e., removing the circularity of aspect) and linearized variables: *Eastness*, the sine of aspect in radians (gradient between -1 = due west to 1 = due east); and *Southness*, the cosine of aspect in radians (gradient between -1 = due south to 1 = due north). I also calculated *Insolation* (Watt Hours per meters squared or W.H./m²) using the "Area Solar Radiation" tool (Rich et al. 1994, Fu 2000, Fu & Rich 2002), representing the total incoming solar radiation (both direct and diffuse) at a given cell during the species' typical overwinter period in Illinois (1 October–1 May). Higher insolation values represent greater amounts of insolation during the overwintering period. I used the following tool parameters: latitude = 39.7° (mean latitude of Illinois); sky size value = 200; day interval = 14; hour interval = 2.

I also calculated three patch-level forest metrics using the software FragStats (McGarigal 1995): forest area (*f_area*), proximity index (*f_prox*; Gustafson and Parker 1992), and shape index (*for_shape*). I defined a forest patch as an expanse of forest bounded entirely by non-forest landcover. I determined Forest patches using a modified version of the "WorldCover" landcover dataset (10 m resolution; Zanaga et al. 2022), whereby I added a "roads" class to the default landcover types using a rasterized road layer of all major and minor roads obtained from OpenStreetMaps (OpenStreetMaps 2015). The inclusion of roads reflected the fragmentation of Illinois forests, which the original landcover raster did not capture, thus more accurately defining individual forest patches. I include the landcover layer and the three forest metrics in the initial set of predictor variables. *f_area* is a forest patch's planimetric area (in hectares; ha). *for_prox* considers the size and proximity of all forest patches whose edges are within a 50 m radius of the

focal forest patch. *for_prox* = 0 if a forest patch has no neighboring forest patches, and *for_prox* ≥ 0 as the neighborhood is increasingly occupied by patches of the same type and as those patches become closer and more contiguous or less fragmented in distribution. *for_index* is a measure of the shape complexity of a forest patch and is calculated as the forest patch perimeter (m) divided by the square root of forest patch area (m²), adjusted by a constant to account for square standard (raster cells). Finally, I included percent canopy cover derived from the United States Forest Service (USFS; Coulston et al. 2012) which I resampled from 30m to 10m resolution. Upscaling from a lower to higher resolution raster should typically be avoided as it can introduce false accuracy, but I made an exception in this instance given the potential importance of canopy cover to hibernacula distribution, and since the USFS raster is the highest resolution and publically available percent canopy cover currently available for Illinois.

Addressing multicollinearity. — My study objectives were to identify the overwintering hibernacula characteristics of *C. horridus* in Illinois and to determine (predict) the distribution of likely hibernacula throughout the state. Thus, I opted to minimize multicollinearity between predictor variables to increase the interpretation of predictor response and importance. I tested for multicollinearity between all continuous predictor variables by calculating pairwise Pearson's coefficients and Variance Inflation Factor (VIF), retaining only one predictor variable from a set of moderate to strongly correlated variables ($|r| \geq 0.6$ or $VIF \geq 5$). Pearson's coefficients also allowed me to interpret the relationship between variables better, even if I did not use them in the final model. Ease of variable interpretation and parsimony (i.e., Occam's razor) are essential for effectively understanding hibernacula habitat drivers and determining likely habitats in the field. Thus, I retained predictor variables with the most biological relevance to the species and ease of

interpretation (Merow et al. 2014) and chose not to pursue dimensionality reduction methods (such as Principal Component Analysis) which would complicate variable interpretability.

Maxent procedures. — A critical assumption of Maxent is the occurrence records used for model building are derived from systematic random sampling; Maxent assumes all locations on the focal landscape are equally likely to be sampled (Kramer-Schadt 2013; Elith et al. 2011). Yet in many cases, including the present study, occurrence data derived from opportunistic observations are not randomly sampled and, therefore, almost always exhibit strong spatial bias and spatial autocorrelation in survey efforts resulting in biased model predictions (Yackulic 2013). I addressed sampling bias and spatial autocorrelation using a common approach described by Phillips et al. (2009) and Elith et al. (2010), whereby a "bias layer" is used to manipulate the selection of background data, so they reflect the same sample selection bias as the occurrence data. In doing so, the effect of sampling bias/spatial autocorrelation cancels out, allowing Maxent to focus on any differentiation between the distribution of the occurrence and the background. Following Elith et al. (2010), I created a bias layer consisting of a Gaussian kernel density of hibernacula locations rescaled from 1 to 20, where values of 1 reflect no sampling bias and higher values represent increased sampling bias. I used the resulting raster grid as a bias layer in all Maxent runs. Like the predictor variables, the background points ($n = 10,000$ random locations from the predictor rasters) and resulting bias layer were prepared with a geographic extent of the 5km-buffered Illinois state boundary.

Since feature classes and the L1-regularization multiplier can affect model fit, transferability, and predictive power (Merow et al. 2013, Elith et al. 2011, Warren & Seifert 2011), I compared models with a combination of feature classes and regularization multiplier. To select appropriate Maxent model parameters, I used the corrected Akaike information criterion

(AICc) in the R package ENMEval (Muscarella et al. 2014, R Core Team 2021). Because of the small number of occurrences records, I omitted P from the model, and thus compared the L, Q, and H features using the combinations "LHQ", "LH", "LQ", "QH", "L-only", "Q-only", and "H-only". I also examined each combination with regularization multiplier values of 1, 2, 5, 10, 15, and 20 (Warren & Seifert 2011, Shcheglovitova & Anderson 2013), resulting in 42 different model configurations for comparison. I chose the most parsimonious model as determined via AICc for further analysis.

I evaluated the relative performance of the most parsimonious model using the area under the curve (AUC) of the receiver operating characteristic (Hanley & McNeil 1982). The resulting AUC performance value ranges from 0–1, with higher values representing better discrimination between presence and background points (Hosmer & Lemeshow 2005). Due to the small sample size, I used a bootstrapping approach to resample the data into training and testing datasets. I selected the training data by randomly sampling the occurrence records with replacement, with the number of samples equaling the total number of occurrence records, and then tested the model on the occurrence records not selected. I repeated the bootstrapping procedure for 100 replicate runs and reported the results for an averaged model $\pm$ one standard deviation of the replicate runs.

I also examined model performance by graphing test omission rates as a function of the cumulative threshold averaged over the 100 bootstrap replicate runs (Phillips et al. 2006). If the model was a good fit, I expected the omission rate of training samples to be similar to the predicted omission rate. I avoided threshold-dependent performance statistics for the reasons Merow et al. (2014) outlined. Foremost, background points used by Maxent are not equivalent to absences (a common misconception in the scientific literature; Guillera-Aroita et al. 2014).

Thus, many threshold-dependent measures, such as the frequently employed Kappa and True Skill Statistic, are inappropriate for presence-only models and should not be used.

I examined how each predictor variable affected the probability of suitable hibernacula habitat and model variability using the final averaged model's marginal response curves (holding all other predictor variables at their mean values). Because I omitted the P (product) feature class from the model, the marginal response curves for each predictor completely defined the model as opposed to those depending on the values (interactions) of other predictors (Merow et al. 2013), thus making variable interpretation easier and reliable. Plot data was output by the Maxent software and then imported into R (R Core Team 2021) to create graphs using the package 'ggplot2' (Wickham et al. 2016). I also examined the relative importance of each variable in predicting suitable hibernacula habitat using jackknife tests and variable percent contributions, both output by Maxent.

Identification of potential hibernacula habitat. — I generated a Clog-log transformed raster from the final (averaged) model representing the predicted probability of suitable hibernacula habitat across Illinois (hereafter probability raster). The probability raster values ranged from 0–100%, with values closer to 100% indicating highly suitable hibernacula habitat and values closer to 0% indicating lower suitability. The high resolution (10 m) of the probability raster presented difficulties in effectively displaying habitat suitability at the scale of Illinois, so I instead presented example maps for two select regions in Jersey and Pope counties, where I and other volunteers conducted surveys using previous model iterations.

I assessed the geographic distribution of potential hibernacula habitat throughout Illinois by calculating each county's total planimetric area (ha) of suitable habitat. It was necessary to make the continuous probability raster binary by choosing a probability value below which I

considered habitat unsuitable for hibernacula and above which I considered suitable to calculate area. While thresholding should be avoided where possible (Merow et al. 2013), creating a binary raster, in this instance, allowed for a useful quantitative evaluation of potential hibernacula distribution. I used the 10-percentile training presence threshold of the final averaged model, which retains 90% of the occurrence records while removing extreme outliers. As an administrative division, calculating the area of suitable habitat by county has little ecological relevance but serves as a simple indication of the spatial distribution of potential hibernacula habitat throughout Illinois in relevant spatial units to management and conservation. I present a map detailing the area of suitable hibernacula habitat in each county.

2.4 RESULTS

Predictor Variables. — Of the initial 10 continuous predictors (9 continuous and 1 discrete; Table 2.1), I retained 5 uncorrelated variables for further analysis: *slope*, *f_area*, *Southness*, *Eastness*, and *TPI* (Table 2.2). The predictor *Slope* was positively correlated with *for_cov* ($r = 0.66$), indicating steeper slopes typically had reduced forest canopy cover. I retained *slope* due to its ecological importance as a proxy for rock outcrops. The predictor variable *f_area* was positively correlated with *f_prox* ($r = 0.82$) and *f_shape* ($r = 0.64$), indicating larger forest patches were in closer proximity to other large forest patches and were less complex in shape (i.e., more contiguous). I retained *f_area* due to its ease of interpretation over the unitless indices of the other two forest patch metrics. The predictor *Southness* was negatively correlated with *insolation* ($r = -0.63$), implying southern aspects receive more solar radiation throughout the typical *Crotalus horridus* overwintering period than northern aspects. I retained *Southness* due to its ease of interpretation and calculation over *insolation* (the computation time to run ArcGIS Pro's solar radiation tool on high-resolution data can be excessive). Preliminary examination of

predictor variables also revealed all hibernacula occurrences were within forests, thus rendering the landcover variable obsolete. Consequently, I removed the landcover predictor from the analysis and clipped the final probability raster to forests. The VIF of the remaining predictor variables were < 4 .

Maxent procedures. — The best-fit Maxent model, with 100% of the cumulative AIC_c model weight, included the quadratic and hinge feature classes with an L1-regularization multiplier of 1. The bootstrap-averaged top model received a mean AUC performance value of 0.96 (SD = 0.62), and the omission rate of training samples (closely resembled the predicted omission rate (i.e., followed a straight line, Figure 2.2), suggesting excellent model fit. Jackknife tests of relative variable importance (Figure 2.3) revealed *slope* was the most important predictor of hibernacula habitat suitability with the highest regularized training gain and percent variable contribution (79.5%; SD = 5.19%) followed by *f_area* (11.0%; SD = 4.84%), *Southness* (7.52%; SD = 3.05%), *Eastness* (1.12%, SD = 0.65%), and *TPI* (0.826%; SD = 0.78; although TPI received higher jackknife support).

Examination of marginal plots revealed hibernacula habitat suitability increased with higher *slope* (Figure 2.4A), with all hibernacula occurrences on slope angles between 17 and 47 degrees ($\bar{X} = 31.15$; SD = 7.72; Table 2.3). The high slope angle suggested outcrops and shallow soils, as all but one hibernaculum were located on or near outcrops and associated talus slopes. Hibernacula were also more likely to occur at higher values of *Southness* (Figure 2.4B) or more southerly aspects and higher values of *Eastness* (Figure 2.4C) or more westerly aspects. The mean *Southness* and *Eastness* values for all hibernacula occurrences were -0.83 (SD = 0.20) and -0.34 (0.43). When translated to cardinal directions, 34 of the 36 hibernacula records were located on south and southwest slopes, one on a south-eastern slope and one on a western slope.

No hibernacula were located on northern (N.E., N, N.W.) or due east slopes. Additionally, hibernacula habitat suitability increased with higher TPI (Figure 2.4D), with all hibernacula occurrences occurring between TPI values of -7 and 36 ($\bar{X} = 10.08$; $SD = 11.22$; Table 2.3) corresponding to upper slopes and ridges. Hibernacula Suitability was also greater with increasing values of f_area (Figure 2.4E), corresponding to larger forest patches. The low variance of each marginal plot indicated hibernacula across Illinois share relatively similar characteristics.

Identification of potential hibernacula habitat. — I used the 10-percentile training presence threshold of the final averaged model ($P \geq 52.69\%$) to create a binary ("suitable" and "unsuitable") map to quantify the area of potential hibernacula habitat in each county throughout Illinois. In general, suitable hibernacula habitat appears to be distributed across southern Illinois and throughout the Mississippi and Illinois River border counties (Figure 2.5). In southern Illinois, Union County appears to have the greatest area of potential habitat (~1139 ha), followed by the neighboring Alexander and Jackson Counties, and extending east through Johnson, Pope, and Hardin Counties (and to a less extent through Williamson, Saline, and Gallatin Counties). Potential habitat continues northward along the Mississippi River to Pike County and then along the Illinois River to LaSalle County. There is a noticeable lack of predicted habit along the Mississippi River from Pike County to Carroll and Jo Daviess Counties in northwest Illinois. Several "outlier" counties in east-central Illinois – primarily Vermilion and Coles Counties – also show a relatively high area of suitable habitat.

Visual examination of the (continuous) probability raster (examples in Figure 2.6) reveals highly probable habitat in topographically rugged forested regions, prominently featuring along south-facing outcrops, ridgelines, and bluffs, identified via bands of high probability habitat

across the landscape. I provide two example maps depicting predicted hibernacula habitat along the Mississippi River bluffs of Jersey County and interior forest outcrops of Pope County (Figure 2.6), both of which were the locations of HSM-guided surveys using previous model iterations (see also habitat photos in Figure A.5).

2.5 DISCUSSION

Suitable overwintering habitat for *Crotalus horridus* in Illinois appears to be located on south to southwest-facing outcrops on the upper slopes and ridges of larger forest patches, distributed primarily in southern Illinois and throughout the Mississippi and Illinois River border counties. My findings are consistent with other reports detailing the species' overwintering requirements across their central to northern range (Martin 1992, Martin 2002, Brown 1993, Browning et al. 2005, Garst 2007) and with anecdotal reports from neighboring states (Breisch et al. 2021). The HSM's ability to predict such habitat throughout the state is invaluable for guiding survey efforts in finding new *C. horridus* hibernacula and populations, as demonstrated during the iterative model-guided surveys in southern Jersey and Pope counties, increasing the number of known hibernacula throughout the state, as of 2023, by 11%.

Slope was the most important variable in predicting *C. horridus* overwintering habitat, corresponding to the findings of other reports (Browning et al. 2005, Garst 2007, Martin 1992, Brown 1993). Steep slopes are highly indicative of outcrops and thin soils, and most of the highly suitable areas predicted by the model, similar HSMs (Browning et al. 2005, Garst 2007), and the model-guided surveys (Appendix A) appear to be located on rocky habitats (e.g., along river bluffs, forest outcrops). Such habitat is presumably advantageous to *C. horridus*; not only does it provide abundant subterranean access via crevices and cracks at the soil-rock interface, it also provides permanent overwintering sites that are reliably available each winter, as opposed to

temporary structures such as animal burrows or root holes that are more ephemeral from year to year. Permanent "on-demand" hibernacula are desirable for *C. horridus*, particularly northern populations, which do not create their hibernacula and whose survival depends on locating these thermally adequate but geographically limited landscape features.

I suspect reliance on rocky habitats may decrease in more southerly U.S. regions where milder temperatures permit overwintering in temporary structures, including mammal burrows and rootholes (Sealy 2002). Although uncommon, anecdotal reports suggest individuals may use temporary structures as hibernacula in southern Illinois (S. Ballard, unpubl. observations; C. Feng, pers. comm.); however, such reports might be mistaking hibernacula with nearby transient habitats used by post-emergent individuals before departure to summer ranges (Brown 1992). For example, VHF-radiotelemetry of *C. horridus* at Principia College in west-central Illinois revealed post-emergent individuals often retreat into hollow trees, logs, and root holes, particularly during periods of colder temperatures (S. Eckert, unpubl. data). It is also possible such hibernacula are associated with subsurface rock crevices covered by loess (wind-blown silt), thus preventing visual outcrop detection. Further investigation into the significance of non-outcrop hibernacula is warranted.

Outcrops on south to south-west-facing aspects appear particularly important to *C. horridus* and the probability of north- or east-facing hibernacula is very low. Other studies support my findings; notably, Browning et al. (2005) found only two of 39 confirmed hibernacula in northwest Arkansas occurred on north and northeast-facing slopes. Further, of the 153 hibernacula studied by Martin (1992) in Virginia, only one faced due north, 10 faced northeast, and only 2 faced due east, with the remaining 140 on a south-southwest axis. While the milder winters in more southerly regions such as Virginia and Arkansas may permit

overwintering in hibernacula on northern or eastern aspects, it appears rare for individuals to do so.

Southern aspects are superior because they are the warmest, as seen via the correlation between the *southness* and *insolation* variables, particularly on steep upland slopes and ridgelines where rocky habitat and shallow soils restrict vegetative growth and canopy cover as indicated by the correlated *slope* and *forest cover* variables. In some cases, such habitat permits the growth of bluff prairies commonly associated with *C. horridus* hibernacula sites (Breisch et al. 2021). South-facing outcrops are, therefore, thermally superior to *C. horridus*, permitting potentially longer activity time and shallower hibernating depths (i.e., more available hibernacula; Martin 1992). Warmer overwintering sites also provide superior thermoregulatory opportunities to pre- and post-emergent snakes, helping prepare individuals for brumation (e.g., purging the gut of food) or "kick-starting" physiological processes (e.g., metabolic rates) for rapid resumption of active season activities. Such habitat also provides a thermal mosaic of rocky features for enhanced thermoregulation and a protective cover for predator evasion, optimal for use by gravid females during the summer months (Gardner-Santana and Beaupre 2009).

The persistence of *C. horridus* depends on suitable overwintering sites and appropriate surrounding habitats to support active-season activities such as mating and foraging. While the sample size is relatively small, my results suggest smaller forest patches are less suitable as hibernacula habitats (*f_area*). Since development of the theory of island biogeography (Wilson and MacArthur 2016) and its applications in mainland systems, the effects of small, fragmented forest patches on reptiles have been well-studied (e.g., Michael et al. 2008, Clark et al. 2011) and have far-reaching implications beyond the scope of my study. However, *C. horridus* and other

large-bodied snakes appear sensitive to smaller forest patches (Robinson et al. 1992, Kjos and Litvaitis 2001), thus warrant special mention.

Life history of *C. horridus* necessitates large tracts of forest to accommodate seasonal departure movements (of up to 7 km) to and from overwintering sites (Brown 1992) and long-distance mate-searching by males for females (Anderson 2015). Smaller patches of fragmented and increasingly isolated forests, therefore, reduce the ability for *C. horridus* to conduct such vital activities by: 1) creating barriers to movement and isolating populations, causing restricted gene flow and inbreeding (Anderson 2010; Clark et al. 2011); 2) direct mortality via roads (Sealy 2002, Clark et al. 2010) or increased human contact (Brown 1993); and 3) removal or restricted access of vital resources (e.g., basking areas, overwintering sites, foraging areas). Thus, while small forest patches might contain a habitat deemed suitable for overwintering, the chance of long-term population viability at such sites is severely reduced; suitable hibernacula habitat for *C. horridus* must have the correct site characteristics and be situated in surrounding forests conducive to active-season activities. Further research into the habitat requirements and spatial ecology of *C. horridus* throughout Illinois is required to elucidate these needs.

Conservation Implications. — Determining hibernacula locations for *C. horridus* is challenging. Because of their conservation status range-wide, people are understandably secretive in revealing specific hibernacula locations. In many cases, rapport and trust must be built between researchers, landowners, government/state agencies, and naturalists to collate known locations for a particular region. Surveying for unknown hibernacula is equally difficult because of harsh survey conditions, the limited time to locate snakes during spring egress and fall ingress, and low detection rates of snakes frequently using inconspicuous locations (e.g., under rocks). While I cannot lessen the challenges of secrecy, my results are valuable for

identifying new hibernacula by characterizing key habitat characteristics and mapping the distribution of likely hibernacula habitat throughout the state.

I advocate using my results to guide future surveys for new *C. horridus* hibernacula and populations, as demonstrated by surveys in Pope and Jersey Counties. Surveyors should maximize the chances of detection by using the HSM to constrain survey efforts to areas with a high probability of suitable hibernacula. Additionally, surveys should be conducted on warmer days (daytime temps > 15–20 °C) during the fall ingress and spring egress periods when individuals congregate at or near hibernacula habitat and are more likely to be surface active. Repeated site surveys are likely necessary to determine site occupancy and hibernacula locations (as well as increase the probability of detection). I encourage examination of lower probability areas whenever possible (time and effort permitted) to not bias survey efforts to only the most "optimal" habitat. For example, while unlikely, hibernacula can occasionally occur on north or east-facing aspects or the outcrops of mid-lower slopes, given all other suitable habitat characteristics. Additionally, visual encounter surveys of potential hibernacula habitat could also be accompanied by VHF-telemetry of snakes, tracking individuals back to hibernacula in the fall, or deployment of field cameras at suspected hibernacula locations, both of which have been used to locate hibernacula in west-central Illinois (S. Eckert, unpubl. data).

I hope future investigators will build on my results by continuing the iterative modeling process to create more robust and informative hibernacula HSMs as new hibernacula locations are located. Future studies should also focus on geographically broader hibernacula HSMs to examine the characteristics and distribution of *C. horridus* hibernacula habitat region-wide, although I acknowledge the logistical challenges in obtaining the number of occurrence records required for such an endeavor and the differences in habitat across their broad geographic range.

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2.7 TABLES AND FIGURES

Table 2.1 The initial set of predictor variables chosen to examine the habitat requirements and distribution of Timber Rattlesnake (*Crotalus horridus*) overwinter hibernacula in Illinois using habitat suitability modeling. Predictor variables were based on *a priori* hypotheses of the drivers of the hibernacula distribution for *C. horridus*.

Variable	Abbrev.	Units	Description
Slope Angle	<i>Slope</i>	Degrees	Maximum elevation change rate over each cell and its eight neighbors with higher slope values represent steeper terrain and can indicate outcrops and shallow soils
Insolation	<i>Insolation</i>	W.H./m ²	Sum of direct and diffuse solar radiation during the typical overwinter period of <i>C. horridus</i> in Illinois (1 October–1 May) with higher insolation values representing greater amounts of insolation
Proximity Index	<i>f_prox</i>	Unitless index	Index considering the size and proximity of all forest patches with edges within a 50 m radius of the focal forest patch. (Gustafson and Parker, 1992)
Forest Area	<i>f_area</i>	Hectares (Ha)	The planimetric area of a forest patch
Forest Shape Index	<i>f_ahape</i>	Unitless index	Measure of forest patch shape-complexity
Forest Cover	<i>f_cov</i>	Percent (%)	Percentage of the forest canopy at a given location derived from 30 m resolution USDM canopy cover data resampled to 10 m x 10 m
Topographical Position Index	<i>TPI</i>	Unitless index	Measure of slope position, positive TPI values represent upper slopes and ridges, while negative TPI values represent lower slopes and valleys/ravines using a neighborhood window size of 25 m to capture small-scale ridge-valley networks throughout Illinois
Aspect Southness	<i>Southness</i>	Cos(Radians)	Linearization of aspect by taking the cosine of aspect (in radians), resulting in a gradient between 1 (due north) and -1 (due south)
Aspect Westness	<i>Eastness</i>	Sin(Radians)	Linearization of aspect by taking the sine of aspect (in radians), resulting in a gradient between 1 (due east) and -1 (due west)

Table 2.2 Pairwise Pearson correlation coefficients between the initial set of continuous predictor variables chosen to examine the habitat requirements and distribution of Timber Rattlesnake (*Crotalus horridus*) overwinter hibernacula in Illinois. Iretained only one variable from a set of highly correlated variables (Pearson correlation coefficient ($|r|$) $\geq$ 0.6 (bold font) or Variance Inflation Factor (VIF) $\geq$ 5) for subsequent habitat suitability modeling. Definitions of each predictor variable are found in Table 2.1.

	area	eastness	for_cov	southness	prox	rad	shape	slope	tpi
area	1.00	-	-	-	-	-	-	-	-
eastness	0.00	1.00	-	-	-	-	-	-	-
For_cov	0.50	0.00	1.00	-	-	-	-	-	-
southness	0.00	-0.01	0.01	1.00	-	-	-	-	-
prox	0.82	0.00	0.59	0.01	1.00	-	-	-	-
rad	-0.11	0.00	-0.18	-0.63	-0.12	1.00	-	-	-
shape	0.64	0.00	0.75	0.01	0.62	-0.16	1.00	-	-
slope	0.34	0.00	0.66	0.02	0.40	-0.28	0.48	1.00	-
tpi	-0.02	0.00	-0.08	0.01	-0.03	0.06	-0.07	-0.01	1.00

Table 2.3 Summary statistics of the five predictor variables retained for suitability modeling (after removal of multicollinear variables) at 36 *C. horridus* hibernacula locations across Illinois. Data derived from the raster cell values (10 m resolution) in which the hibernacula were located. Definitions of each predictor variable are found in Table 2.1.

Statistic	Predictor Variable				
	<i>TPI</i>	<i>Slope</i>	<i>Southness</i>	<i>Eastness</i>	<i>f_area</i>
Mean	10.08	31.15	-0.82	-0.34	1613.36
SD	11.22	7.72	0.20	0.43	1068.96
min	-7.00	17.00	-1.00	-0.93	173.21
max	36.00	47.00	-0.36	0.39	2829.54

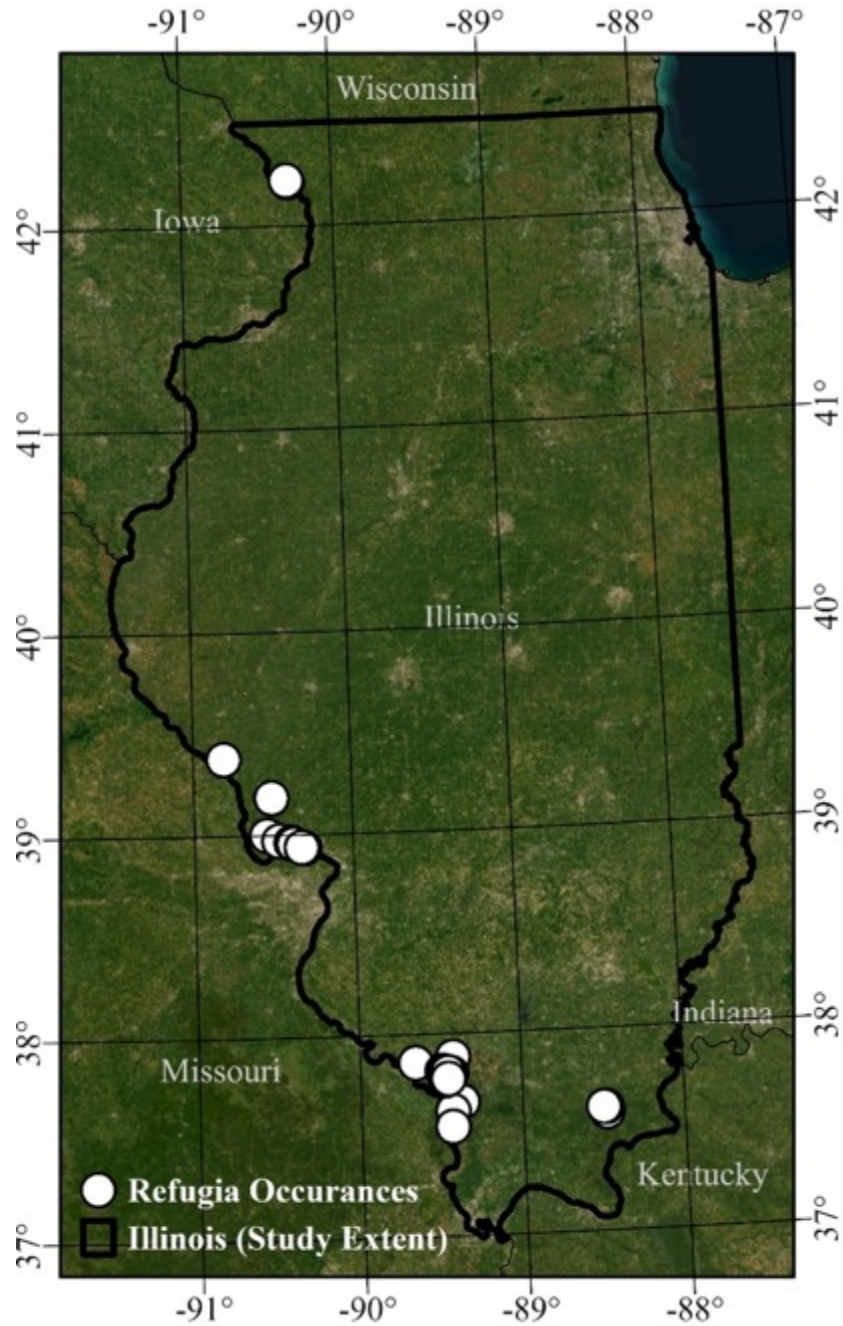


Figure 2.1 The locations of 36 *C. horridus*' hibernacula occurrences in Illinois used in the final Maxent model. Each point has been enlarged to obscure specific hibernacula locations.

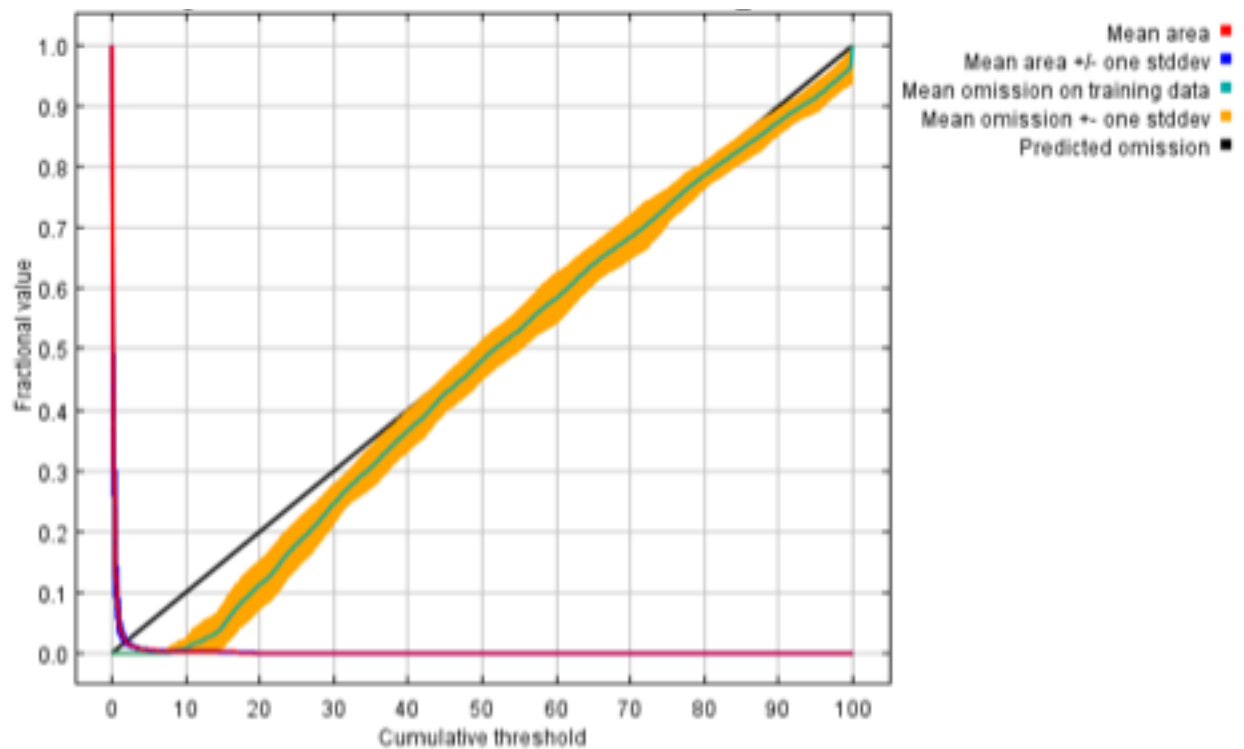


Figure 2.2 Test omission rates and predicted area for the final model as a function of the cumulative threshold averaged, over the 100 bootstrap replicate runs. The omission rate of training data should be similar to the predicted omission rate (black line), the definition of the cumulative output format.

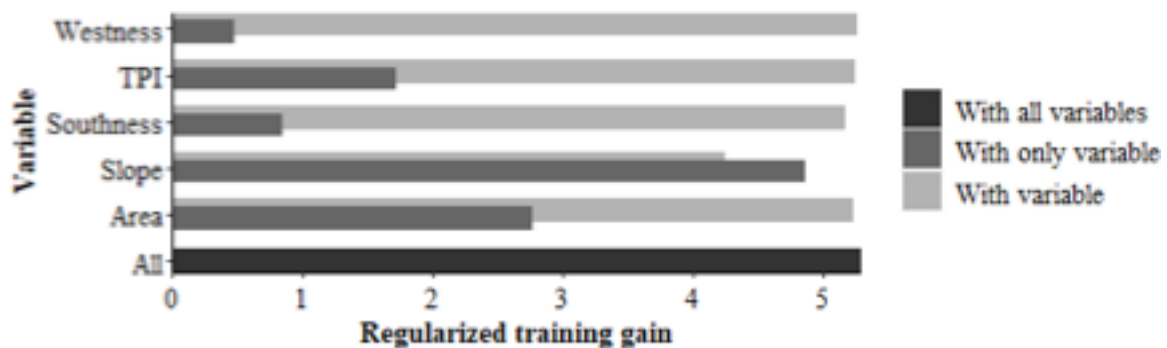


Figure 2.3 Jackknife plot of training gain for the final averaged Maxent model of 100 bootstrapped replicate runs. Dark and light gray bars indicate the training gain for a given variable when it was or was not included in the model, respectively. The black bar indicates the training gain of the global model with all variables included.

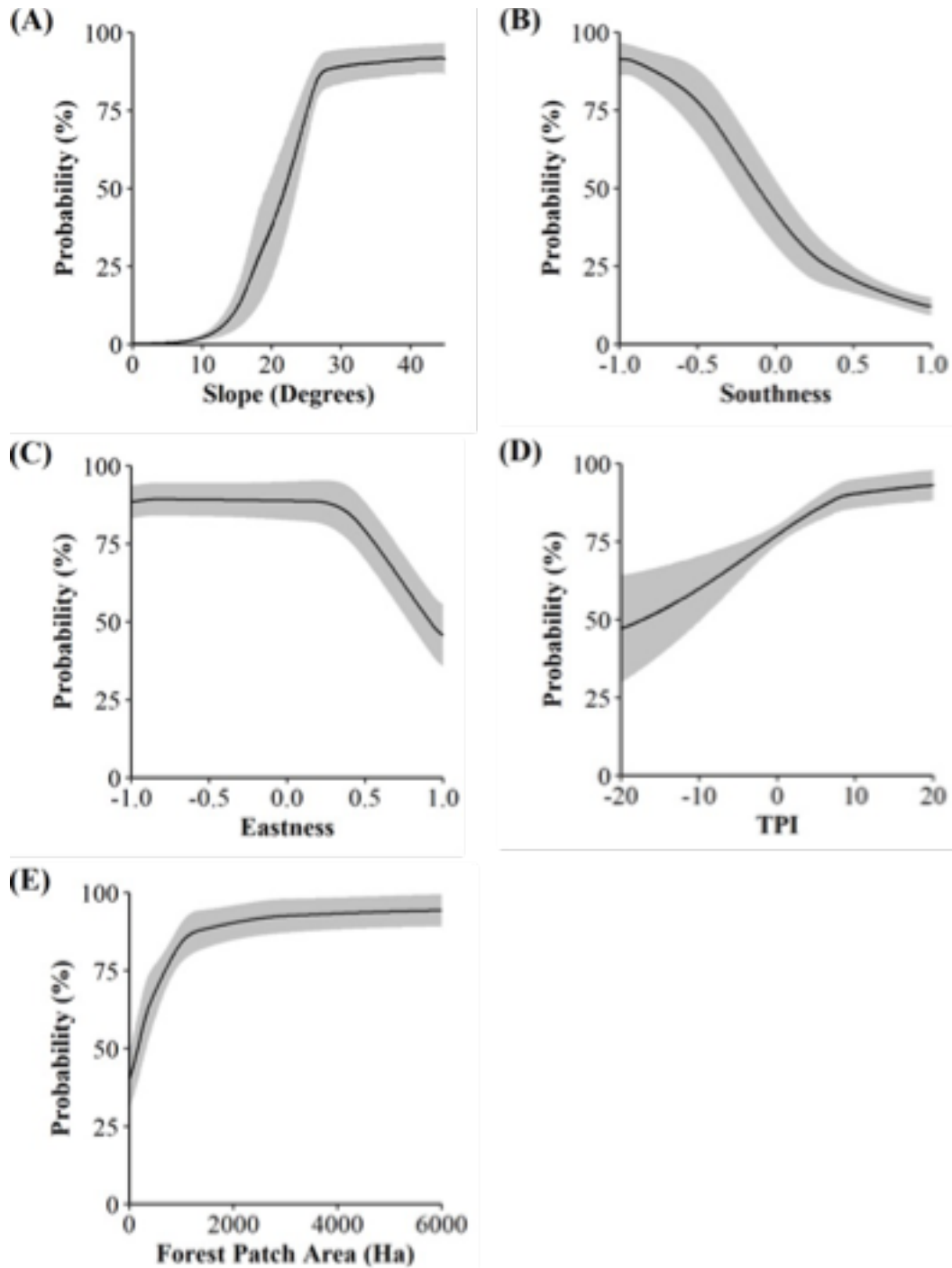


Figure 2.4 Marginal response plots for the final Maxent model, depicting the predicted probability of suitable hibernacula habitat for each predictor variable while holding all other predictor variables at their mean values. Each plot shows the mean response of 100 bootstrapped replicate runs (black lines) $\pm$ one standard deviation (grey ribbons).

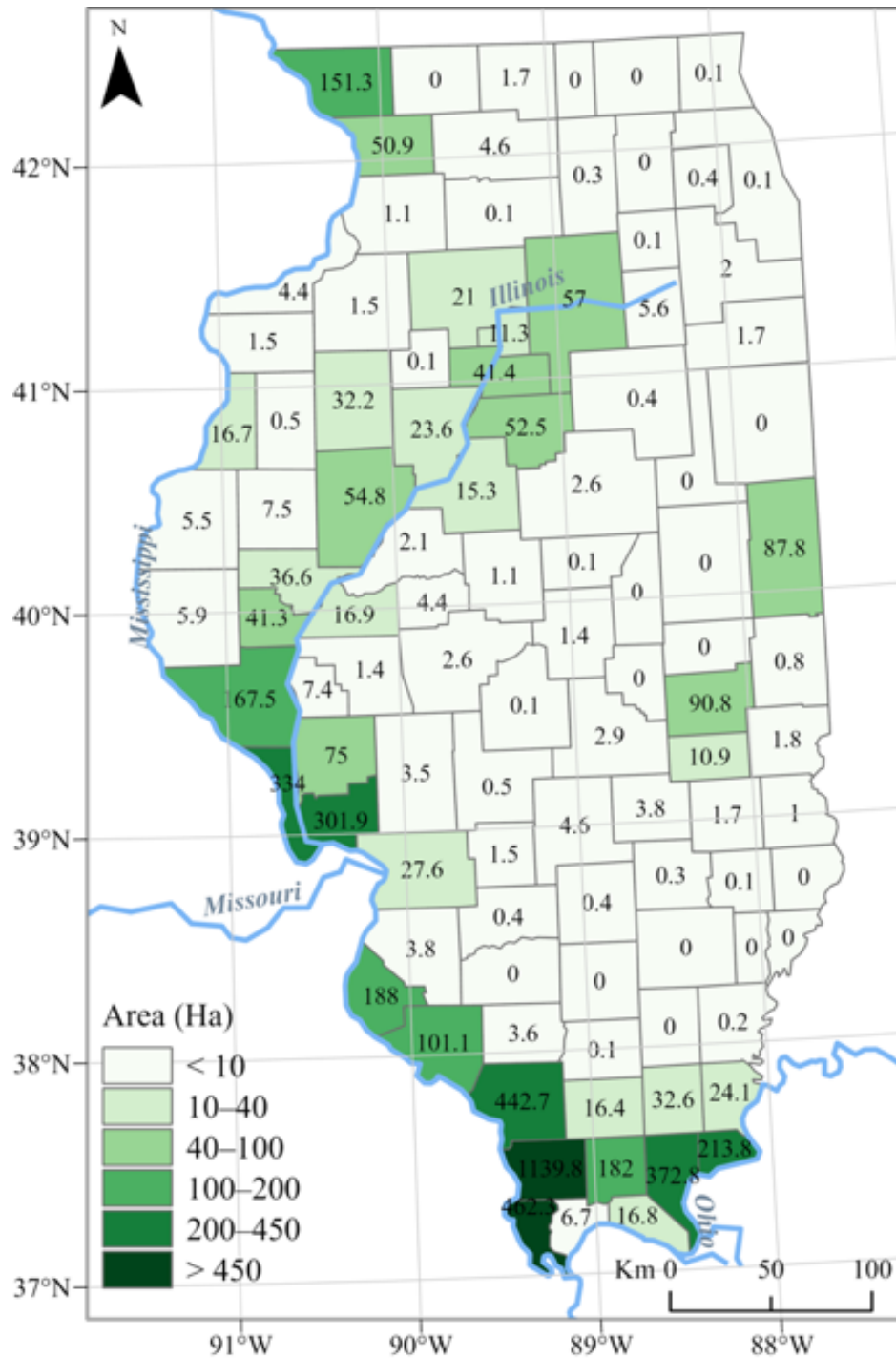
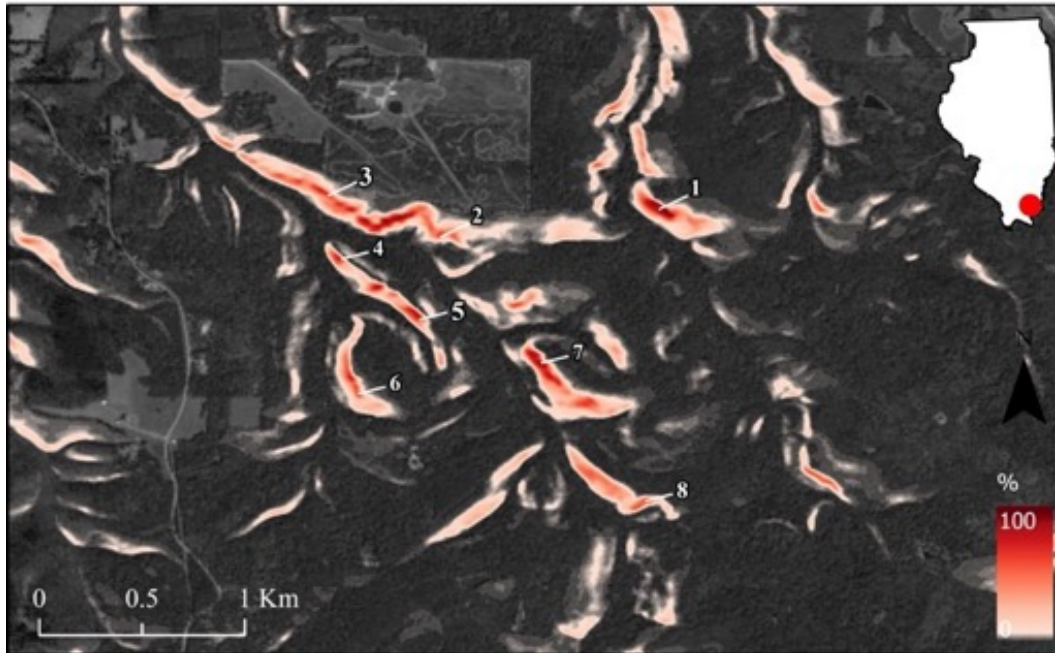


Figure 2.5 Map of Illinois detailing the total area (ha) of suitable hibernacula habitat for Timber Rattlesnakes (*Crotalus horridus*) in each county, as estimated by the final (averaged) Maxent model. Suitable habitat is defined as locations with a probability value of > 52.69%, corresponding with the 10-percentile training presence C-loglog threshold (see text). Legend breaks are colored using natural jenks to account for unequal data distribution. Major rivers (blue lines) are included as a reference.

A) Interior forest outcrops of Pope County



B) Mississippi bluffs of Jersey County

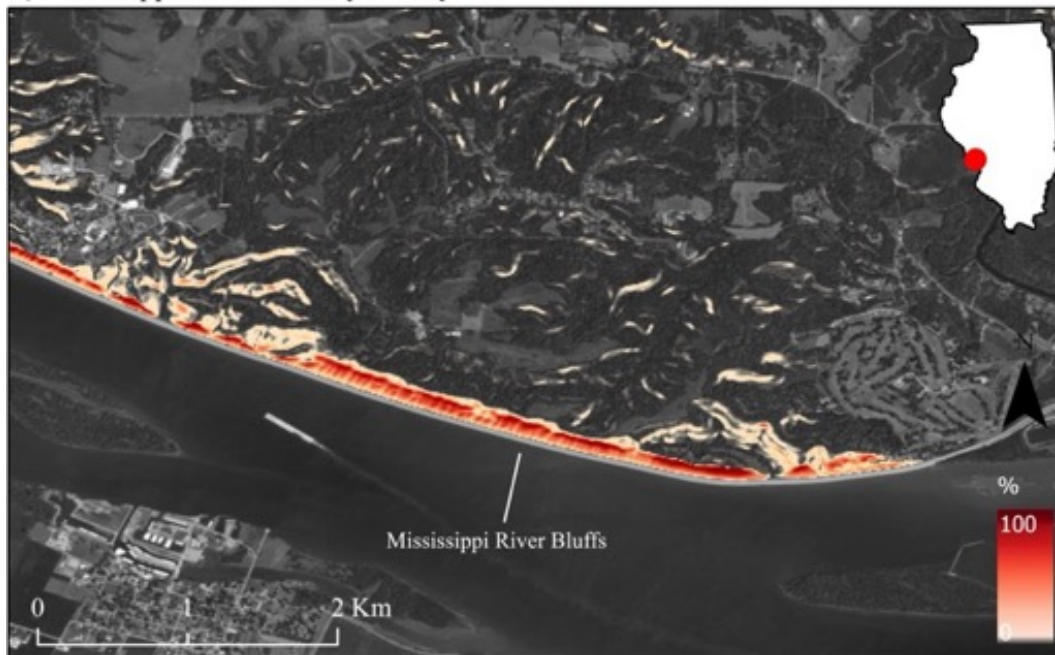


Figure 2.6 Hibernacula habitat suitability maps for the Timber Rattlesnake (*Crotalus horridus*) across two selected regions of Illinois: (A) interior forest outcrops in Pope County (southern Illinois); and (B) Mississippi River bluffs in Jersey County (west-central Illinois). The probability raster was determined using the Clog-log output of the final Maxent model with probability values of 0% made transparent to aid in map interpretation. Number labels in subplot A correspond to the locations of photos in Figure A.5, taken during model-guided surveys.

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CHAPTER THREE: PHENOLOGY AND PREDICTORS OF SPRING EMERGENCE FOR THE TIMBER RATTLESNAKE (*CROTALUS HORRIDUS*)

3.1 ABSTRACT

Many temperate reptiles survive winter by using subterranean refugia until external conditions become suitable for above-ground activity. Determining when to emerge from refugia relies on the ability to interpret when above-ground environmental conditions are survivable. If temperate reptiles rely on specific environmental cues such as temperature to initiate emergence, should expect emergence phenologies to be predictable using local climatic data. However, specific predictors of emergence for many temperate reptiles, including the Timber Rattlesnake (*Crotalus horridus*), remain unclear, limiting understanding of their overwintering phenology and restricting effective conservation and management. My objectives were to identify environmental cues of spring emergence for *C. horridus* in Illinois to determine the species' emergence phenology, and to examine the applicability of identified cues in predicting emergence phenology across the species' range. I used wildlife cameras and weather station-derived environmental data to observe and predict the daily surface presence of *C. horridus* throughout the late winter and early spring at communal refugia in west-central and northern Illinois. The most parsimonious model for predicting surface presence included the additive effects of maximum daily temperature, accumulated degree days, and latitude. With notable exceptions in the southeastern U.S., the model accurately predicted the average emergence day for eight other populations range wide, emphasizing the importance of temperature in influencing the phenological plasticity observed across the species' range. The apparent broad applicability of the model to other populations suggests it can be a valuable tool in predicting

spring emergence phenology. My results provide a foundation for further ecological enquiries and improved management and conservation strategies.

3.2 INTRODUCTION

Seasonally colder periods in temperate regions expose reptiles to temperatures exceeding critical thermal minima (Gregory 1982). A common strategy to survive such critically low temperatures is to retreat into subterranean refugia until external conditions become suitable for above-ground activity. Though buffered from external conditions, occupants of refugia are often still subject to thermal regimens that are lower than preferred (Brown 1993; Claussen et al. 1991; Firth and Belan 1998), resulting in a cold-induced dormancy (brumation) characterized by restricted physiological, behavioral, and cellular-level functions (Angilletta 2009). Brumation poses several challenges to reptiles. Despite highly depressed metabolic rates, brumation creates an energy deficit, and reptiles must survive solely on stored energy reserves. Brumation also restricts a reptile's ability to conduct vital active-season processes such as foraging, reproduction, or basic physiological maintenance (Blouin-Demers et al. 1982; Prior and Weatherhead 2000; Smits and Yorke 1980; Viitanen 1967). Reptiles must therefore limit the duration of brumation and balance the inherent risks of emerging too early with the advantages of maximizing active duration out of the refugia.

Determining when to emerge from refugia relies on the ability to interpret when above-ground conditions are survivable. Such a decision is particularly complicated in temperate climates because of their stochastic environmental conditions. While studies have proposed several cues for emergence, including physiological thresholds (Angilletta 2009), endogenous rhythms (Lutterschmidt et al. 2006; Weatherhead 1989), rainfall/humidity (Viitanen 1967), and photoperiod (Rismiller and Heldmaier 1982), the most prevalent for temperate reptiles is

temperature. Reptiles generally emerge as air temperatures rise in the spring, a phenomenon correlated with several covariates, including maximum, minimum, and mean daily temperatures (Bishop and Echternacht 2004; Brown 1992; Graves and Duvall 1990), accumulated degree days (ADD; Hoffman 2021; Turner and Maclean 2022) and moving "lagged" average temperatures (DeGregorio et al. 2017). Many species emerge only when specific threshold temperatures are surpassed (Blouin-Demers et al. 2000; Burger 2019; DeGregorio et al. 2017; Sexton and Marion 1981), although significant inter-individual variation has often confounded the identification of a reliable thermal trigger. Such thresholds likely reflect the thermal dependency of many physiological, behavioral, and cellular-level functions, which dictate the lower thermal limits of surface activity (Angilletta 2009).

If spring emergence in temperate reptiles is prompted by specific environmental cues such as temperature, emergence phenologies should be predictable using local climatic data. Wide-ranging species subject to latitudinal and elevational clines might exhibit phenological plasticity, similar to what is observed for insects (Cayton et al. 2015; Herms 2004; Uelmen et al. 2016), plants (Aslam et al. 2017) and mammals (Boutin and Lane 2014). However, local adaptation or study methods have often confounded the identification of such spatial patterns (Andrews and Waldron 2017; Blouin-Demers et al. 2000). While environmental variables, primarily temperature, likely dictate the timing of emergence, specific predictors of emergence for many temperate reptiles remain unclear. Furthermore, no study has examined the applicability of their results across populations, particularly for wide-ranging species exhibiting a high degree of phenological plasticity (Blouin-Demers et al. 2000; DeGregorio et al., 2017; Gregory 1982; Martin 1992). The lack of phenological schedules for such species limits our

understanding of overwintering ecology and restricts effective conservation and land management (e.g., prescribed burns, tree thinning).

The imperiled Timber Rattlesnake (*Crotalus horridus*) is a wide-ranging pitviper that depends on subterranean refugia for overwinter survival. Dependency on refugia throughout higher latitudes within its distribution dictates the species' ecology – exemplified by communal overwintering of up to 200 individuals (Brown 1993) and seasonal movements between refugia and summer habitat in the fall and spring (Brown 1992; MacGowan et al. 2017; Sealy 2002). *Crotalus horridus* has the largest geographic range of any rattlesnake and exhibits considerable phenological plasticity in its overwintering ecology (Andrews and Waldron 2017; Brown 1992; Martin 2002). For example, populations in warmer southern climates emerge up to 3 months earlier (March/April in South Carolina; Andrews and Waldron 2017) than northern climates (May/June in New York; Brown 1992).

Despite studies identifying various temperature-related drivers of emergence (Andrews and Waldron 2017; Brown 1992; Martin 1992), the range-wide applicability of specific cues remains unknown. Consequently, the timing of spring emergence for most populations, such as in Midwest states including Illinois, remains undefined. Such inquiries are particularly relevant for *C. horridus* throughout northern regions where philopatric individuals congregate at refugia during spring emergence and are consequently susceptible to threats including human persecution (Galligan and Dunson 1979) and management activities such as prescribed burns (Beaupre and Douglas 2012). As a slow-maturing species with infrequent reproductive events (Aldridge and Brown 1995; Bielema 2022; Brown 1991), *C. horridus* cannot rapidly recover from population declines. Therefore, knowledge of spring emergence phenology is invaluable for management and conservation purposes.

My objectives were to: 1) identify environmental cues of spring emergence for *C. horridus* to determine emergence phenology; and 2) examine the applicability of identified cues in predicting the emergence phenology across the species' range. I used wildlife cameras and weather station-derived environmental data to construct a predictive model for the daily surface presence of *C. horridus* during spring egress at two sites in Illinois. I then examined the applicability of the model in correctly determining the spring egress of other *C. horridus* populations by comparing my predictions with the average egress dates reported by other studies. I also used the model to predict surface presence across the latitudinal gradient of Illinois, allowing examination of the intra- and inter-annual differences in predicted (untested) surface presence across a finer latitudinal scale. My results provide a foundation for further ecological enquiries and effective management and conservation strategies.

3.3 MATERIALS AND METHODS

Study Site and Data Collection. — I conducted research at two over-wintering sites located ~350 km (>3° latitude) apart in western Jo Daviess County (northern Illinois; Figure 3.1) and Principia College in Jersey County (west-central Illinois; Figure 3.1), Illinois, USA. I performed all research under an approved Illinois Department of Natural Resources Endangered and Threatened Species Permit (#05-11S) and approved University of Illinois Institutional Animal Care and Use Committee protocols (IUCUC: 22167 and 22168). Determining refugia locations for the enigmatic *C. horridus* is difficult, and consequently only a few refugia locations are known throughout the state. Such refugia are typically found along larger river valleys and unglaciated regions in northwestern and southern Illinois where outcrops and ridgelines intersect with most of the states' remaining forests (Bielema 2022; Phillips, Crawford and Kuhns 2022; Smith 1961). I chose the two sites because: 1) the latitudinal difference presented an opportunity

to examine phenological differences between populations; 2) refugia were situated on private or protected lands which I had permission to access; and 3) the exact location of refugia entrances were known (Bielema 2022; S Eckert 2023, unpublished data).

Knowledge of *C. horridus* at Jo Daviess County dates to the 1930s, with locations of refugia discovered via visual encounter surveys in 1991 (Bielema 2022). Known occupancy at Principia College also dates to the 1930s with the acquisition of the land for the College, with specific entrances identified and reconfirmed via visual encounter surveys and VHF-radiotelemetry of individuals beginning in 2010 (S Eckert 2023, unpublished data). Both sites consist of upland mesic forest bounded to the south (Jersey County) and southwest (Jo Daviess County) by Mississippi River limestone-dolomite bluffs, covered with a vegetational matrix of remnant hill prairies and oak-hickory dominated woodlands. Crevices, talus, and holes along the bluff fronts at both sites provide overwintering refugia for *C. horridus*.

I monitored *C. horridus* activity at all identified refugia entrances using Bushnell HD Trophy Cameras (Model #119736) fitted with external 12V batteries for extended life. The cameras were in remote, topographically rugged locations away from local communities, and thus no people, apart from the researchers, visited the sites or were photographed. Each camera's position depended on substrate and habitat but were generally set ~1–2 m from refugia and at the same elevation as the main entrances to afford a satisfactory field of view. Preliminary investigation revealed cameras occasionally failed to photograph *C. horridus* because the passive infrared sensors (PIR) did not detect slow-moving individuals. Therefore, I supplemented PIR-triggered photos with the camera's time-lapse feature to take date and time-stamped photographs at 5 min intervals for the deployment periods. A 5 min photo interval provided high-resolution monitoring of refugia while also maximizing battery life and camera uptime. One exception to

the camera schedule occurred at Jo Daviess County during spring 2018, where cameras photographed the entrances at 1 hr intervals alongside PIR-triggered photos.

Data Analysis. — I visually inspected photographs for *C. horridus* and recorded the dates and times of snake sightings. I then converted the snake sightings of each refugium-year combination into a binomial response variable representing the presence of snakes on the surface at refugia (hereafter “surface presence”), where 1 = snake present and 0 = no snake present. I omitted days when cameras malfunctioned or when they were deployed or retrieved. I then determined differences between the days of surface presence for each refugium-year combination using bootstrapped means and 95% confidence intervals (CIs). Specifically, I resampled the ordinal days of surface presence for each refugium-year combination with replacement 10,000 times, calculated the mean for each resample, and then determined the mean and 95% CIs based on the resulting bootstrapped resampling distributions (2.5% quantile = lower CI limit; 97.5% quantile = upper CI limit). Non-overlapping CIs indicated informative differences in the effects between refugium-year combinations.

I used Generalized Logistic Mixed Effects Models (GLMMs) in the R package 'lme4' (Bates et al. 2015) to examine the effects of environmental variables (Table 3.1) on the probability of surface presence; that is, the probability of one or more *C. horridus* being present on the surface during a given day. For this study, I limited the analysis to variables derived from weather stations ≤ 30 km from each site, allowing for relative comparisons across different spatial and temporal extents. Due to the prevalence and apparent importance of temperature as a cue for emergence within the literature, I focused primarily on temperature-derived variables found to be important drivers of surface presence for *C. horridus* and other temperate reptiles, including maximum, minimum, and mean daily temperatures, five-day rolling minimum and

maximum daily temperature, day of year, latitude, and accumulated degree days of base 5 °C (ADD). Accumulated degree days is a phenological measure of seasonally increasing cumulative mean daily temperature above a selected threshold and is frequently used to predict phenological events and organismal developmental stages for a variety of taxa (Boutin and Lane 2014; Cayton et al. 2015; Herms 2004; Uelmen et al. 2016; Hoffman 2021). I calculated degree days for each day using the formula: $((T_{\max} + T_{\min})/2) - T_{\text{base}}$, where $T_{\max}$ and $T_{\min}$ are the maximum and minimum temperature for a given day, and T_{base} is a selected threshold (base) temperature. The selection of the base temperature of 5 °C represented the lowest temperature I observed surface presence of snakes throughout my study. I then summed ("accumulated") the degree day values for each sequential day, starting from 1 January, to calculate ADD over the study period. Before modeling, I Z-transformed (centered and scaled) all variables and tested them for multicollinearity using Variance Inflation Factor (VIF) analysis, removing highly correlated (VIF ≥ 5) covariates from the same model.

I created a suite of candidate models (Table B.1), including a null model (intercept and random effect only) and a fully additive global model, based on *a priori* hypotheses of drivers of surface presence. The dependent variable for each model was daily presence, the random effect structure was refugium nested within the year, and the fixed effects were a combination of the environmental variables. I included latitude as a fixed effect in all models, serving as a numerical proxy for the site, to examine potential differences between the two populations. the candidate set also included additive and two-way interactive models of the same fixed effect configurations to account for different hypotheses. For example, a significant interaction between latitude and a temperature-related variable might imply that the effect of temperature on surface presence depended on latitude (Jersey County snakes might be present at the surface at different

temperatures than Jo Daviess County). In contrast, an additive model might imply both populations responded equally to temperature, although the probability of surface presence between the two sites might differ. Finally, as demonstrated by other studies, I specified all continuous variables as quadratic terms to account for potential curvilinear relationships (Hoffman 2021).

Examination of candidate models using the R package 'AER' (Kleiber and Zeileis 2008) revealed no overdispersion, and therefore I ranked all models using Akaike's Information Criterion adjusted for small sample sizes (AICc) in the R package 'AICcmodavg' (Mazerolle 2020), and then examined the marginal and conditional effects of the most parsimonious model(s) using the R package 'effects' (Fox and Weisberg 2018). I considered parameters with 95% CIs not broadly overlapping zero as informative predictors of daily surface presence. I back-transformed the top model for interpretation and graphed the predicted values and 95% CIs using the R package 'ggplot2' (Wickham 2009). I then examined model fit via marginal and conditional coefficients of determination using the R package 'MuMin' (Barton and Barton 2015).

I examined the applicability of the top-ranked AIC_c model in correctly determining the spring emergence of other *C. horridus* populations by comparing my predictions with the average emergence dates reported by other studies. Examination of other studies also allowed me to examine the extent of phenological plasticity in spring emergence across the species' geographic range. I limited the comparisons to studies providing a detailed assessment of *C. horridus* emergence (e.g., Andrew and Waldron 2017; Brown 1992; Martin 2002) instead of briefly mentioning general dates with little empirical evidence. For each study, I used the top ranked AIC_c model to calculate predicted probabilities of surface presence for each ordinal day

from day 1 to 243, comfortably spanning the entire emergence period at each site, using environmental data gathered from National Oceanic and Atmospheric Association (NOAA) weather stations nearest to the study sites. Because missing data were present in the NOAA datasets, I calculated 15 year "normals" (averages) for estimation using the top model. Doing so afforded complete datasets and determined the typical climatic conditions and probability of surface presence on a given day for each site. Because I was interested in population-level predictions, I held the fixed effect "latitude" at its mean and set the random effects of refugium and year to zero. If the model predictions were accurate, I expected the day of year with the highest (peak) probability of surface presence at each site to correspond to the average emergence day reported by each study. To aid interpretation, I performed a simple linear regression between each study's reported average emergence day and latitude and graphed the results with the model predictions.

Given the model predictions were accurate, I also used the top-ranked AIC_c model to predict surface presence across the latitudinal gradient of Illinois for each year of the study period (2018–2020). Unlike the previous model predictions at sites which were verifiable by the results of other studies, the predictions across Illinois were untested and as such should be treated as hypothetical until verified with empirical data. Nevertheless, they served to examine the potential intra- and inter-annual differences in predicted surface presence across a finer latitudinal scale, while also providing preliminary phenological estimates for potential conservation and management schedules. To determine the site predictions, I derived the same environmental variables as before from weather stations within each degree of latitude in Illinois (37–42°) and used the top model to generate predicted probabilities of surface presence for each latitude-year combination. As before, because I was interested in population-level predictions, I

held the fixed effect latitude at its mean and set the random effects of den and year equal to zero. I determined predicted values and 95% confidence intervals using the R package 'lme4' (Bates et al. 2015). Using the 'bootMer' function, I refit the model by resampling the dependent variable, daily presence, with replacement 10,000 times, and calculated the predicted values and 95% CIs based on the resulting bootstrapped resampling distributions (2.5% quantile = lower CI limit; 97.5% quantile = upper CI limit). I present graphs of the daily predictions and 14 day moving averages (for examination of general phenological patterns) against ordinal date for each latitude-year combination and averaged across all three years.

3.4 RESULTS

Cameras were deployed at six overwintering refugia (three in Jersey County and three in Jo Daviess County) for one or more years from 2018–2020 (Table 3.2), accumulating ~473,000 photos throughout the study. In Jersey County, cameras monitored two refugia for three years and a third refugia for two years after being discovered in 2019. All refugia in Jersey County were <1km apart and situated on tree-covered talus slopes near bluff prairies. In Jo Daviess County, cameras monitored all three refugia for two years in 2018 and 2019, although I removed data from one refugium in 2019 because vegetation restricted the cameras' view and obscured observations. Jo Daviess County refugia were < 0.5 km apart and were located on open-canopy outcrops.

The dates and durations of camera deployment varied between refugia and years (Table 3.2), but all deployments successfully spanned the snake emergence periods at their respective sites. Generally, most camera records showed several weeks of no snake presence on either end of the deployment periods, although some cameras in Jo Daviess County photographed several post-emergent *C. horridus* remaining near refugia entrances at the tail-end of the emergence

periods. The individuals typically coiled in crevices within the camera's field of view and were distinguishable from other emerging snakes as they often occupied the same location each day, were the only snakes seen on the photographs at the end of the emergence periods, and in almost all cases, appeared to return to the refugia after several days of no snake sightings (see gap in days of surface presence in Figure 3.2). I suspect the snakes were gravid females who frequently remain near refugia after spring emergence until parturition. Because I was interested only in activity related to refugia use, I removed these observations from further analysis (Table 3.2; Figure 3.2).

Examination of bootstrapped 95% means and CIs revealed the daily presence of *C. horridus* at all refugia in Jo Daviess County occurred later in the spring than in Jersey County (Figure 3.2). The mean county-level presence for Jo Daviess County occurred on day 136 (16-May) compared to day 103 (13-Apr) for Jersey County. The 95% CIs also indicated intra-county differences in presence days between some, but not all, refugium-year combinations in Jersey County (Figure 3.2). Early "one-off" surface presence occurred at all refugia in Jersey County in most years (Figure 3.2), with the earliest activity occurring on day 55 (24-Feb). Cameras observed no such early surface presence in Jo Daviess County. Despite early activity, refugia in Jersey County usually exhibited fewer days of surface presence each year (range = 13–27) than in Jo Daviess County (range = 20–41; Table 3.2).

I used 1525 camera-deployment days in the analysis to predict the surface presence of *C. horridus* during the late fall and spring from the six refugia (Table 3.2). Three candidate models received 100% of the AIC_c weights and included additive or two-way interactive effects between ADD, maximum daily temperature, and latitude (Table 3.3). The most parsimonious model included the additive effects between model covariates, accounted for 72% of model weights,

and was used for all further analyses. The 95% CIs of ADD and maximum daily temperature (quadratic terms) in the top model did not span zero, signifying they had strong explanatory power and were strongly related to the surface presence (Table 3.4). Conversely, latitude narrowly spanned zero, indicating the parameter had weaker explanatory power.

The additive-only top model, as opposed to the interactive, implied the surface presence of *C. horridus* at both counties occurred at the same values of maximum daily temperature and ADD. However, the probabilities of surface presence were higher in Jo Daviess County than in Jersey County for both variables (Figure 3.3). The marginal effects of ADD (holding maximum daily temperature constant; Figure 3.3A) revealed an increase in the probability of surface presence to a peak at 277.24 °C, decreasing thereafter, with the high value of ADD reflecting the accumulation of degree days from day of year 1 (1-Jan). The marginal effects of maximum daily temperature (holding ADD constant; Figure 3.3), revealed that the probability of surface presence increased with higher temperatures. I did not observe snakes in Jo Daviess County when the maximum daily temperature fell below 11 °C. In Jersey County, snakes remained present on the surface at temperatures as low as 5 °C; however, such occurrences represented only 2.5% (4/155) of all days occurring below 11 °C. The combined effect of both variables implies the probability of surface presence increases with higher ADD and temperatures (Figure 3.3C and 3.3D).

I used seven other studies (8 sites) to examine how accurately the top AIC-ranked model (holding latitude constant; Figure 3.4) predicted spring emergence for other *C. horridus* populations (Table 3.5). Three of the studies determined the timing of spring emergence using daily VHF-radiotelemetry of transmitted individuals (Andrew and Waldron 2017; Bauder et al. 2011; Hoffman 2021), three using visual encounter surveys (Brown 1992; Martin 2002; Sealy

2002), and one via indicative spikes in body temperature (Nordberg and Cobb 2017). The studies reported the average day of spring emergence spanning latitudes from $\sim 32.4^\circ$ to $\sim 43.8^\circ$ (Table 3.5; Figure 3.5). Simple linear regression (Figure 3.5) revealed a later date of spring emergence as latitude increased ($r^2 = 0.81$). The model predicted the average emergence day at each site within 10.2 (SD = 13.1) days. Closer inspection revealed the model failed to accurately predict two sites in Hampton (-23 day difference) and Beaufort Counties (-42 day difference), east-central South Carolina (Andrews and Waldron, 2017), which inflated prediction error (Figure 3.5; Table 3.5). If I removed the two sites in South Carolina, the model predicted the day of peak emergence for the remaining sites within 4.6 days (SD = 4.2).

I also predicted the probability of surface presence across each latitude-year combination of Illinois (Figure 3.6). Visual inspection of each latitude-year subplot suggests the probability of surface presence is highly stochastic throughout late winter and spring, with intra- and inter-year phenological differences within each degree of latitude, particularly in more southerly regions. However, a general unimodal trend is apparent, characterized by a steady increase in the probability of surface presence to a peak as individuals emerged from refugia, followed by a decline in surface presence as snakes departed to nearby basking habitat or summer ranges. The peak probability of surface presence between the southernmost (37° ; peak probability = day 95) and northernmost latitudes in Illinois (43° ; peak probability = day 137), averaged over the three study years, were approximately 42 days apart. Thus, the model suggests a 1° increase in latitude shifted the predicted peak probability of surface presence approximately seven days later into spring, although substantial annual differences in peak surface presence occurred across latitudes, likely due to local climatic variation (Figure 3.6). Additionally, comparison between subplots suggests an increased probability of daily surface presence earlier in the season at

progressively lower latitudes with early spikes of probability on warmer days, perhaps indicating the greater potential for early "midwinter" emergences (longer left tails and probability spikes; Figure 3.6).

3.5 DISCUSSION

I used wildlife cameras and weather station-derived environmental data to successfully observe and predict the daily surface presence of *C. horridus* throughout the late winter and early spring at communal refugia in Jersey and Jo Daviess Counties, Illinois. The most parsimonious model for predicting surface presence included the additive effects of maximum daily temperature, accumulated degree days, and latitude. With a notable exception in the southeastern U.S., the model accurately predicted the average emergence day for other populations as reported by studies located across the species range, emphasizing the role of temperature in influencing the substantial phenological plasticity observed across the species' range. The apparent applicability of the model to other populations suggests it can be a valuable tool in generating fine-scaled predictions of the timing of spring emergence for unknown populations across much of the species' geographic range.

My results suggest temperature-related variables are strong drivers of spring emergence for *C. horridus*. Accumulated degree days allowed the model to capture the general increase in temperature occurring at refugia throughout the late winter and early spring. Accumulated degree days have a long history in phenological predictions of plants (Boutin and Lane 2014), invertebrates (Cayton et al. 2015; Herms 2004; Uelmen et al. 2016), and to a lesser extent, reptiles (Hoffman 2021; Turner and Maclean 2022). Unlike other time-related variables such as ordinal date and photoperiod (Martin 1992), ADD allows for flexible predictions of daily surface presence by accounting for temperature variation across spatial (latitude and elevation) and

temporal (years) extents. For example, degree days will accumulate faster in years and regions exhibiting earlier spring warming. Incorporating such climatic variation in phenological studies is particularly important for species occupying large geographic ranges that are subject to varying thermal regimes and exhibit substantial phenological plasticity.

Including maximum daily temperature with ADD allowed the model to capture the highly stochastic thermal regimes characteristic of temperate climates during gradual spring warming. Other studies have implied daily air temperatures are highly influential and suggest surface activity occurs only once thermal thresholds are surpassed (Andrews and Waldron 2017; Brown 1992; Martin 1992). For example, spring emergence was associated with a maximum air temperature of ~15 °C in New York, South Carolina, and Virginia (Andrews and Waldron 2017; Brown 1992; Martin 1992). My results concur with past findings, suggesting 15 °C corresponds to a 50% probability of surface presence, above which surface presence was more likely than not.

It is apparent *C. horridus* only remains present on the surface until ~11 °C, with some exceptions, perhaps indicating the lower thermal limits of the species and the onset of physiological changes which inhibit surface activity. Agugliaro (2011) showed temperature-dependent metabolic rate depression in *C. horridus* occurred at 5 °C and 9 °C, with a steep temperature sensitivity in metabolic rate between 9 °C and 13 °C. Similar metabolic sensitivity was found in Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*) between ~10°C and 15 °C (Aleksiuk 1971). Such metabolic responses likely promote energy conservation during brumation and serve as a mechanism to rapidly return to activity with increasing temperatures (Agugliaro 2011). Snakes tend not to exhibit activity close to their critical thermal limits because of the risks associated with lower performance (Angilletta 2009; Gregory 1982). Thus, the

warmer temperature of 15 °C may represent the species' voluntary thermal minima, below which most snakes remain within refugia. However, laboratory-based thermal selection studies on *C. horridus* that emphasize responses to thermal extremes are required to elucidate such thresholds.

Including latitude as a model parameter allowed us to examine the effects of temperature-related variables between sites and across latitudes. Acknowledging that the model included only additive effects, not interactive, between latitude, maximum daily temperature, and degree days is critical for correct interpretation. At both sites, *C. horridus* responded equally to temperature, but the probability of surface presence was higher in Jo Daviess County than in Jersey County. Such a difference likely reflects the distinct habitat configurations at each site, which influenced the post-emergent behavior of *C. horridus* and the cameras' subsequent ability to detect surface activity. Specifically, all refugia in Jo Daviess County were on sun-exposed outcrops with abundant crevices and rock shelves, providing a thermally superior basking habitat and protective cover. Post-emergent *C. horridus* would frequently use such basking habitat, remaining within the camera's view and thus increasing the probability of surface presence.

Conversely, refugia in Jersey County were on closed-canopy, loess-covered talus slopes with a notable lack of undergrowth or rocks near the entrances. Post-emergent *C. horridus* did not linger at the entrances but departed from the camera's view to nearby "transient" open habitats such as the bluff front or adjacent hill prairies. Such behavior resulted in fewer daily observations and a lower probability of surface presence. Thus, the model's observed effect of latitude implies the probability of observing snake presence via cameras can differ depending on proximate site characteristics rather than intra-population differences in response to temperature.

With notable exceptions in the southeastern U.S., the top model accurately predicted the average date of emergence for six other populations across the species' geographic range. Some

discrepancy between reported versus predicted values should be expected due to potential sources of error, including the precision of weather station data used for model predictions and the study's sample size and sampling methods providing emergence estimates. Despite these potential sources of error, the model provides accurate predictions for most of the sites examined and indicates temperature is largely responsible for the phenological plasticity of spring emergence exhibited by *C. horridus*. Consequently, there is a strong correlation between spring emergence and latitude, with spring warming occurring progressively later in northern sites (Figure 3.5), resulting in delayed *C. horridus* emergence. Delayed emergence has also been associated with higher, colder elevations (Martin 2002). The reported average mean emergence day 135 (15-May) at a high-elevation site at 1,075 m on the Allegheny Plateau, West Virginia, resembles the northern extreme of the species range, approximately 5° latitude north.

Prolonged cold temperatures and subsequent delays in spring emergence at higher latitudes and elevations (Brown 1992; Martin 1992;) result in increasingly shorter active seasons with later emergence and earlier ingress, directly impacting life histories. Shorter active seasons reduce the time dedicated to foraging, thus limiting yearly energy acquisition and subsequent adjustment of energy budgets between growth, maintenance, and reproduction (Brown 2016; Martin 1993). Consequently, alongside potentially cooler temperatures during the active season, *C. horridus* in colder climates tend to exhibit slower growth rates, delayed sexual maturity, longer intervals between reproductive events, smaller offspring sizes, and lower reproductive success (Aldridge and Brown 1995; Brown 2016; Martin 2002). Ultimately, prolonged cold temperatures are likely responsible for a reduction in overall fitness, as seen in the apparent vulnerability of northern populations, including those extirpated from Canada and the northeastern U.S. (Brown 1992; Environment Canada 2010)

The model failed to predict the average emergence day at the two southernmost sites in coastal South Carolina (Andrews and Waldron 2017). I am uncertain about the reasons for the difference but suspect the region's distinct climate might permit an alternate dormancy strategy that the model does not account for. The study by Andrews and Waldron (2017) falls between 32–33° latitude and represents the only populations I examined within the humid subtropical climate of the southeastern coastal plain. Relative to the other study locations, winter temperatures throughout the Lower East Coast are warmer, with average maximum daily temperatures of 15–17 °C in the region's coldest months of January and February (Andrews and Waldron, 2017). Because of the warmer climate, degree days in Hampton and Beaufort Counties accumulate faster and reach the model's peak predictions earlier in the season than the observed emergence. According to Andrews and Waldron (2017), a maximum daily temperature of ~15 °C corresponded with the 50% probability threshold of daily surface presence, suggesting surface activity of *C. horridus* could occur on most days throughout the winter months, although they did not comment on any midwinter surface activity.

While relatively rare, cameras observed occasional midwinter surface activity in Jersey county throughout all months except January. They also witnessed the winter emergences of non-target species, including Northern Copperheads (*Agkistrodon contortrix*), North American Racers (*Coluber constrictor*), and Common Garter Snakes (*Thamnophis sirtalis*). Other studies note winter emergences in several other snake species, including *A. contortrix* (Sanders and Jacob, 1981), *C. horridus* (Nordberg and Cobb 2016), Prairie rattlesnakes (*C. viridis*; Jacob and Painter 1980), Eastern Hognose Snakes (*Heterodon platirhinos*; Plummer 2002), and Pigmy Rattlesnakes (*Sistrurus miliarius barabouri*; May et al. 1996). Notably, Nordberg and Cobb (2016) identified 60 winter emergence events from 13 *C. horridus* in Tennessee based on spikes

in body temperature. However, despite the number of observations, relatively little is known regarding the frequency and ecological significance of winter surface activity in snakes. Presumably, such activity becomes progressively less frequent in higher latitudes where persistently low temperatures restrict surface activity, a hypothesis that is partially supported by the lack of early winter emergences in Jo Daviess compared to Jersey County, and the lack of observed winter activity at other high-latitude sites (Brown 1992). Furthermore, my predictions of surface activity reveal a progressively higher probability of early surface presence at lower latitudes in Illinois.

While I can only speculate on the significance of such winter surface activity without further study, I suspect such activity is the exception and not the rule. Most early emergences in Jersey County were "one-off" events, typically characterized by a single snake emerging and basking at a refugium entrance on warmer days which permitted surface activity. The snakes perhaps attempted to elevate body temperature to fight disease or infection (Clark et al. 2011; Nordberg and Cobb 2016). Nordberg and Cobb (2016) observed over 60 emergence events from 12 *C. horridus* that were surgically implanted with radio transmitters only a few days before their ingress into refugia. Thus, it is possible the surgical incision sites did not fully heal before the onset of brumation and necessitated above-ground basking. Additionally, Clark et al. (2011) and Nordberg and Cobb (2016) noted snakes emerging with skin lesions early in the spring were not uncommon and may have indicated Snake Fungal Disease. I also observed *C. horridus* in Jersey County with severe skin lesions and contusions of unknown origin that may have motivated snakes to emerge and bask. Although the causes behind early emergences are unknown, the surprising frequency of such events warrants further investigation.

Conservation implications. — Spring emergence is a vulnerable period for *C. horridus*, particularly in northerly latitudes where post-emergent and lethargic individuals congregate at communal refugia and are consequently susceptible to local threats, including management activities (Beaupre and Douglas 2012). Reducing the risk of such threats is vital for effective conservation; for example, scheduling prescribed burn regimes to occur when snakes are less likely to be surface present to reduce potential fire-induced mortality. Yet, the enigmatic nature of *C. horridus*, paired with the apparent phenological variation across both latitudinal and elevational clines, makes determining site-specific spring phenologies difficult and consequently limits conservation. My model's ability to generate the probability of surface presence for any given day during spring emergence is, therefore, a valuable tool for defining conservation and management schedules. However, predictions about emergence dates at unstudied sites should be treated as hypotheses until verified with empirical data.

One strategy to direct management schedules, once predictions have been verified, is to define specific probability thresholds which can be translated into dates that are useful for management and conservation. For example, management activities could be conducted near refugia until the probability of surface presence exceeds a selected threshold. From a management perspective, the probability of surface presence is synonymous with risk; a higher probability indicates a greater potential for snake surface presence and exposure to management activities. Therefore, the selection of appropriate thresholds depends on the amount of risk deemed acceptable given a specific application. I provide a variety of date thresholds for each latitude in Illinois (Table B.2), as determined from general probability trends (i.e., 14 day averages across all years; Figure 3.6) to aid in preliminary conservation and land management scheduling.

Ideally, harmful activities would occur only when there is minimal risk of snake presence (e.g., probabilities < 5%; Table B.2), corresponding to sustained temperatures below the species' suspected thermal limits of 11 °C. However, such thresholds would likely limit management schedules, particularly in warmer southern regions (below 39° latitude) where warmer temperatures increase the probability of surface presence earlier in the season. In such cases, effective cutoffs must balance the risk of snake exposure with time allocated to management activities. I also encourage flexible scheduling whenever possible to account for intra-year and latitudinal climatic differences, although I recognize such scheduling would require the frequent calculation of model predictions based on current temperatures, which are not as readily accessible or practical as a single fixed date threshold.

I also recognize the need for similar statistical models to determine the drivers and phenology of the species' fall ingress. Like spring emergence, fall ingress is a vulnerable period because snakes congregate around refugia prior to brumation (Brown 1993; Martin 1992). While temperature likely influences the timing of ingress (Brown 1993; Martin 1992), I suspect other factors may also play a role (e.g., the timing of arrival to refugia from active-season habitats), which are examined further in the third data chapter (chapter four).

An obvious disadvantage of my study methods is the inability to determine the abundance of snakes present on the surface at refugia using only wildlife cameras. Insight into the number of surface-present snakes would afford a more detailed assessment of the spring emergence phenology of *C. horridus* and the implementation of more effective conservation strategies by incorporating population-level risk assessments. Knowledge of snake abundance would also help differentiate between early "one-off" emergence events by a single snake, particularly in more southern regions, and general spring emergence when most snakes emerge

and resume active season pursuits. Both differ in associated risk, which I cannot currently distinguish between. Anecdotal observations of the amount of surface activity seen on the cameras (not reported here) suggest the probability surface presence is positively correlated with surface abundance, although such evidence could be misleading as identifying individuals via photographs was not possible. Future research should focus on determining snake abundance to examine population-level risk, but I acknowledge obtaining such information for this enigmatic species would be time- and energy-intensive, as shown by Brown (1993) and Martin (1993) who spent upward of a decade obtaining such data.

3.6 ACKNOWLEDGEMENTS

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3.7 TABLES AND FIGURES

Table 3.1 Environmental variables used to construct 32 candidate models predicting the daily surface presence of Timber Rattlesnakes (*Crotalus horridus*) during spring emergence at two sites in Illinois. I obtained climatic data from two weather stations located < 30 km from the six overwinter refugia in Jo Daviess (northern Illinois; n = 3 refugia) and Jersey (west-central Illinois; n = 3 refugia) Counties, monitored during the late winter and spring of 2018–2020.

Predictor	Description (unit)
Min. Temp	Minimum daily temperature (°C)
Max. Temp	Maximum daily temperature (°C)
Mean. Temp	Mean daily temperature (°C)
Min ₅ . Temp	Five-day rolling minimum daily temperature (°C)
Max ₅ . Temp	Five-day rolling maximum daily temperature (°C)
Accumulated Degree Days (ADD)	Cumulative mean daily temperature above 5 °C (°C)
Day of Year	Ordinal date (1 = January 1 st) (day)
Latitude	Numerical proxy for study site (degrees)

Table 3.2 Refugium by year deployment summaries of 6 field cameras installed at Timber Rattlesnake (*Crotalus horridus*) overwinter refugia during spring emergence at two sites in Illinois, USA. Bushnell HD Trophy cameras (Model #119736) were deployed at 6 refugia in Jo Daviess (northern Illinois; n = 3 refugia) and Jersey (west-central Illinois; n = 3 refugia) Counties during late winter and spring of 2018–2020. Columns represent camera deployment locations (County, Refugia); the year of camera deployment (Year); dates of camera deployment (First, Last, Duration); total number of photos taken during deployment (Photos); and the number of days *C. horridus* were photographed (Presence Days) or not (Absence Days).

County	Refugia	Year	Deployment Dates			Photos	Presence Days	Absence Days
			First	Last	Duration			
Jersey	1	2018	2/24	6/1	98	27,376	27	71
		2019	1/1	6/6	157	69,260	19	138
		2020	1/1	5/31	152	72,002	19	133
Jersey	2	2018	2/24	5/31	97	17,914	19	78
		2019	1/1	6/7	158	46,243	22	136
		2020	1/1	5/31	152	50,230	20	130
Jersey	3	2019	1/1	6/7	158	36,283	13	115
		2020	1/1	5/29	150	67,045	24	126
Jo Daviess	4	2018	4/8	6/23	77	7,041	35*	32*
		2019	3/17	7/13	119	34,165	35*	64*
Jo Daviess	5	2018	4/8	6/13	67	4,567	20	47
Jo Daviess	6	2018	4/8	6/13	67	6,431	38	29
		2019	3/17	7/10	116	33,719	41*	47*

*Values represent the number of observed presence days after the removal of days of presumed gravid females (see text).

Table 3.3 The top-ranked models examining the effect of environmental variables on the daily surface presence of Timber Rattlesnakes (*Crotalus horridus*) during spring emergence at two sites in Illinois, USA. I used Akaike's Information Criterion corrected for small sample sizes (AICc), to rank 32 Generalized Logistic Mixed Effects Models (GLMMs; Table B.1) based on a priori hypotheses of drivers of surface presence during spring emergence. The top ten models are shown. I derived surface presence of snakes using field cameras at 6 refugia in Jo Daviess (northern Illinois; n = 3 refugia) and Jersey (west-central Illinois; n = 3 refugia) Counties during late winter and spring of 2018–2020. Columns represent the number of parameters (k); Akaike score (AICc); difference in Akaike score from the top model (ΔAICc); Akaike Weights (w_i); log-likelihood (LL); marginal coefficient of determination (R^2_m); and conditional coefficient of determination (R^2_c).

Model	<i>k</i>	AIC _c	ΔAIC_c	w_i	LL	R^2_m	R^2_c
Latitude + ADD ² + Max. Temp ²	8	788.03	0.00	0.72	-385.97	0.83	0.84
Latitude * Max. Temp ² + ADD ²	10	791.21	3.18	0.15	-385.53	0.84	0.84
Latitude * ADD ² + Max. Temp ²	10	791.47	3.44	0.13	-385.66	0.84	0.84
Latitude + ADD ² + Mean. Temp ²	8	801.19	13.16	0.00	-392.55	0.83	0.83
Latitude * Mean. Temp ² + ADD ²	10	804.12	16.10	0.00	-391.99	0.83	0.84
Latitude * ADD ² + Mean. Temp ²	10	804.73	16.70	0.00	-392.29	0.83	0.83
Global	16	842.46	54.43	0.00	-412.17	0.81	0.82
Latitude + ADD ² + Min. Temp ²	8	850.43	62.40	0.00	-417.17	0.82	0.83
Latitude * Min. Temp ² + ADD ²	10	852.12	64.09	0.00	-415.99	0.83	0.84
Latitude * ADD ² + Min. Temp ²	10	853.22	65.19	0.00	-416.54	0.82	0.83

Table 3.4 Parameter estimates for the top model examining the effect of environmental variables on the daily surface presence of Timber Rattlesnakes (*Crotalus horridus*) during spring emergence in Illinois. I used Akaike's Information Criterion corrected for small sample sizes (AICc), to rank 32 (Table B.1) Generalized Logistic Mixed Effects Models (GLMMs) based on a priori hypotheses of drivers of surface presence during spring emergence. The top-ranked model included the additive effects of accumulated degree days (ADD), maximum daily temperature (Max. Temp), and latitude. I derived surface presence of snakes using field cameras at 6 refugia in Jo Daviess (northern Illinois; n = 3 refugia) and Jersey (west-central Illinois; n = 3 refugia) Counties during late winter and spring of 2018–2020. I obtained climatic data from weather stations located < 30 km from the six overwinter refugia. Columns represent the model parameter (Parameter); the parameter estimate (Estimate); and the standard error (SE); and the upper and lower 95% confidence intervals (CI) of the parameter estimates.

Parameter	Estimate	SE	Upper CI	Lower CI
Intercept	−4.61	0.36	−5.27	−3.90
ADD	−167.80	17.47	199.26	−133.40
ADD ²	−151.52	13.99	−177.20	−124.71
Latitude	0.36	0.28	0.03	0.63
Max. Temp	80.69	10.20	60.56	99.89
Max. Temp ²	−23.02	8.24	−38.37	−6.53

Table 3.5 Summary of the studies I used to examine the applicability of the top-ranked model in correctly determining the spring emergence of other timber rattlesnake (*Crotalus horridus*) populations. Columns represent the citation ID corresponding to the citation indexes in Figure 3.5 (ID), the study citation (Citation); the study location (Study Location), the year(s) in which the study was conducted (Year(s)); the method used to determine of egress (Method); the day of peak probability of surface presence as predicted by the model (Predicted egress); the average day of emergence as reported by the study (Reported egress); and the difference in days between the reported and predicted values (Diff).

ID	Citation	Study location (county, state)	Year(s)	Method	Predicted egress	Reported egress	Diff
a	Brown (1992)	Warren, NY	1981-1988	Visual encounter	133	137	4
b	Bauder et al. (2011)	Rutland, VA	2011	Radiotelemetry	132	145	13
c	Current study	Jo Daviess, IL	2018-2020	Cameras	136	135	-1
d	Hoffman (2021)	Vinton, OH	2017 -2020	Radiotelemetry	114	122	8
e	Martin (2002)	Grant, WV	1989-2001	Visual encounter	135	137	2
f	Current study	Jersey, IL	2018-2020	Cameras	103	109	6
g	Sealy (2002)	Stokes/Surry, NC	1990-1997	Visual encounter	102	103	1
h	Nordberg and Cobb (2017)	Rutherford, TN	2011-2013	Body temperature	98	100	2
i	Andrews and Waldron (2017)	Hampton, SC	2002-2004	Radiotelemetry	82	59	-23
j	Andrews and Waldron (2017)	Beaufort, SC	2006-2008	Radiotelemetry	90	48	-42

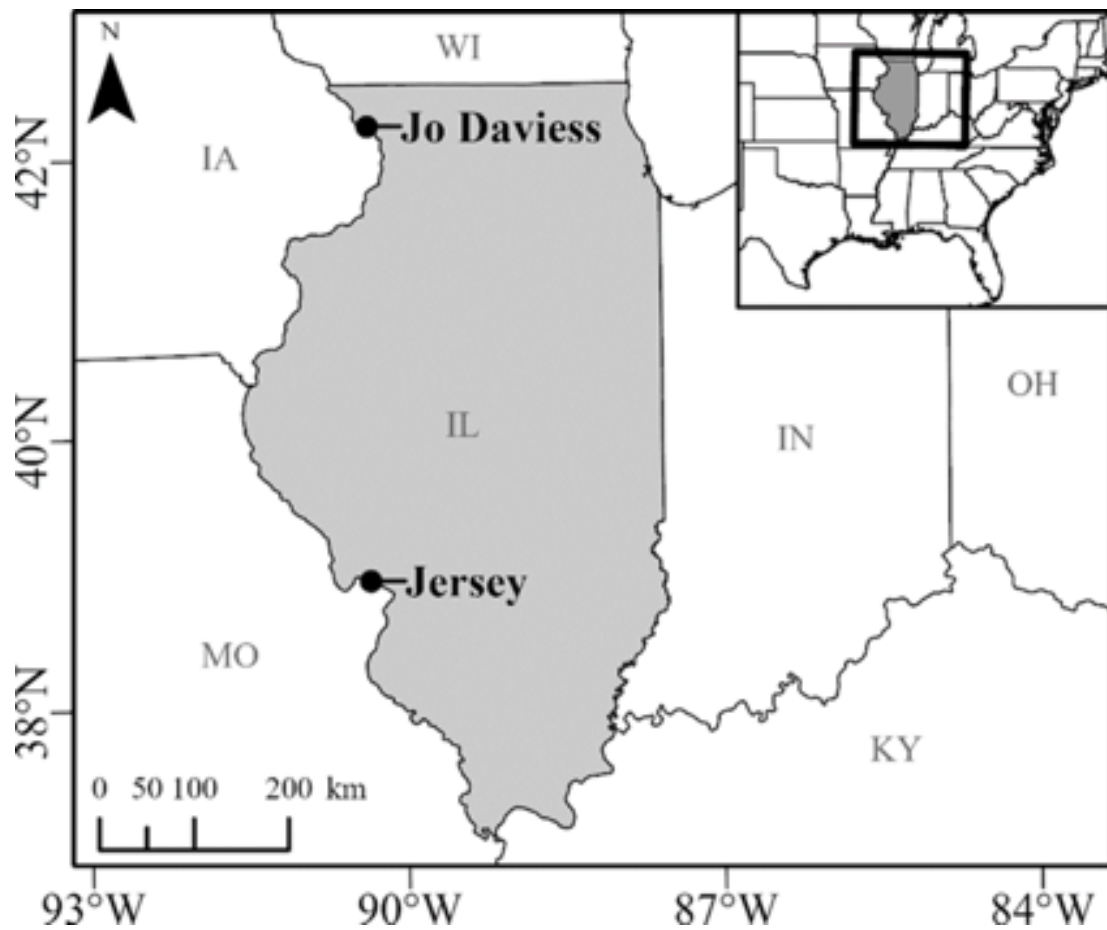


Figure 3.1 The location of the Timber Rattlesnake (*Crotalus horridus*) overwintering refugia I studied in Jo Daviess (n = 3 refugia) and Jersey (n = 3 refugia) Counties in northern and west-central Illinois. I monitored the daily surface presence of snakes at all 6 refugia during the late winter and spring of 2018–2020. Map created in ArcGIS Pro Version 2.7.3 (Esri, 2020)

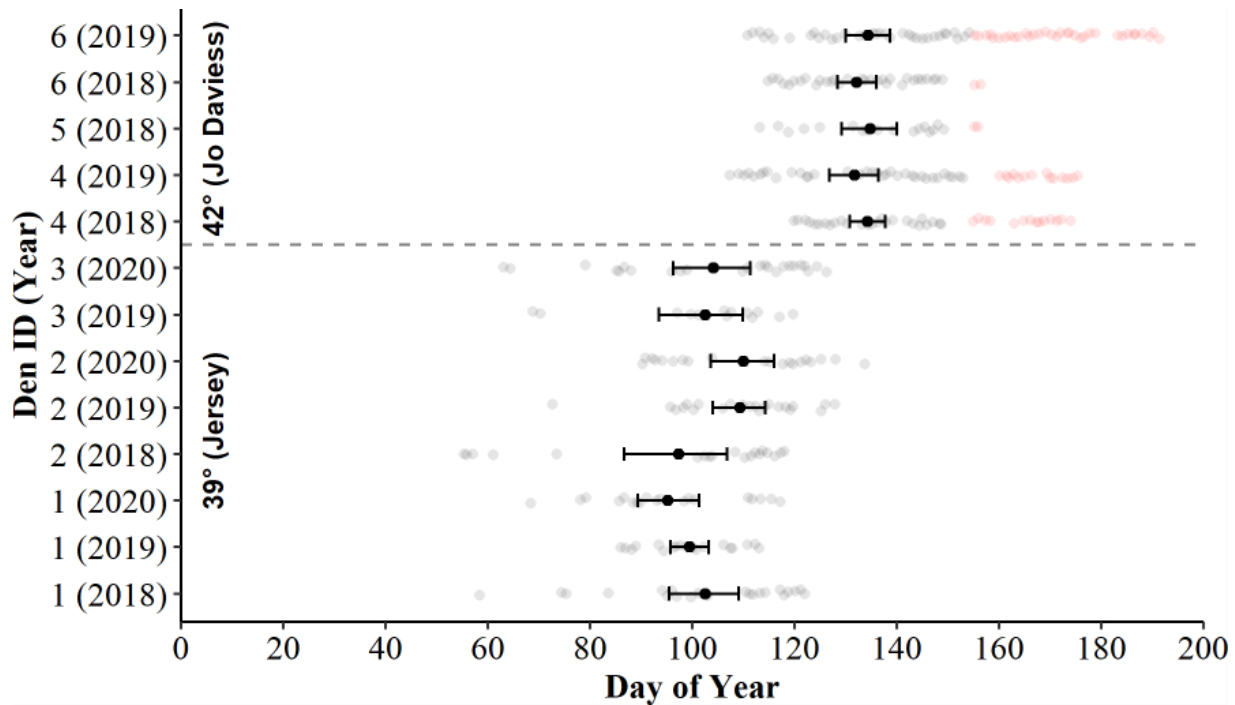


Figure 3.2 Days of surface presence for Timber Rattlesnakes (*Crotalus horridus*) during spring emergence at six refugia in Jo Daviess (n = 3 refugia) and Jersey County (n = 3 refugia), Illinois. I determined days of surface presence (grey and red dots) using Bushnell HD Trophy cameras (Model #119736) deployed at refugia entrances during late winter and spring of 2018–2020. I removed days of suspected gravid females (red dots) from further analysis. The mean day of emergence and bootstrapped 95% confidence intervals (solid black dot and error bars) are displayed for each refugium-year combination.

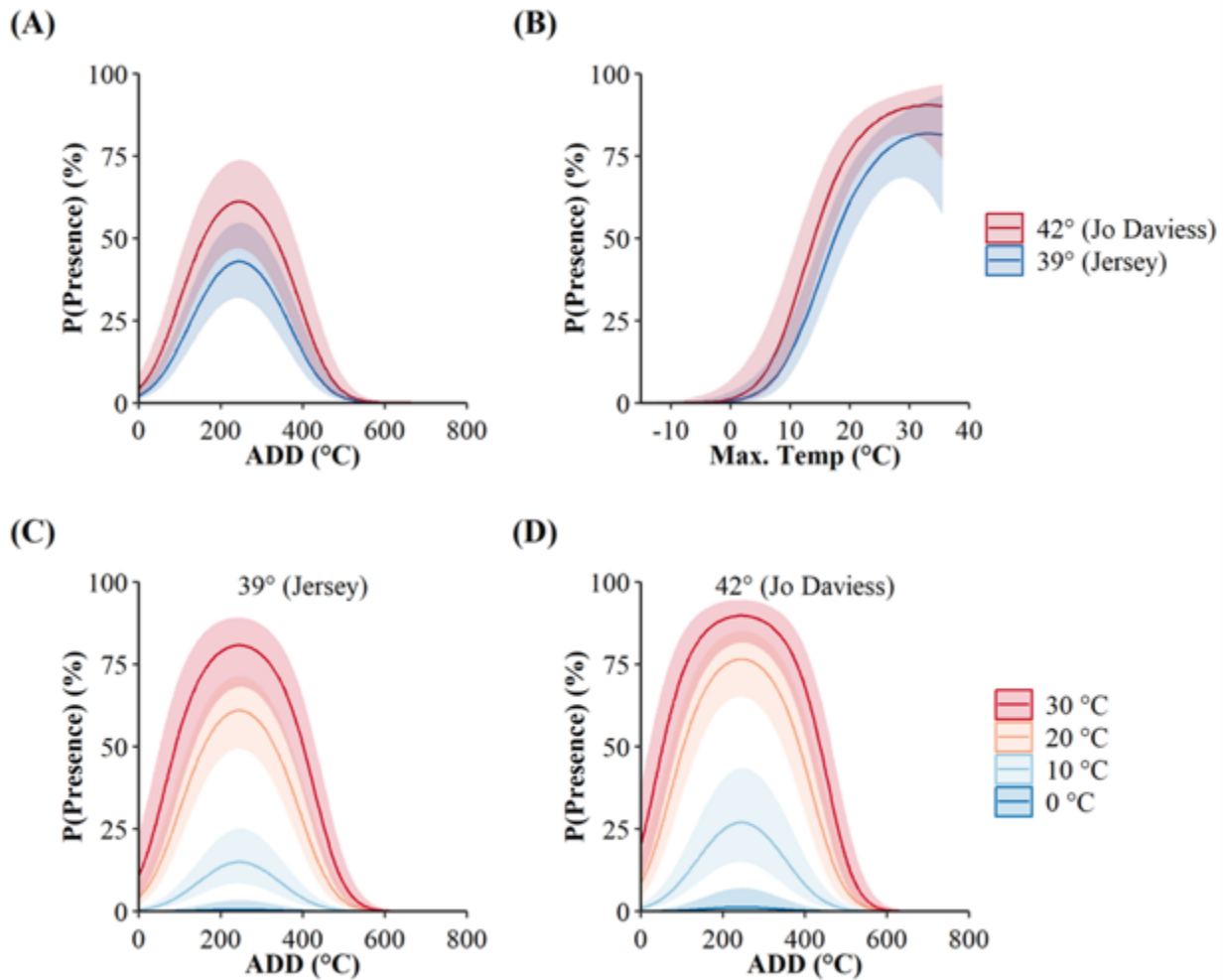


Figure 3.3 Site-level probabilities of the daily surface presence of Timber Rattlesnakes (*Crotalus horridus*) at refugia in Jersey (n = 3 refugia) and Jo Daviess (n = 3 refugia) Counties, Illinois. Predicted probabilities (solid lines) and 95% confidence intervals (ribbons) of the top candidate model as ranked using Akaike's Information Criterion corrected for small sample sizes (AICc). Subplots represent the individual effects of accumulated degree days (ADD; holding maximum daily temperature constant at its mean of 15.95 °C; subplot A), maximum daily temperature (holding ADD constant at its mean of 248.88 °C; subplot B), and the additive effects of both variables for each county, in maximum daily temperature increments of 10 °C (subplots C and D). Data collected during late winter and spring of 2018–2020.

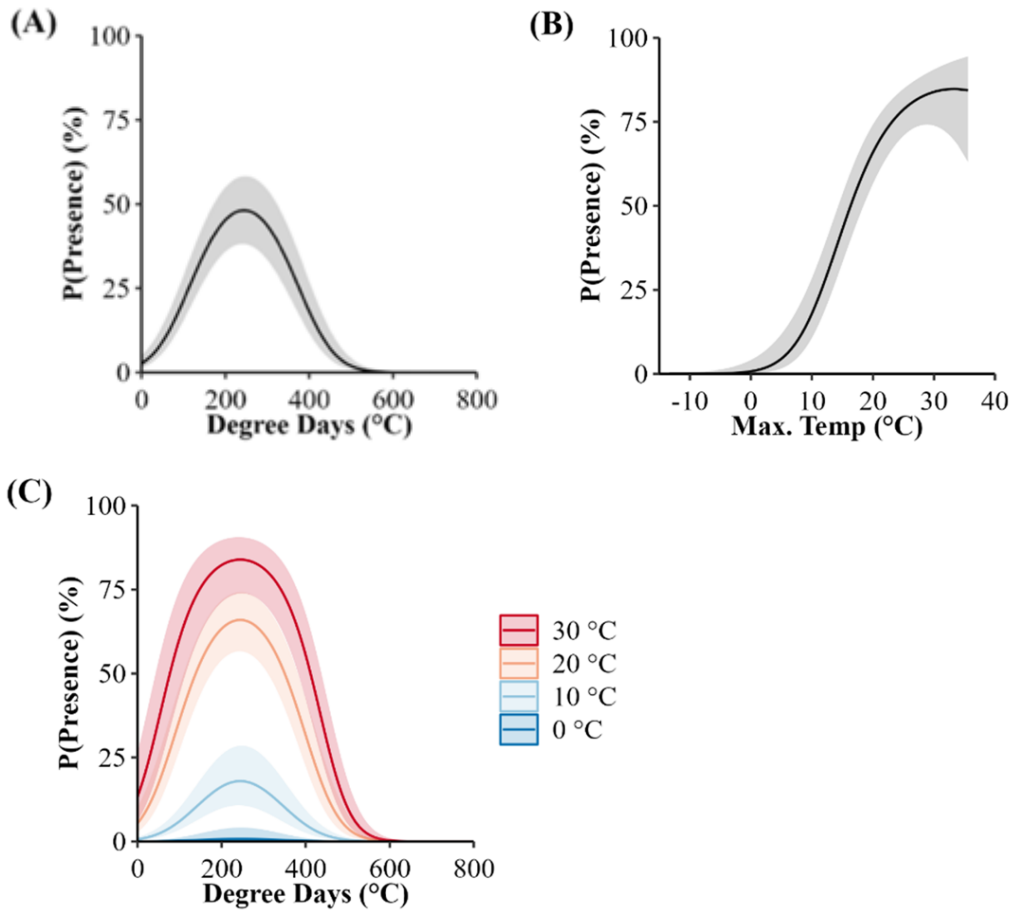


Figure 3.4 Population-level probabilities of the daily surface presence of Timber Rattlesnakes (*Crotalus horridus*) at refugia in Jersey ($n = 3$ refugia) and Jo Daviess ($n = 3$ refugia) Counties, Illinois. Predicted probabilities (solid lines) and 95% confidence intervals (ribbons) of the top candidate model, as ranked using Akaike's Information Criterion corrected for small sample sizes (AICc), while holding the effect of latitude constant. Subplots represent the individual effects of accumulated degree days (ADD) (holding maximum daily temperature constant at its mean of 15.95 °C; subplot A) and maximum daily temperature (°C) (holding ADD constant at its mean of 248.88 °C; subplot B), and the additive effects of both variables (subplot C). Data collected during late winter and spring of 2018–2020.

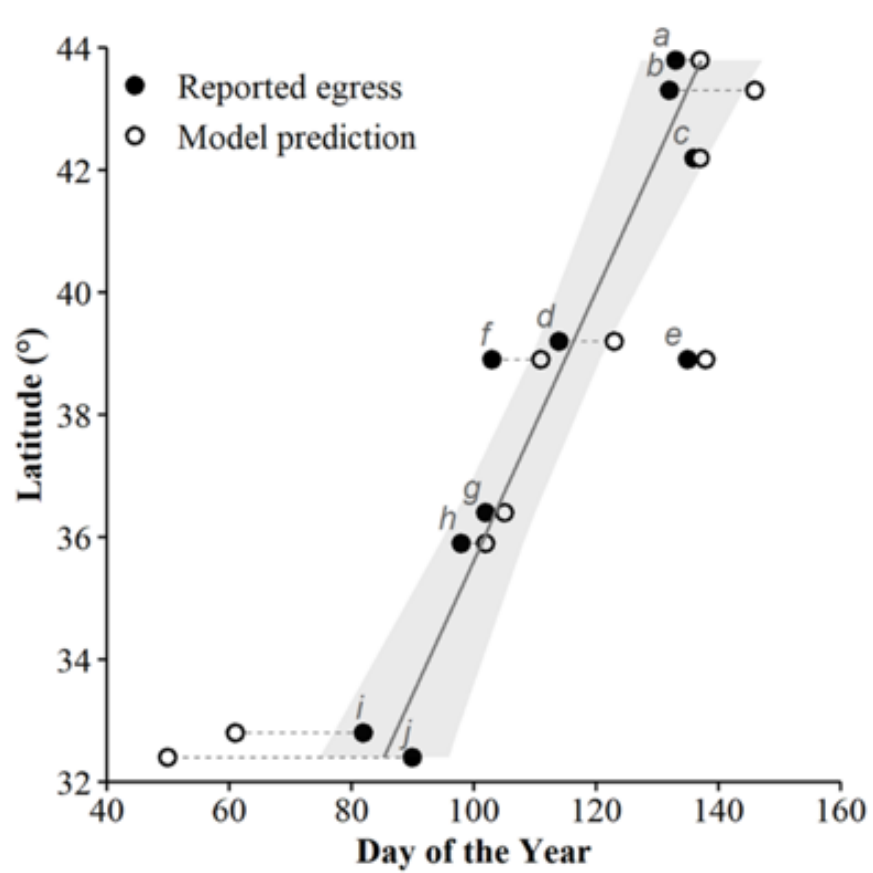


Figure 3.5 The applicability of the top-ranked model in determining the spring emergence of other timber rattlesnake (*Crotalus horridus*) populations. If my predictions were accurate, I expected the day of year with the highest (peak) predicted probability of surface presence at each site (white dots) to correspond to the average emergence day reported by each study (black dots). Dashed lines connect each study site's reported emergence day and corresponding model prediction. I included a simple linear regression (black line = fitted line; grey ribbon = 95% CIs) between the reported average emergence day and the latitude of each study to examine phenological shifts in emergence timing. Letters correspond to the citation ID of each study detailed in Table 3.5. I derived predictions using the top AICc model examining surface presence as a function of the additive effects of accumulated degree days (ADD; base 5 °C) and maximum daily temperature (°C) while holding latitude constant. My predictions were based on data derived from weather station data located within each degree of latitude.

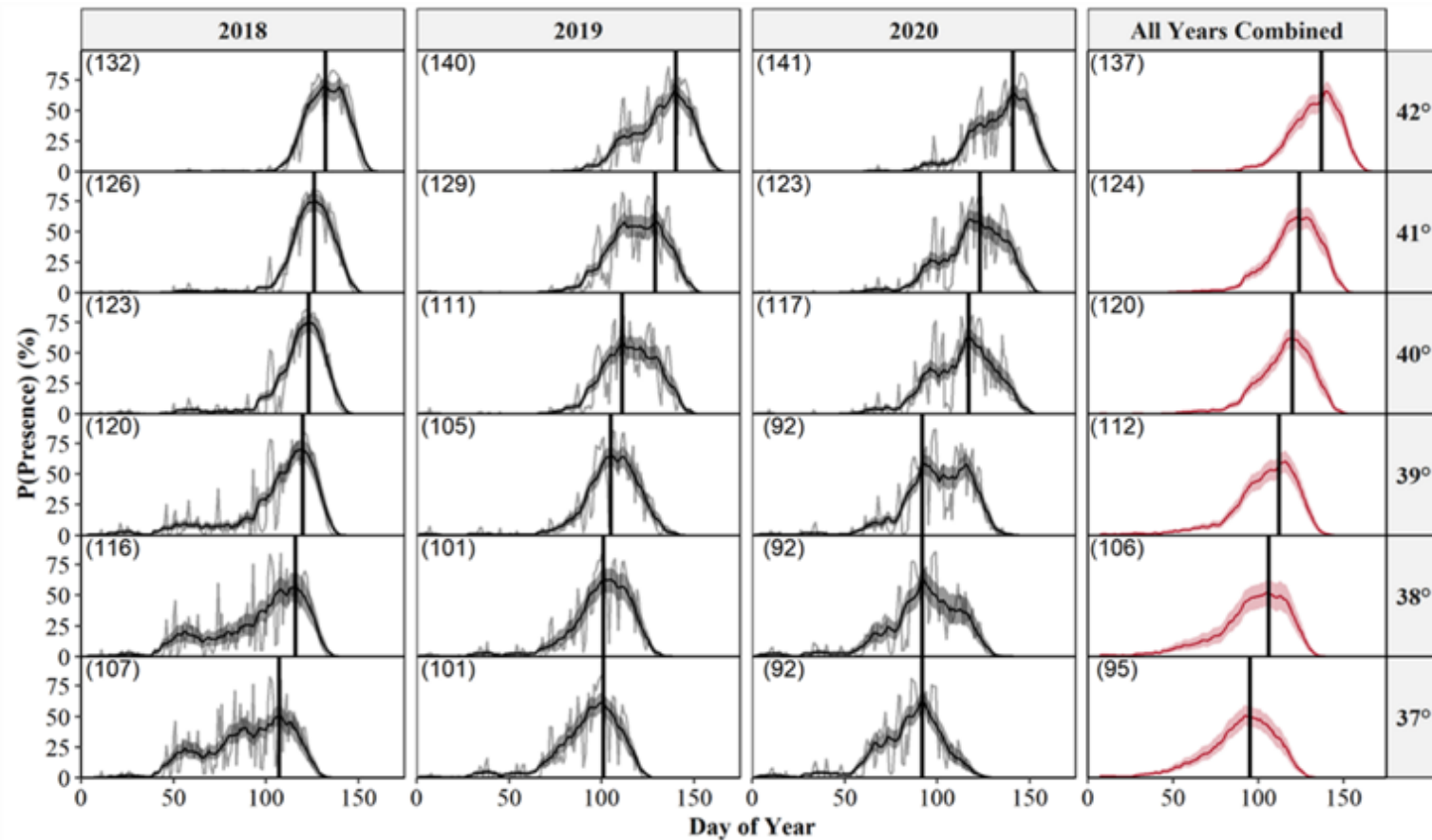


Figure 3.6 Predicted daily probabilities of surface presence for the Timber Rattlesnake (*Crotalus horridus*) across the latitudinal gradient of Illinois during the late winter and spring of 2018–2020. Predicted probabilities are displayed in 1° increments of latitude for each year of the study (2018–2020) and averaged across years. I displayed probabilities for each day (grey lines) and averaged across a 14 day moving window (black or red lines) with bootstrapped 95% confidence intervals (ribbons). Vertical black lines and parenthesized numbers within each subplot represent the day with the peak probability of surface presence. I derived predictions using the top AICc model examining surface presence as a function of the additive effects of accumulated degree days (ADD; base 5 °C) and maximum daily temperature (°C) while holding latitude constant. My predictions were based on data derived from weather station data located within each degree of latitude.

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CHAPTER FOUR: OCCURRENCE OF TRANSIENT PERIODS IN REFUGIA-DEPENDENT AND MIGRATORY RATTLESNAKES

4.1 ABSTRACT

Migratory and refugia-dependent snakes face a complex phenological challenge each winter cycle. Individuals must time their fall ingress and spring egress into and from overwintering refugia, alongside their inbound and outbound migrations to and from summer ranges, to maximize restricted active seasons while avoiding critically low temperatures. To aid in the transition between the active period and overwintering period, some snakes appear to have adapted to the use of fall and spring “transient” periods whereby individuals arrive at refugia earlier in the fall to avoid being caught too distant from refugia when temperatures decrease and depart from refugia later than they egress in spring for similar reasons. While plausible, such a hypothesis is rarely investigated, and whether transient periods are a common or required phenomenon is poorly documented. Here, I demonstrate the existence, phenology, and likely purpose of the fall and spring transient periods for Timber Rattlesnakes (*Crotalus horridus*) in west-central Illinois. My results suggest transient periods for *C. horridus* can last upwards of a month, but their duration is phenologically flexible in response to variable annual temperatures. Spring transient periods result from post-emergent snakes waiting at overwintering sites for a second thermal trigger—alongside the initial cue for egress—which signals the arrival of consistently warm temperatures appropriate for summer activity. Fall transient periods result from snakes intentionally arriving at refugia at higher temperatures, and thus typically earlier than they ingress to avoid being caught in summer habitats when temperatures fall below thermal limits. However, the role of temperature appears more nuanced in the fall, as snakes must anticipate falling temperatures while in summer habitats. Overall, the fall and spring transient

periods serve as necessary buffers during stochastic shoulder seasons, ensuring snakes have reliable and swift access to thermally adequate refugia when temperatures surpass lower thermal tolerances. My findings complement and expand previous work on the overwintering strategies of *C. horridus* and provide further insights into an understudied, but important, aspect of the annual life cycle of refugia-dependent and migratory snakes.

4.2 INTRODUCTION

Migration is necessary for many animals facing spatiotemporal fluctuations in resources and environmental conditions (Chapman et al. 2014, Dingle 2014, Milner-Gulland et al. 2011). Moving between suitable habitats during different life stages or seasons may improve an animal's survivability and future reproductive success by increasing resource acquisition (e.g., food, water, shelter, and mates; Avgar et al. 2014, Fudickar et al. 2021) or avoiding adverse environments (e.g., extreme temperatures, predation, and pathogens; Chapman et al., 2015; Russell et al. 2005). Migratory animals must balance the advantages of such movements with potential fitness trade-offs, including the risk of predation (Hebblewhite & Merrill 2009, Middleton et al. 2013), exposure to diseases and parasites (Altizer et al. 2011, Reed et al. 2003), and the time-energy constraints of the migratory movement itself or the duration of residency in disjunct environments (Fudickar et al. 2021, Shepard et al. 2013). How long to remain within different environments is particularly important for seasonal migrators evading adverse climates, most commonly temperate winters (Kubelka et al. 2022), which constrain annual resource acquisition and limit time-energy budgets (Brown et al. 2023, Buehler et al. 2008). Such animals maximize fitness by avoiding lethally cold temperatures while allowing enough time for adequate energy acquisition, reproduction, and development (Southwood & Avens 2010, Winger

et al. 2019). Therefore, optimizing migratory phenology within the scope of an animal's life history is essential for increased survival and reproduction (Bauer et al. 2016).

Animals use a variety of strategies to optimize the timing of migrations and balance prolonged winters with resource acquisition and reproduction. For example, several migratory birds exhibit quiescence before migration to warmer climates (Cochran et al. 2004, Ramenofsky et al. 2008, Schofield et al. 2018) or use *en route* stopover sites (Cohen et al. 2021), likely to balance movement between habitats with resource and mate availability, favorable flight conditions, and adequate temperatures. Many insects undergo reproductive diapause (Tougeron et al. 2019), probably to preserve time and energy for their migrations (Tenger-Trolander et al. 2023) or to maximize migratory success of the proceeding generation by delaying reproduction until adequate spring resources and temperatures are available for optimal larvae development (Reppert & De Roode 2018). Some fish species migrate to warmer waters in discrete temporal pulses characterized by pauses in movement until specific environmental conditions are met (e.g., adequate water temperatures or river currents; Smith 2012). Terrestrial reptiles, primarily snakes, appear to exhibit similar pauses or “transient” periods (*sensu* Brown 1992) between their spring and fall migrations between summer habitats and overwintering refugia, likely for similar reasons (Brown 1992, Dyugmedzhiev et al. 2019, Martin 1992, Viitanen 1967)

A shared characteristic of such migratory strategies involves the existence of some form of staging period (e.g., diapause in insects; Gill et al. 2017, quiescence in birds; Schofield et al. 2018, transient or basking periods in reptiles; Brown 1992, pre- and post-hibernation swarming in bats; Muthersbaugh et al. 2019). Temporary pauses in movement before or after migration are often thought to represent temporal buffers used to fine-tune migratory phenology with adequate environmental conditions, physiological or behavioral processes (Reppert & Roode 2018,

Southwood & Avens 2010), predator avoidance (Brönmark et al. 2008, Lank et al. 2003), resource acquisition (Monteith et al. 2011, Moussy et al. 2013), reproduction (Muthersbaugh et al. 2019), or social interactions (Aikens 2022, Helm et al. 2006). Yet, despite their apparent importance in determining migratory success and survival, few studies explicitly examine migratory-related staging periods (but see Brown 1992, Muthersbaugh et al. 2019, Schofield et al. 2018). Consequently, the causal factors, function(s) (if any), and the existence of such phenomena in many taxa remain anecdotal or unknown.

Staging periods appear particularly important to several terrestrial animals which migrate seasonally between summer mating and foraging habitats, and overwintering refugia (e.g., Custer et al. 2023, Muthersbaugh et al. 2019, Tetzlaff et al. 2023). Migration is often necessary for such animals by permitting access between geographically limited overwintering sites and critical resources distributed throughout adjacent landscapes (Fleming et al. 2003, Gnanadesikan et al. 2016, Harvey & Larsen 2020). However, movement between seasonal habitats while dependent on overwintering sites is phenologically challenging. Individuals must find and access refugia during low temperatures while timing their spring and fall migrations to and from distant summer ranges (Brown 1992, Martin 1992, Russell et al. 2005). In response, several animals reportedly use staging periods to fine-tune migration phenology to maximize active-season pursuits while ensuring reliable access to refugia (Brown 1992, Custer et al. 2023, Dyugmedzhiev et al. 2019, Martin 1992, Viitanen 1967). Specifically, staging periods may result from animals arriving at the refugia early in the fall to avoid being caught too far from the refugia when temperatures decrease and when departing from the vicinity of the refugia later than they egress in spring for similar reasons (Brown 1992, Martin 1992). While plausible, such a hypothesis is rarely investigated, and thus, whether staging periods are a common or required

phenomenon for refugia-dependent and migratory animals and what animals are doing during such periods is poorly documented.

Here, I examine the movements of Timber Rattlesnakes (*Crotalus horridus*) to examine whether staging or “transient” periods (Brown 1992) are a potential strategy used by refugia-dependent and migratory animals to fine-tune their migration phenology in response to adverse and prolonged winters. Endemic to the eastern USA, central to northern populations of *C. horridus* spend more than seven months per year—collectively greater than half of their lives—within refugia (Brown 1992). Such dependency dictates the species’ movement ecology, characterized by migrations of typically 1–3km between summer foraging and mating habitats and communal overwintering sites to which individuals show high fidelity (Brown 1992, Eckert and Jesper 2024, Petersen et al. 2019). The species reportedly exhibits fall and spring transient periods, although information regarding their duration and phenology is sparse and varies dramatically from over a month to not at all (Brown 1992, Martin 1992). Such phenological plasticity suggests the transient periods are phenologically flexible in response to stochastic inter-annual temperatures, which characterize temperate spring and fall periods.

My objectives were to (1) demonstrate the existence—or lack of— fall and spring transient periods *C. horridus*, (2) describe the phenology, associated movements, and behavior of individuals during the two periods, and (3) examine whether transient periods (if they do exist) are coincidental to a strategy used by refugia-dependent and migratory snakes to navigate stochastic fall and spring temperatures. My results elucidate a relatively overlooked but important aspect of the annual lifecycle of *C. horridus* and similar species, and characterize a strategy used to fine-tune the timing of migratory-related movements in the face of seasonal adversity

4.3 MATERIALS AND METHODS

Study Site and Data Collection. — I conducted research at Principia College in Jersey County, west-central Illinois, USA. The site consists of an upland mesic forest bounded to the south by Mississippi River limestone bluffs covered with a vegetational matrix of remnant hill prairies and oak-hickory dominated woodlands. The ~ 7km of bluff front constitute all major outcrops on the property with crevices, talus, and holes serving as overwintering refugia for *C. horridus*. Knowledge of refugia dates to the 1930s, with specific entrances identified in recent years via visual encounter surveys (Jesper et al. 2024; chapter two), wildlife cameras (Jesper et al. 2023; chapter three), and VHF-radiotelemetry (Eckert and Jesper 2024).

From 2015–2021, researchers captured snakes at refugia using visual encounter surveys during the species' suspected fall ingress (Sep-Oct) and spring egress (Apr-May) periods or opportunistically throughout the active season. To increase the sample size, in spring 2021, we fitted several refugia entrances with enclosure-traps consisting of PVC pipe frames (~1.5m³) and galvanized steel mesh walls (Figure C.1) and checked them daily to retrieve trapped snakes. We then weighed, sexed, and measured all captured snakes and implanted a Passive Integrated Transponder (PIT) tag (BioMark Inc., Boise, Idaho) subcutaneously in the distal one-third of the body. We also chose a subset of captured individuals for VHF-radiotelemetry based on their ability to support a transmitter and to achieve equal sex and size distributions. Following Reinert & Cundall (1982), veterinarians at the Saint Louis Zoo surgically implanted temperature-sensitive radio transmitters (Holohil SI-2T, Carp, Ontario) into the peritoneal cavities of snakes under inhalation anesthesia (isoflurane). We released snakes at their original capture locations within one week of surgery.

We used two primary methods to monitor the movements and behavior of *C. horridus* during the fall and spring transient periods. First, we monitored the refugia-use of PIT-tagged snakes at three communal refugia using small-scale PIT tag detection systems (Biomark, Inc., Boise, Idaho; hereafter "PITT systems") comprising an IS1001-12V PIT tag reader capable of connecting to 18 circular antennae (25.4 cm internal diameter; 15 m cable length). We inserted a clay pipe (15cm x 60cm) at each refugia entrance and positioned an antenna at either end (Figure C.2). We then partially covered the internal antennae and clay pipe with soil and limestone rubble from the surrounding habitat to mimic natural conditions and to prevent refugia access by alternate (unmonitored) routes. Using two antennae on either end of the pipe allowed me to evaluate the snake's directionality of movement (egress or ingress). PITT systems remained deployed throughout the overwintering period to monitor all refugia activity.

We also located VHF radio-transmitted snakes to examine pre-fall ingress and post-spring egress movements and behavior near refugia. We tracked snakes daily using a three-element folding yagi and receiver and recorded each snake's location with a handheld GPS (Garmin GPSMAP 66i) or Apple iPad and Bluetooth GPS combination. We used the same GPS coordinates when snakes were in the same location for multiple sequential days to remove GPS errors which might incorrectly infer movement. We tracked snakes throughout the spring transient period between their emergence date from refugia (identified via the PITT systems) to departure to summer habitats. We defined departure as the first of several long-distance and continuous migratory movements as determined by the Euclidian straight-line distance between a snake's refugia and its recorded location for each day of the tracking duration (i.e., displacement). Departure also represented the first move from distinct south-facing bluff habitat into the old-growth forest interior. In the fall, tracking commenced once we detected a snake

with VHF telemetry near the refugia, and it continued daily until the snake entered its refugia (identified via the PITT systems). In all cases, fall tracking included the terminus of a snake's migration and subsequent arrival to the vicinity of its refugia, demarking the start of the fall transient period. We defined a snake's arrival using the same methods as spring departure but in reverse. I provide figures of each snake's movement (maps created in ArcMap 10.7; ESRI 2011) and displacement from refugia in Appendix C (Figures C.3–6) for reference.

Analysis. — I defined the fall transient period as the number of days between a snake's arrival to the overwintering sites, signifying the end of its inbound migration from summer habitats ("arrival"), and its final entry into refugia ("ingress"). Similarly, I defined the spring transient period as the number of days between a snake's emergence from refugia ("egress") and subsequent departure, signifying the start of the outbound migration to summer habitats ("departure"). I first compiled the dates of each "movement type" (fall = arrival and ingress; spring = egress and departure) for all monitored snakes as determined via VHF telemetry and automated PITT data. I then used two generalized linear mixed models (GLMM; R package 'nlme' Pinheiro and Bates 2023)—one for each transient period—to calculate the marginal (mean) day of each movement type and to test for informative differences between them and across years. Note the number of days between the movement types of each season represented the length of the transient period. For easier reference and distinguishability, I refer to the two models as the *fall_day* model and the *spring_day* model.

I specifically used GLMMs to specify snake ID as both a random intercept and slope to account for (1) pseudoreplication of the same snake across years and (2) partially paired data of the same snakes across movement types; for example, I did not have dates of fall arrival and ingress, or spring egress and departure, for all snakes (some individuals were monitored using

both PITT readers and VHF telemetry while others were monitored using one or the other only). For the latter, setting snake ID as a random effect allowed me to use all data to calculate population-level estimates instead of limiting my sample size by removing unpaired data points (Zuur et al., 2009).

For each model, I set the response variable as the ordinal date (day of year) and the explanatory variables as an interaction between movement type (*fall_day* model = arrival and ingress; *spring_day* model = egress and departure) and year. A significant interaction suggests the timing of one or both movements differed between years (e.g., the mean day of egress or departure (in the spring), or arrival and ingress (in the fall), occurred on different days each year), indicating a phenologically flexible transient period. Conversely, an insignificant interaction suggests no inter-year differences in movement types and evidence of a potentially temporally fixed transient period. I checked for informative estimates of the beta coefficients (additive and interactive) using 95% CIs, with CIs not broadly overlapping zero indicating informative parameters. I then determined estimated marginal values—the estimated mean day of each movement type—for each model using the R package 'emmeans' (Lenth 2023) and determined informative differences using pairwise contrasts. I provide plots (R package 'ggplot'; Wickham et al. 2016) of both the marginal (mean) ordinal dates $\pm$ 95% CIs of the movement types for both the fall and spring transient periods, alongside pairwise contrasts for comparison of the difference of the mean estimates.

I also report several summary metrics (Mean $\pm$ 95% CI; range) to broadly describe the movements and behavior of VHF-telemetered snakes during each transient period. I calculated maximum linear Euclidean distance from refugia (displacement), average number of locations used during the transient periods, and average duration of stay at each location. To account for

pseudoreplication, I calculated each summary metric's estimated mean values and 95% CIs by fitting generalized linear mixed models (r package 'lme4'; Bates et al. 2015) with Snake ID as a random effect. I report the resulting model intercept estimates ($\pm$ 95% CIs) representing the mean of each summary metric.

Given any inter- and intra-year phenological differences between the transient periods, I examined the temperature at which snakes conducted each transient movement type (i.e., arrival, ingress, egress, and departure) to examine if they were thermally motivated. Like the above analysis determining the phenology of the transient periods, I used two GLMMs to generate estimated marginal mean temperatures of each movement type, and then tested for informative thermal differences between movement types in each transient period. For easier reference and distinguishability, I refer to the two models as the *fall_temp* ("fall temperature") and *spring_temp* ("spring temperature") models.

I repeated the modelling procedures described above, specifying snake ID as both a random intercept and slope, but instead specified the response variables as an interaction between year and minimum daily temperature. Minimum daily temperature was derived from Principia College's weather station located ~1km from the overwintering sites and was chosen as it represented the lowest temperature snakes would be subject to on a given day and thus closely reflected the lower thermal limits of a snake compared to other common temperature variables (e.g., maximum or mean daily temperature). The interaction between the explanatory variables allowed me to examine potential differences in the temperature of movement types across and within years. Specifically, a significant interaction would suggest the mean temperature of one or both movements differed between years (e.g., snakes departed or egressed at different temperatures each year), indicating the phenological schedule was not thermally motivated.

Conversely, an insignificant interaction would suggest no inter-year differences in temperature and evidence the phenological schedule was thermally motivated (e.g., snakes arrived or departed at the same temperature each year). I provide plots of the marginal means $\pm$ 95% CIs of each movement type against minimum daily temperature for the fall and spring transient periods, alongside pairwise contrasts.

Model assumptions. — All models were checked for the validity of assumptions. I checked for normality of residuals using quantile-quantile plots and Shapiro-Wilk tests and homogeneity of residual variance across each level of the independent variables using boxplots, Levene tests, and Pearson residual plots. While none of the models violated normality assumptions, all showed heteroskedastic group variance across movement type and year. Thus, I refit the models using a within-group variance structure using the `varIdent` function in the R package 'nlme' (Pinheiro and Bates 2023), with year and movement type as grouping factors allowing for different variances across each level of the models.

4.4 RESULTS

Fall Transient Period. —I monitored 31 *C. horridus* for one or both years during the fall of 2021 and 2022. After removing the data of three snakes due to aberrant behaviors (See below), the fall dataset comprised 46 ingress events from 28 snakes and 27 arrival events (and associated pre-ingress movements) from 17 snakes. I used all arrival and ingress records to construct the *fall_day* model. The 95% CIs of all the model's parameter estimates (both additive and interactive) did not span zero (Table 4.1), indicating informative differences between the mean day of arrival and ingress within and across years. Specifically, the marginal effects and pairwise contrasts (Figure 4.1) revealed, on average, snakes in 2021 arrived at overwintering refugia ($\bar{x}$ = day 256, 13-Sep) 29.23 days earlier than they ingressed into refugia ($\bar{x}$ = day 285,

12-Oct), while snakes in 2022 arrived ($\bar{x}$ = day 267, 24-Sep) 8.22 days earlier than they ingressed ($\bar{x}$ = day 276, 3-Oct).

Of the 27 snake records (from 17 snakes) with full tracking records (i.e., paired arrival and ingress dates, and transient movements), six egressed into refugia on the same day they arrived, while 21 remained on the surface each year and used leaves, rocks, and hollow logs close to refugia ($\bar{x}$ distance from refugia = 58.17 m; CI = 39.67–76.23) for concealment (Appendix C3-4). Surface-active snakes moved little during the transient period, occupying an average of 1.91 locations (CI = 1.65–2.17) and remaining at each location for an average of 12.29 days (CI = 4.75–9.88). Snakes entered refugia primarily at night (Figure 4.2), with a noticeable increase in activity beginning after sunset. Furthermore, of the 28 snakes detected via PITT systems, 19 entered refugia each fall and did not re-emerge until the following spring (Figure 7), nine snakes re-emerged shortly after ingress during one or both years before re-entering refugia (typically on the same day), and three snakes shuttled frequently (59 shuttling events) throughout one or both overwintering periods (Figure 4.6). The three snakes, one with a visible injury around the incision site of the VHF transmitter (snake ID 10) and two which were later found dead (snake IDs 12 and 37), typically emerged in the afternoon ($\bar{x}$ = 01:30 p.m.; SD = 1.87 hrs.) and re-entered refugia in the evening of the same day ($\bar{x}$ time = 05:18 p.m.; SD = 2.21 hrs.). All records of these snakes were removed from further analysis due to their apparent physiological impairment and aberrant behavior.

I used the *fall_temp* model to examine the minimum daily temperature at which snakes arrived at, and ingressed into, refugia to determine if the inter- and intra-year phenological differences observed in the *fall_day* model were thermally motivated (i.e., if snakes arrived and ingressed in response to the same temperatures). The 95% CIs of Movement Type did not span

zero (Table 4.1), indicating informative differences between the temperature snakes arrived at overwintering habitat and ingressed into refugia. Conversely, both year and the interaction between movement type and year broadly spanned zero, indicating no annual differences between arrival or ingress temperatures. The estimated marginal effects and pairwise contrasts revealed that, on average, snakes arrived at overwintering habitat at ~ 19.0 °C each year ($\bar{x}$ in 2021 = 19.7 °C; $\bar{x}$ in 2022 = 19.2 °C), around 5.0 °C higher than the temperature they typically ingressed into refugia at ~ 14.0 °C 2021 = 14.2 °C; $\bar{x}$ in 2022 = 13.5 °C; Figure 4.3).

Visual examination of the fall temperature profiles of both years indicated snakes began entering refugia only after a rapid decline in temperature (Figure 4.4). In fall 2021, all monitored snakes had arrived at overwintering sites before the temperature decrease ($\sim$ day 265; Figure 4.4A); thus, there was no overlap between days of arrival and ingress. Conversely, in 2022, the temperature fell while snakes were still arriving, thus arrival and ingress dates overlapped considerably (Figure 4.4B).

Spring Activity. — I monitored 27 *C. horridus* for one or both years during the springs of 2022 and 2023. After removing outlier snakes, the spring dataset comprised 40 ingress records from 26 snakes and 27 departure records from 20 snakes. Discrepancies in sample sizes between the fall and spring result from mortality or VHF transmitter failure. Like the *fall_day* model, the 95% CIs of the *spring_day* model's parameter estimates did not span zero, indicating informative differences between the mean day of egress and departure within and across years. (Table 4.1). The model's marginal effects and pairwise contrasts (Figure 4.1) revealed lengthy spring transient periods with intra-year phenological plasticity, although note the relatively similar departure dates. On average, snakes in 2022 departed from overwintering sites ($\bar{x}$ = day 133; 13-

May) 21.81 days later than they egressed ($\bar{x}$ = day 111; 21-Apr), and in 2023, departed ($\bar{x}$ = day 129; 9-May) 26.35 days later than they egressed (day = 103; 13-Apr).

The egress of snakes in both years occurred during highly stochastic spring temperatures, prompting a staggered emergence pattern as groups of snakes emerged on warmer days before colder temperatures precluded further activity (see temperature profile in Figure 4.5). Snakes monitored using PITT systems emerged primarily at night (Figure 4.2) and did not return to refugia even during highly stochastic post-emergent temperatures (Figure 4.5). Upon emergence, all VHF-equipped snakes moved to nearby south-facing bluff habitat (Figure C.5–6), where they used leaves, rocks, and hollow logs near overwintering refugia until departure (mean distance = 75.50 m; CI = 45.3–105.9). Like the fall transient period, individuals were largely immobile, occupying an average of 2.4 locations (CI = 1.96–2.93; range = 1–6) and remaining at each location for an average of 10.63 days (CI = 8.74–12.52; range = 1–46).

I used the *spring_temp* model to examine the minimum daily temperature at which snakes egressed from refugia, and departed to summer habitats, to determine if the inter- and intra-year phenological differences observed in the *spring_day* model were thermally motivated (i.e., if snakes egressed and departed in response to the same temperatures). The 95% CIs of Movement Type did not span zero (Table 4.1), indicating informative differences between the temperature snakes egressed from refugia and departed to summer ranges. Conversely, both year and the interaction between movement type and year broadly spanned zero, indicating no annual differences between arrival or ingress temperatures. The estimated marginal effects and pairwise contrasts revealed, on average, snakes departed to summer habitat at ~ 19.0 °C each year ($\bar{x}$ in 2022 = 19.7 °C; $\bar{x}$ in 2023 = 18.4 °C) around 5.0 °C higher than the temperature they typically ingressed into refugia, at ~ 14.0 °C ($\bar{x}$ in 2022 = 14.1 °C; $\bar{x}$ in 2023 = 14.0°C; Figure 4.3). Visual

examination of the spring temperature profiles indicated departure in both years occurred only after a sharp and persistent rise in temperatures over several consecutive days (Figure 4.5).

4.5 DISCUSSION

Overview. — Although there were considerable inter-year phenological differences, my results, averaged across years, suggest *C. horridus* remained within refugia for an average of six months and seven days (6 Sep–17 Apr). If I include the fall and spring transient periods, the overwintering period averaged seven months and 23 days (18 Sep–11 May), leaving only four months (~ 36%) of their annual time budget for active-season pursuits. Such restricted active seasons reduce overall fitness because limited energy acquisition results in slower growth rates, delayed sexual maturity, longer intervals between reproductive events, smaller offspring sizes, and lower reproductive success (Aldridge and Brown 1995, Brown 2016, Martin 2002). Thus, the fall and spring transient periods, which restrict active seasons further, appear ecologically necessary. My data supports the hypothesis they likely represent a survival strategy used by migratory and refugia-dependent snakes to navigate the stochastic thermal transitions during temperate fall and spring periods. In short, transient periods appear to serve as necessary buffers, ensuring snakes have reliable and swift access to refugia when temperatures surpass lower thermal tolerances.

Spring Transient Period. — Northern populations of *C. horridus* face important and risky decisions during the spring. First, individuals must decide when to abandon the relative thermal safety of refugia and emerge onto the surface. Temperature has been identified as a spring egress trigger for *C. horridus* and other species by several studies (Andrews and Waldron 2017, Brown 1992, Jesper et al. 2023, Martin 1992), and they generally agree egress occurs at ~15 °C with minimal activity outside refugia below 10 °C. Like the second data chapter, my results support

the proposed egress temperature thresholds but notes a "stop-start" egress pattern resulting from the interruption of warmer temperatures by cold fronts, thus protracting the emergence period until the resumption of higher temperatures. Other studies on *C. horridus* report similar patterns with staggered emergence peaks occurring whenever temperatures exceeded 15 °C (Brown 1992, Martin 1992). A "stop-start" emergence pattern may seem an obvious and necessary thermal response to fluctuating temperatures, but studies often question the reasons for prolonged asynchronous spring egress in temperate reptiles (Blouin-Demers et al. 2000, DeGregorio et al. 2017, Gregory 1982) without fully attributing the effects of climatic stochasticity.

Once a snake has emerged from refugia, it must decide when to depart from overwintering habitat and migrate to summer ranges. Departing too early risks cold exposure during thermally stochastic springs without the relative protection of overwintering habitats, while delaying departure reduces valuable time spent pursuing active-season activities. My results suggest snakes balance the opposing selective pressures by waiting for a second thermal trigger, ~5 °C higher than egress, which signals the arrival of consistently warm temperatures appropriate for summer activity. Few other studies have reported the phenology and thermal triggers of departure in snakes, but those that have generally supported my findings, stating the beginning of spring migrations begin with the onset of the first warm and humid "summer-like" evenings (e.g., Martin 1992). Consecutive days of warmer weather prompt snakes to terminate the spring transient period and commence outbound migrations.

While the timing of spring departure was informatively different across years, it occurred only three days apart and consequently, I cannot conclusively uncouple the effects of temperature from a potentially fixed departure trigger, including a photoperiod or a physiological zeitgeber (Gregory 1982). However, given departure occurred at the same temperature each year

and the anecdotal reports of other studies (Martin 1992), I suspect temperature is the departure trigger, and the observed difference stems from similar inter-year thermal regimes. If temperature is the primary cue for departure, as it appears to be for egress, we should expect consistently warm spring temperatures to truncate the spring transient periods; snakes may even emerge and depart to summer habitats immediately, as demonstrated or suggested by other studies (Martin 1992, Sealy 2002). Thus, the spring transient period likely represents a behavioral adaption to the stochastic temperatures of temperate springs, suggesting prolonged spring transient periods may be primarily a northern latitude phenomenon. Further study in warmer southern regions is required to elucidate my findings.

One question I cannot directly answer is why snakes risk emerging early when temperatures are unsuitable for departure. Similarly, why did snakes not return to refugia during colder temperatures throughout the spring transient periods, as other studies have suggested (e.g., Brown 1993)? Such behavior seems counterintuitive as individuals leave the relative safety of refugia and become exposed to the surface. At a minimum, abandoning refugia early suggests nearby south-facing bluff habitats, such as crevices, leaf piles, and talus, are thermally adequate to survive the stochastic post-emergent temperatures but might also suggest alternative motivations. Snakes are likely taking advantage of the warmer south-facing environment to aid post-egress lethargy and improve physiological functions, including pre-departure ecdysis and possibly even acclimation. However, whether a basking period is required before the resumption of active season activities, as it is for other reptiles—notably those exposed to hypoxic conditions such as turtles (Janzen et al. 1992, Van Houtan et al. 2015)—is unknown and warrants further study.

Fall Transient Period. — Like the spring, *C. horridus* face similar risky decisions during the fall, which appear more nuanced. Specifically, snakes must anticipate falling temperatures in summer habitats to ensure they arrive at and ingress into refugia before environmental temperatures exceed lower thermal tolerances. My results suggest snakes intentionally arrive at overwintering sites at higher temperatures, and thus typically earlier in the fall, to avoid being caught in summer habitats when temperatures drop below thermal limits consistently. Anecdotally, snakes in both years began arriving at the vicinity of refugia on warmer days only after a noticeable decrease in temperature below $\sim 15^{\circ}\text{C}$, which most studies suggest is the voluntary thermal minima for the species and corresponds to the same temperature of egress. I suspect the initial temperature decrease served as the cue for snakes to return near refugia during the proceeding warmer periods, a hypothesis supported by other studies (Brown 1992, Martin 1992, Sealy 2002). However, the initial temperature decreases occurred only after snakes were already migrating toward overwintering refugia, as we began tracking only once snakes entered the telemetry range when standing near the refugia. Thus, while the temperature appeared to prompt snakes to finish their inbound migrations quickly in preparation for ingress, another trigger is responsible for initiating the fall migration in summer habitats. Further studies are required to examine such triggers.

Regardless of the arrival cues, most snakes did not enter overwintering refugia immediately. Instead, individuals waited until the last available opportunity to ingress as temperature permitted, resulting in a lengthy fall transient period in years remaining warm after arrival (e.g., 2021–2022) and a shorter fall transient period in years becoming cold soon after arrival (e.g., 2022–2023). Like the spring transient period, I am unsure why most snakes remained on the surface near refugia after arriving instead of immediately entering refugia,

although I suspect snakes have different motivations that are not present during the spring. Individuals are likely attempting to extend the time for active-season pursuits such as foraging, with some studies suggesting digestion of prey can delay ingress (Martin 1992, Sealy 2002). While I did not observe any foraging activity, I expect individuals basked to purge the intestinal tract of food before ingress (enhanced by the warm south-facing bluffs), as cold temperatures would prevent digestion. Additionally, physiologically impaired snakes may also be using the fall transient period to fight infection or disease (see below), a behavior which may continue throughout the winter months and into the spring (Jesper et al. 2024, Nordberg et al., 2016, Chapter 3).

Snakes typically entered refugia on days when temperatures fell below 15 °C, supporting the findings of other studies (Brown 1992, Martin 1992) and mirroring the average spring egress temperature (see “Spring Transient Period” section above), again suggesting the lower thermal limits of the species. Except for three individuals exhibiting consistent post-ingress / winter activity, once snakes entered refugia, they typically did not emerge until the spring. The shuttling individuals all appeared to be physiologically compromised, one with a visible injury around the incision site of the VHF transmitter and two later found dead. I suspect the abnormal behavior displayed by the snakes was prompted by the need to bask to fight infection, with snakes emerging exclusively during the warmer hours of warmer days to thermoregulate effectively. Thus, compared to other reports (Nordberg and Cobb 2016), midwinter emergences do not appear common in west-central Illinois, and thus, ingress into refugia conclusively ends the fall transient period.

Conclusions. — Migration is necessary for many refugia-dependent snakes to access often restricted resources while reducing conspecific competition of individuals using communal

refugia (Harvey and Larson 2020). However, to survive, individuals must navigate stochastic spring and fall temperatures by determining when to conduct seasonal migrations to access refugia without overly restricting already limited active seasons. My study suggests fall and spring transient periods serve as necessary buffers to ensure snakes have reliable and swift access to refugia and adjacent, thermally superior overwintering habitats when temperatures surpass lower thermal tolerances. Spring transient periods result from post-emergent snakes waiting at overwintering sites for a second thermal trigger—alongside the initial cue for egress—which signals the arrival of consistently warm temperatures appropriate for summer activity. Fall transient periods result from snakes intentionally arriving at refugia at higher temperatures, and thus typically earlier than they ingress to avoid being caught in summer habitats when temperatures fall below thermal limits.

The timing and duration of transient periods depend on seasonal stochasticity and are, therefore, phenologically flexible. As such, I suspect prolonged transient periods are a temperate latitude phenomenon motivated by stochastic fall and spring temperatures. Other factors may also encourage surface activity during the two periods (e.g., shedding, purging the gut of food, acclimation), which are beyond the scope of this study and warrant further study. My work complements and expands previous work on the overwintering strategies of *C. horridus* (e.g., Jesper et al. 2023; chapter three) and provides further insights into an understudied aspect of the annual life cycle of refugia-dependent and migratory snakes.

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4.7 TABLES AND FIGURES

Table 4.1 Parameter estimates for two generalized linear mixed models (GLMM) I used to determine and compare the mean day of 1) arrival and ingress into refugia in the fall (“*fall_day*” model) and 2) egress and departure to summer ranges in the spring (“*spring_day*” model), by Timber Rattlesnakes (*Crotalus horridus*) in Jersey County, Illinois. I set the response variable as the day of year and the explanatory variables as an interaction between the discrete variables “movement type” (Fall = arrival and ingress; Spring = egress and departure) and study year. Columns represent the model parameter (Parameter), the parameter estimate (Estimate), and the upper and lower 95% confidence intervals (CI) of the parameter estimates.

Parameter	Estimate	Upper CI	Lower CI
<i>fall_day model</i>			
Intercept	255.71	252.07	259.34
Movement Type	28.06	22.74	33.38
Year	11.59	5.62	17.56
Movement Type * Season	-19.86	-28.25	-11.47
<i>Spring_day model</i>			
Intercept	110.58	107.65	113.51
Movement Type	21.88	19.17	24.59
Year	-7.76	-10.73	-4.80
Movement Type * Season	4.44	1.14	7.73

Table 4.2 Parameter estimates for two generalized linear mixed models (GLMM) I used to determine and compare the mean minimum daily temperature of 1) arrival and ingress into refugia in the fall (“*fall_temp*” model) and 2) egress and departure to summer ranges in the spring (“*spring_temp*” model), by Timber Rattlesnakes (*Crotalus horridus*) in Jersey County, Illinois. I set the response variable as minimum daily temperature and the explanatory variables as an interaction between the discrete variables “movement type” (Fall = arrival and ingress; Spring = egress and departure) and study year. Columns represent the model parameter (Parameter), the parameter estimate (Estimate), and the upper and lower 95% confidence intervals (CI) of the parameter estimates.

Parameter	Estimate	Upper 95% CI	Lower 95% CI
<i>fall_temp</i> model			
Intercept	19.70	18.47	20.93
Movement Type	-5.18	-7.33	-3.04
Year	-0.52	-2.55	1.50
Movement Type * Year	-0.49	-3.83	2.86
<i>Spring_temp</i> model			
Intercept	14.00	12.06	15.93
Movement Type	5.63	3.51	7.75
Year	-0.09	-2.16	1.98
Movement Type * Year	-1.22	-3.76	1.33

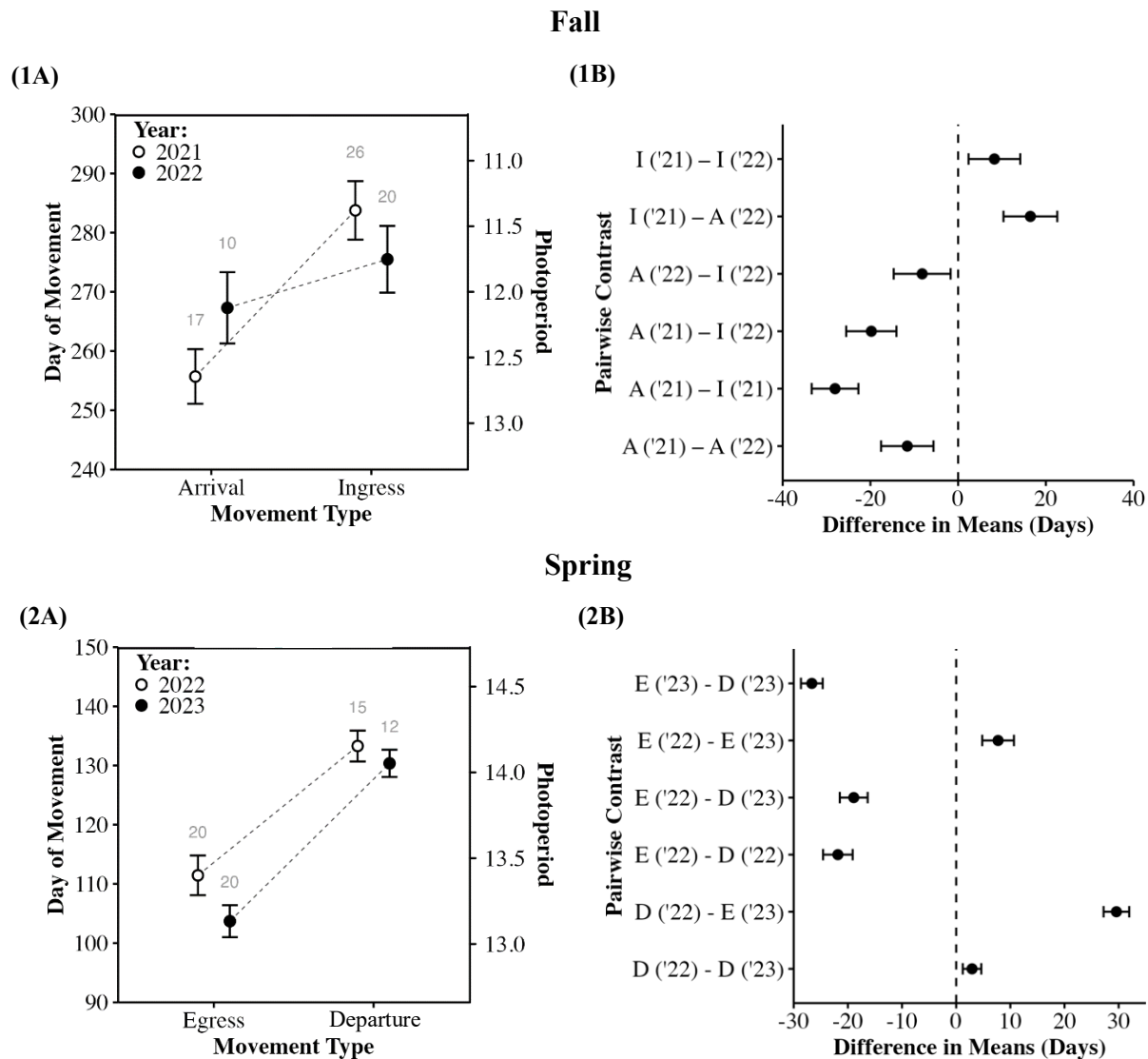


Figure 4.1 Marginal (mean) day of fall and spring transient period related movements by Timber Rattlesnakes (*Crotalus horridus*) in Jersey County, Illinois. Plots show the marginal day of arrival to overwintering habitat ("Arrival") and subsequent ingress into refugia ("Ingress") in the fall (Subplot 1A), alongside corresponding pairwise contrasts of the difference between values (Subplot 1B) and the marginal day of egress from refugia ("Egress") and subsequent departure to summer habitats ("Departure") in the spring (subplot 2A), alongside corresponding pairwise contrasts of the difference between values (Subplot 2B). Error bars represent 95% confidence intervals. Subplots 1A and 2A also show sample sizes above each group, and a secondary axis with corresponding photoperiod values.

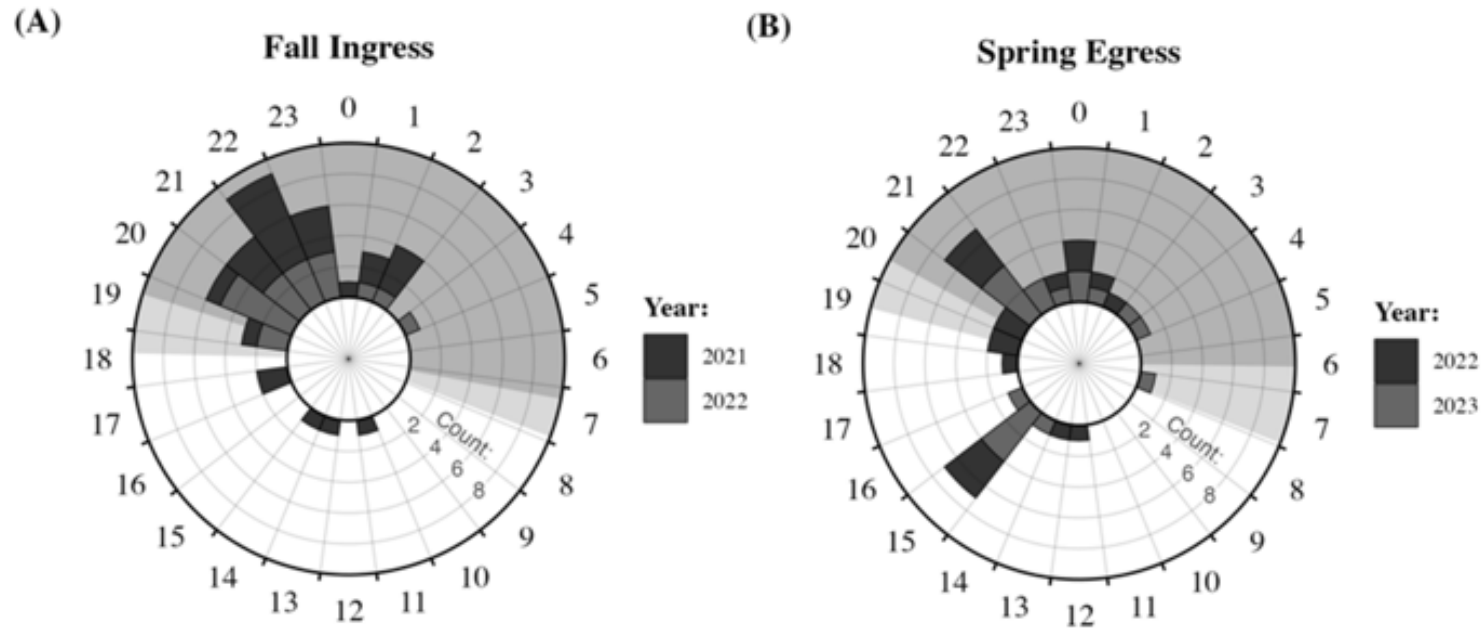


Figure 4.2 The time of fall ingress into refugia (subplot A; n = 46 ingress events) and spring egress from refugia (subplot B; n = 40 egress events) by PIT tagged Timber Rattlesnakes (*Crotalus horridus*) in Jersey County, Illinois. Each plot represents a 24-hr circular "clock" (e.g., 0 = 12 a.m.) with grey bars showing the number of ingress/egress events observed within each hour. Data pooled across refugia. Shaded sections depict hours of darkness (dark-grey shade) and dawn/dusk (mid-grey shade). Data collected using BioMark Passive Integrated Transponder (PIT) tag detection systems.

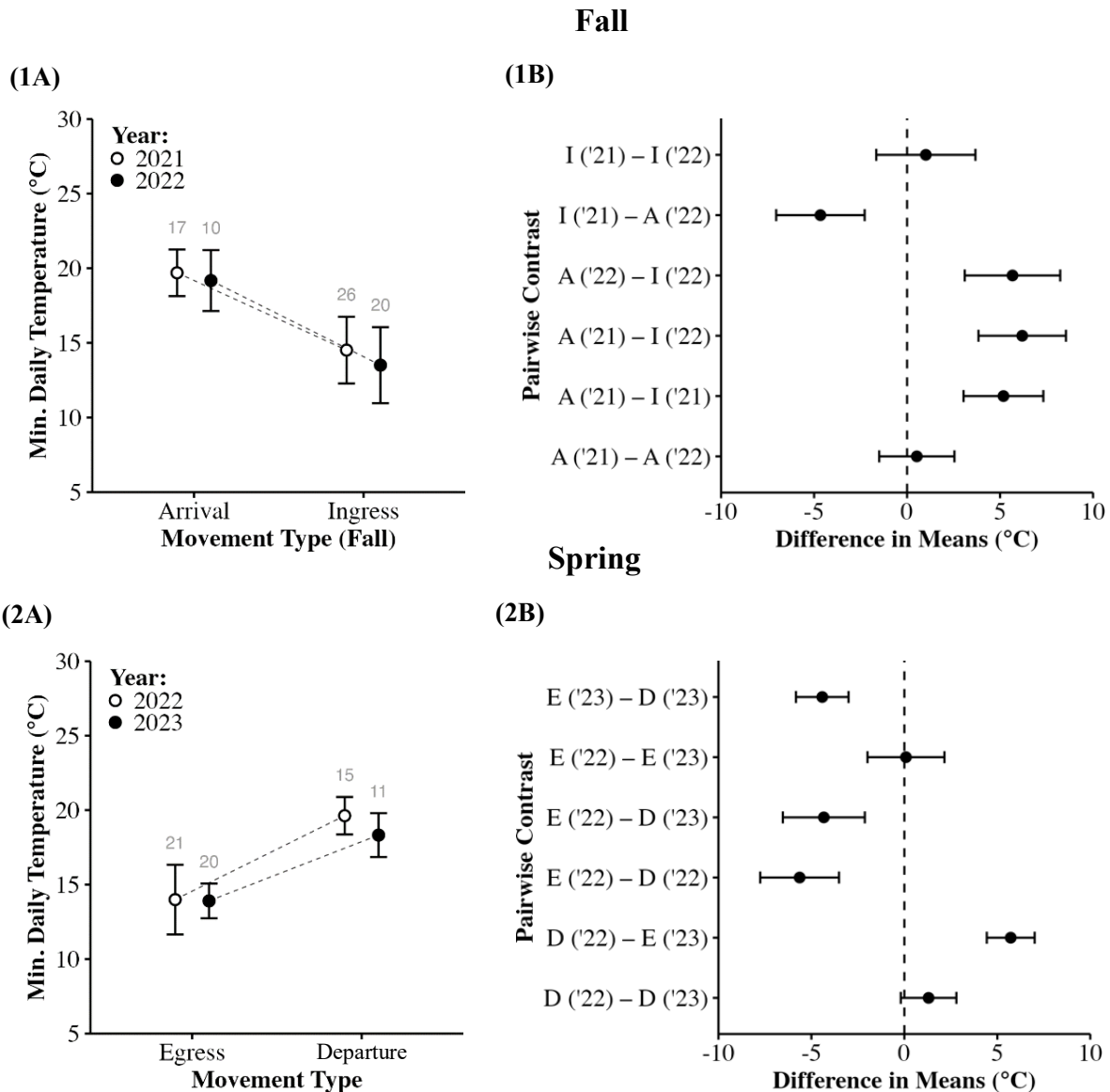


Figure 4.3 Marginal (mean) minimum daily temperature of fall and spring transient habitat-related movements by Timber Rattlesnakes (*Crotalus horridus*) in Jersey County, Illinois. Plots on the top row show the marginal temperature of arrival to overwintering habitat ("Arrival") and subsequent ingress into refugia ("Ingress") in the fall (Subplot 1A), alongside corresponding pairwise contrasts of the difference between values (Subplot 1B). Plots on the bottom row show the marginal temperature of egress from refugia ("Egress") and subsequent departure to summer habitats ("Departure") in the spring (subplot 2A), alongside corresponding pairwise contrasts of the difference between values (Subplot 2B). Error bars represent 95% confidence intervals. Subplots 1A and 2A also show sample sizes above each group.

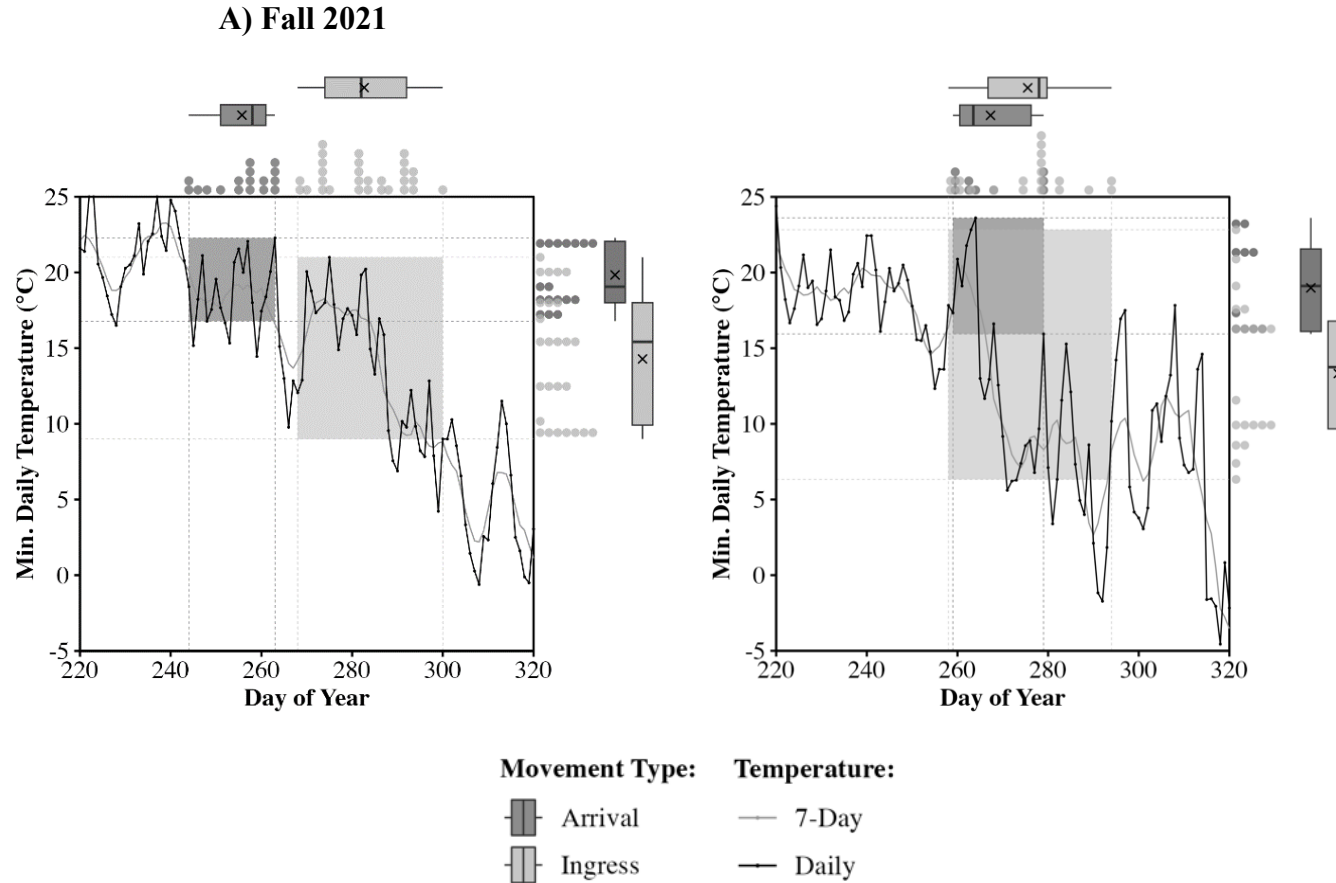


Figure 4.4 The daily minimum temperature and day of arrival to overwintering habitat and ingress into refugia by Timber Rattlesnakes (*Crotalus horridus*) in Jersey County, Illinois, during fall 2021 (subplot A) and 2022 (subplot B). Dot and box plots display the data distribution for the day (top) and temperature (right) of arrival and ingress by all monitored snakes. Each boxplot displays the range (whiskers), interquartile range (boxes; middle 50% of data), median (vertical line), and mean (cross) of the data for each group. The bin size for each dot plot is set to 1-unit increments (i.e., 1 day or 1 °C). Translucent boxes (and dashed lines) spanning the data range for both axes are shown for reference.

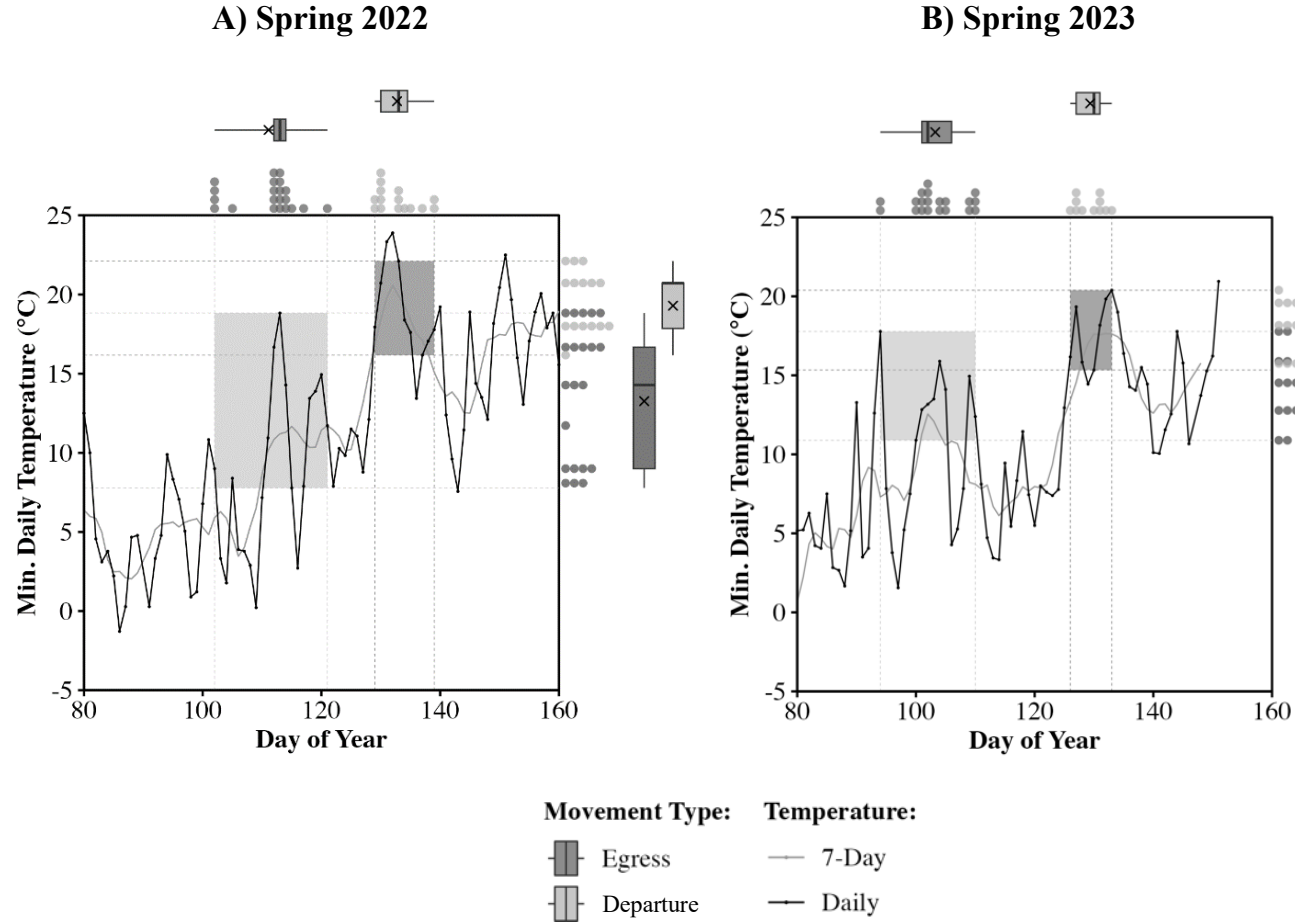


Figure 4.5 The daily minimum temperature and day of egress from refugia and subsequent departure to summer habitats by Timber Rattlesnakes (*Crotalus horridus*) in Jersey County, Illinois, during spring 2022 (subplot A) and 2023 (subplot B). Dot and box plots display the data distribution for the day (top) and temperature (right) of egress and departure by all monitored snakes. Each boxplot displays the range (whiskers), interquartile range (boxes; middle 50% of data), median (vertical line), and mean (cross) of the data for each group. The bin size for each dot plot is set to 1-unit increments (i.e., 1 day or 1 °C). Translucent boxes (and dashed lines) spanning the data range for both axes are shown for reference.

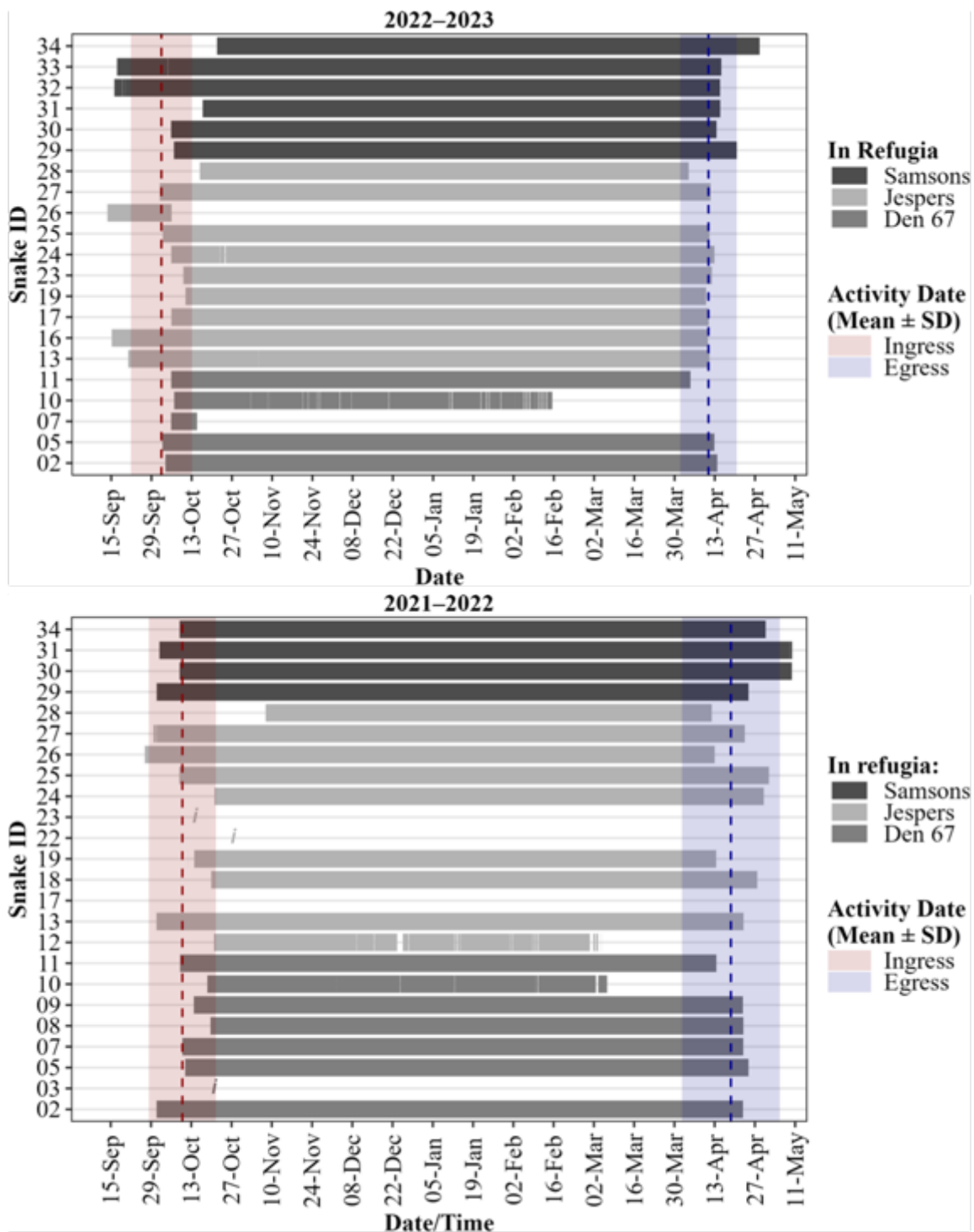


Figure 4.6 Refugia-use of Timber Rattlesnakes (*Crotalus horridus*) during the 2021-2022 (n = 21) and 2022-2023 (n = 24) overwintering periods. Horizontal bars indicate when snakes were in refugia and breaks in the bars (white vertical lines) indicate surface activity. The letter "i" represents the time of a recorded ingress without subsequent egress. Data collected using small-scale PITT detection systems deployed at the entrances of three refugia in Jersey County, IL.

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CHAPTER FIVE: SUMMARY

My dissertation aimed to elucidate several behavioral strategies used by refugia-dependent reptiles—focusing on enigmatic and imperiled Timber Rattlesnakes (*C. horridus*)—to navigate and survive harsh temperate winters successfully.

In data chapter one (chapter two), I identified the overwintering habitat characteristics of *C. horridus* throughout Illinois and determined the distribution of likely hibernacula habitats throughout the state. Through iterative habitat suitability modeling consisting of three distinct rounds of Maxent, I identified slope angle, topographical position index, forest patch area, and aspect as important drivers of *C. horridus* hibernacula habitat. The five variables and site surveys suggest a suitable overwintering habitat for *C. horridus* in Illinois is located on south to southwest facing outcrops on upper slopes and ridges of larger forest patches. Such habitat is distributed primarily in southern Illinois and throughout the Mississippi and Illinois River border counties, where rugged terrain intercepts the state's remaining forest land unsuitable for conversion to agriculture.

I advocate using the high-resolution distribution maps to guide future surveys for new hibernacula and populations and hope future investigators will build on my results by continuing the iterative modeling process to create more robust and informative hibernacula HSMs as new hibernacula locations are located. I also hope further studies will focus on constructing geographically broader hibernacula HSMs to examine the characteristics and distribution of *C. horridus* hibernacula habitat region wide.

In data chapter two (chapter three), I used wildlife cameras and weather station-derived environmental data to observe and predict the daily surface presence of *C. horridus* throughout the late winter and early spring at communal refugia in west-central and northern Illinois. The

top model for predicting the surface presence of snakes included the additive effects of maximum daily temperature, accumulated degree days, and latitude. With notable exceptions in the southeastern U.S., the model accurately predicted the average emergence day for eight other populations range-wide, emphasizing the importance of temperature in influencing the phenological plasticity observed across the species' range.

The apparent broad applicability of the model to other populations suggests it can be a valuable tool in predicting spring emergence phenology. Consequently, I also provided predictions of surface presence for each latitude of Illinois to serve as preliminary hypotheses of site-level spring emergence phenology. I encourage flexible scheduling whenever possible to account for intra-year and latitudinal climatic differences, although I recognize such scheduling would require the frequent calculation of model predictions based on current temperatures, which are not as readily accessible or practical as a single fixed date threshold. Such difficulties could be remedied by making the predictive model accessible by managers and researchers (e.g., hosted online), allowing them to generate predictions based on automated data collected from nearby weather stations. I also recognize the need for similarly available models to determine the drivers and phenology of the species' fall ingress, which future studies should focus on.

In data chapter three (chapter four), I demonstrated more clearly the existence, phenology, and likely purpose of the fall and spring transient periods for Timber Rattlesnakes (*Crotalus horridus*) in west-central Illinois. My results suggest the transient periods for *C. horridus* can last upwards of a month, are phenologically flexible in response to variable annual temperatures, and serve as necessary buffers, ensuring snakes have reliable and swift access to thermally adequate refugia when temperatures surpass lower thermal tolerances. My findings complement and expands previous work on the overwintering strategies of *C. horridus* and

provides further insights into an understudied aspect of the annual life cycle of refugia-dependent and migratory snakes.

In many regards, the findings in my dissertation are foundational and fill knowledge gaps in the ecology of *C. horridus* (and similar species), which are relatively well-defined in other taxonomic groups. Like many snakes, studying *C. horridus* during the overwintering period is challenging given the low detection rates of enigmatic and fossorial individuals, tough surveying conditions in topographically rugged terrain, and strict secrecy measures resulting from their historic persecution rates and threatened conservation status. Consequently, relatively simple but fundamental information for effective conservation and study—namely when, where, and how refugia are being used—is unavailable across much of the species' range, thus severely restricting conservation and management in response to ongoing population declines.

My hope is the findings of my dissertation will be used to better understand the overwintering ecology of *C. horridus* and similar species, with the ultimate goal of providing a foundation for further study and enhanced protection of hibernacula and their occupants during one of the most vulnerable periods in the life cycle of these unique forest-dwelling pitvipers.

APPENDIX A: SUPPLEMENTARY MATERIALS FOR CHAPTER TWO

Text A.1 Habitat Suitability Modelling for the initial Maxent model iteration (round one, 2019).

Maxent modeling. — I used 13 refugia occurrences in the initial Maxent model iteration (round one, 2019). Due to the small sample size, I limited model complexity by using only the variables *slope*, *topographical position index* (TPI), and *aspect* (See main text and Table 2.1) and specified only the linear (L) feature class in the Maxent. All other Maxent settings remained at their default values. I evaluated the relative performance of the model using the area under the curve (AUC) of the receiver operating characteristic (Hanley & McNeil, 1982) and examination of observed versus predicted test omission rates (See main text for description). Due to the small sample size, I used a bootstrapping approach. The training data was selected by randomly sampling the occurrence records with replacement (with the number of samples equaling the total number of occurrence records) and testing the model on the occurrence records not selected. I repeated the bootstrapping procedure for 100 replicate runs and reported the results for an averaged model $\pm$ one standard deviation of the replicate runs. Finally, I generated a Clog-log transformed raster from the averaged model representing the predicted probability of suitable refugia habitat across Illinois.

I examined how each predictor variable affected the probability of suitable refugia habitat using the final averaged model's marginal response. The plot data was output by the Maxent software and then imported into R (R Core Team 2021) to create graphs using the package 'ggplot2' (Wickham et al., 2016). I also examined the relative importance of each variable in predicting suitable refugia habitat using jackknife tests and variable percent contributions, both output by Maxent.

The bootstrap-averaged top model received a mean AUC performance value of 0.92 (SD = 0.54), and the omission rate of training samples closely resembled the predicted omission rate (i.e., followed a straight line, Figure A.2), both suggesting good model fit. I provide the results for jackknife tests of variable importance (Figure A.2), the omission rate and predicted area as a function of the cumulative threshold (Figure A.2), and marginal response plots (Figure A.3).

Survey procedures. – To bolster sample sizes and to field-validate the model, I conducted model-guided surveys throughout southern Jersey County, IL (Figure A.1). I selected southern Jersey County because I knew *C. horridus* were present throughout the region, but specific refugia locations were unknown, thus warranting further investigation. To maximize the chances of finding refugia, I constrained survey efforts to areas with a probability $\geq 50\%$ (as determined by a Maxent-derived C-loglog probability raster) and searched during the species' spring egress period when snakes were likely to be surface active at refugia. I randomly selected 24 sites from across the region (Figure A.1) using the "random points" tool in ArcGIS Pro 3.0, with the constraining extent set to areas with a Probability $\geq 50\%$ and the minimum distance between points (sites) set to 0.5 km to increase coverage of the region.

I designed the initial survey procedures to fit within a detection and occupancy survey framework, whereby I split each site into eighteen 50 x 10 m transects (three rows of six; Figure A.1) positioned along the top, middle, and bottom of suspected outcrops and shallow slopes as determined via bands of high probability habitat. Teams of three surveyors (six volunteers) surveyed each site at least once during the spring egress period, beginning at one end of the site and navigating through the pre-determined transects using GPS devices (which also tracked the time spent in each transect). To reduce search bias, each surveyor switched rows (see map inset in Figure A.1) every two transects, thus searching the top, middle, and bottom of the potential

outcrops twice per site (each with varying habitat and topographic characteristics, such as the abundance of outcrop/crevices).

Overall, between 6–25 April, surveyors spent 135.34 person-hours across 10 days searching for *C. horridus* (three sites per day with some repeat site visits). Twenty-three of the 24 sites (96%) comprised bluff or interior forest outcrops with associated talus slopes and boulder fields, while one set consisted of highly sloped shallow soils with minimal surface outcrops. Surveyors found two additional refugia; the first which was identified by three snakes (2 adults and 1 juvenile) coiled near a large crevice and the second by a large male egressing from a hole at the soil-rock interface.

Text A.2. Habitat Suitability Modelling for the initial Maxent model iteration (round two, 2022).

Maxent modeling. — In 2022 (model round two), I created a revised Maxent model using 34 refugia locations (21 new refugia). Alongside the two refugia discovered from the first model iteration, I included eight refugia located by IDNR staff and in-state naturalists during surveys in southern Illinois in 2020. I also included 11 refugia determined via opportunistic information from landowners and in-state naturalists who had located refugia in the past 20 years. The modeling procedures (e.g., predictor variables used, MaxEnt settings, and model evaluation) for the second model iteration were identical to the third (final) iteration explained in the main text.

With 100% of the cumulative AIC_c model weight, the best-fit Maxent model included the quadratic and hinge feature classes with an L1-regularization multiplier of 1. The bootstrap-averaged top model received a mean AUC performance value of 0.94 (SD = 0.23), and the omission rate of training samples closely resembled the predicted omission rate (i.e., followed a straight line, Figure A.6), suggesting excellent model fit. I provide the results for jackknife tests of variable importance (Figure A.6), the omission rate and predicted area as a function of the cumulative threshold (figure A.6), and marginal response plots (figure A.7) below.

Survey procedures. — In April 2023, I conducted model-guided surveys in northern Pope County, southern IL, to bolster sample sizes and field-validate the second model iteration. I selected Pope County because, like Jersey County, I knew *C. horridus* were present throughout the region, but specific refugia locations (besides one location) were unknown, thus warranting further investigation. Surveying in Pope County also allowed me to validate model predictions in other regions of Illinois (Pope County is ~230 km southeast of Jersey County). Adapting from previous survey efforts in Jersey County, I conducted surveys only on warmer days (daytime

temps > 20 °C) during the spring egress period and made repeat visits to habitats deemed highly suitable ($P \geq 75\%$) by the HSM to maximize detection chances. Additionally, I did not constrain surveys to the detection-occupancy survey (transect) procedures adopted during the Jersey County surveys because they limited time in an already restricted search window (i.e., between snake egress and departure to summer habitat). Thus, surveys in Pope County consisted of searching all high-probability habitats throughout the region, hiking the same route each day between 1000–1800 hrs (see survey route; Figure A.4).

Between 10–20 April, I searched for 30.22 hr over 5 days (mean = 6.04 hours/day). All high-probability habitats consisted of south-facing outcrops and ridgelines on upper slopes and ridges, which resembled habitat at known refugia locations throughout Illinois. Searches were conducted around outcrops, associated talus slopes, and boulder fields alongside nearby logs and brush piles. I found two new *C. horridus* refugia during the surveys ~ 1.5 km apart, revealed as *C. horridus* emerged from holes at the soil-rock interface. Two adult snakes (unknown sex) were located at the first refugia ~ 10 m apart; one coiled under a rock slab and one emerging from a hole under a large outcrop. I located the second refugia as a large male *C. horridus* emerged from a hole under a rock slab and returned to the refugia shortly after the surveyor approached.

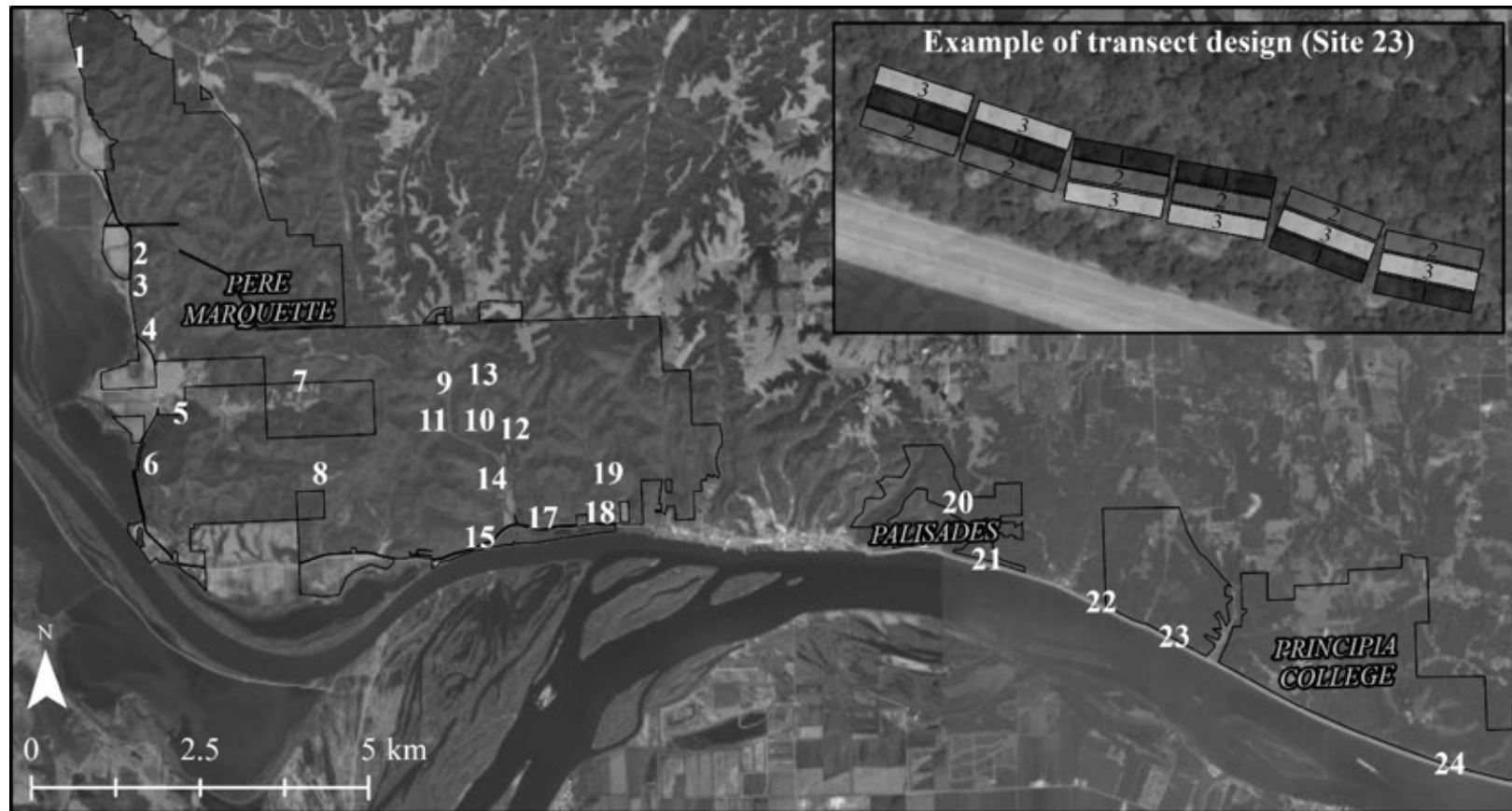
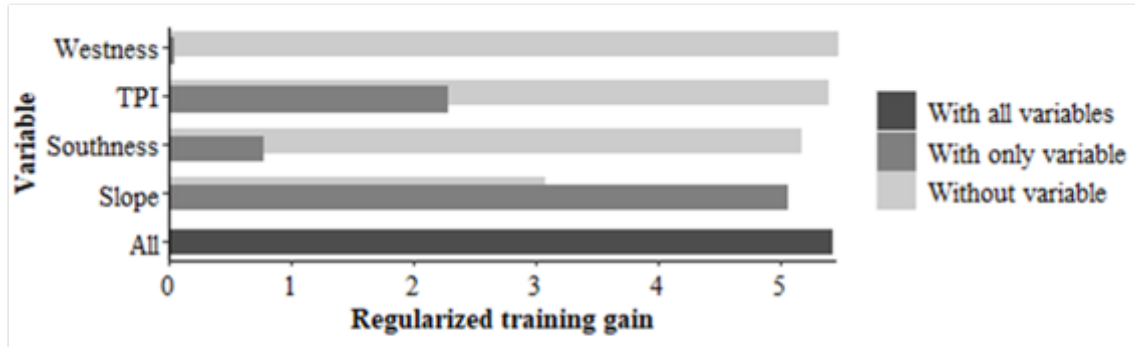


Figure A.1 Sites ($n = 24$) surveyed for *Crotalus horridus* and refugia during the first model iteration (round one) in spring 2019 at Pere Marquette State Park, Palisades Nature Preserve, and Principia College, in southern Jersey County, IL. White numbers represent each site's location, and black polygons denote property boundaries. The map inset shows an example transect design carried out by three surveyors at each site, with numbers in each transect denoting the surveyor ID (1, 2, or 3).

(A)



(B)

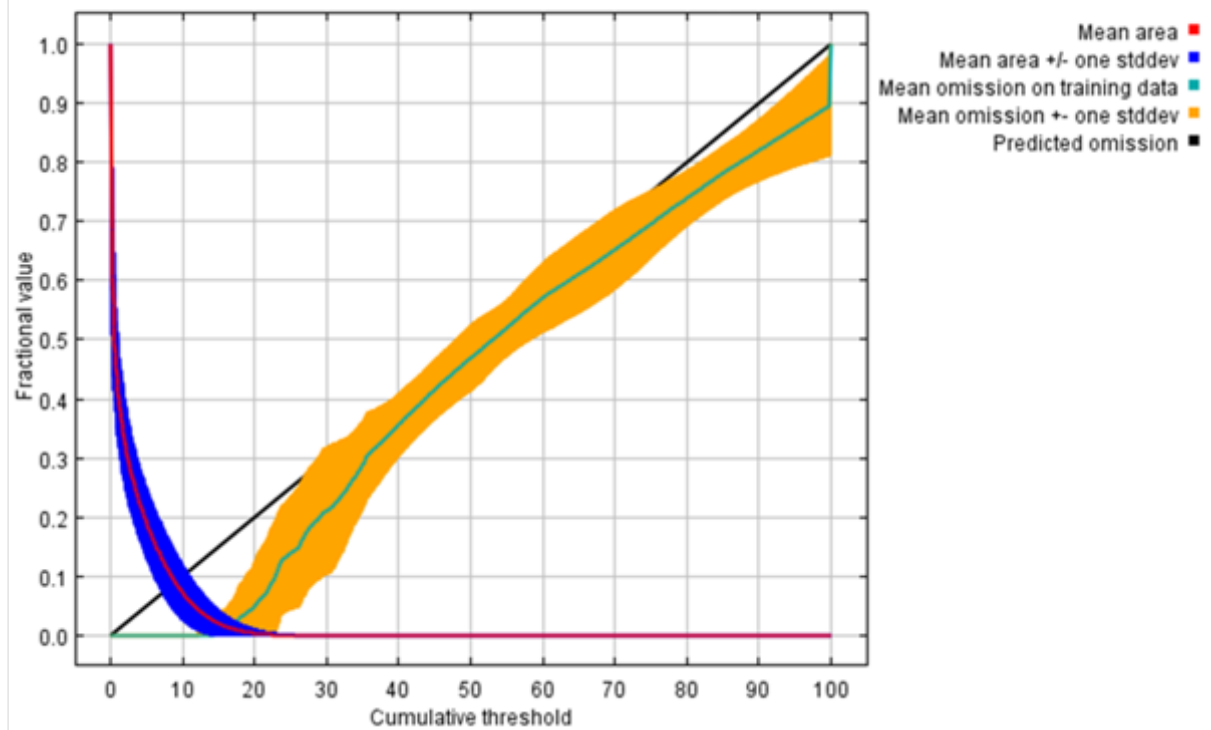


Figure A.2 Model performance metrics for the first Maxent model iteration (Round One) in 2019 averaged over 100 bootstrapped replicate runs: A) Jackknife plots of variable importance; and B) the omission rate and predicted area as a function of the cumulative threshold.

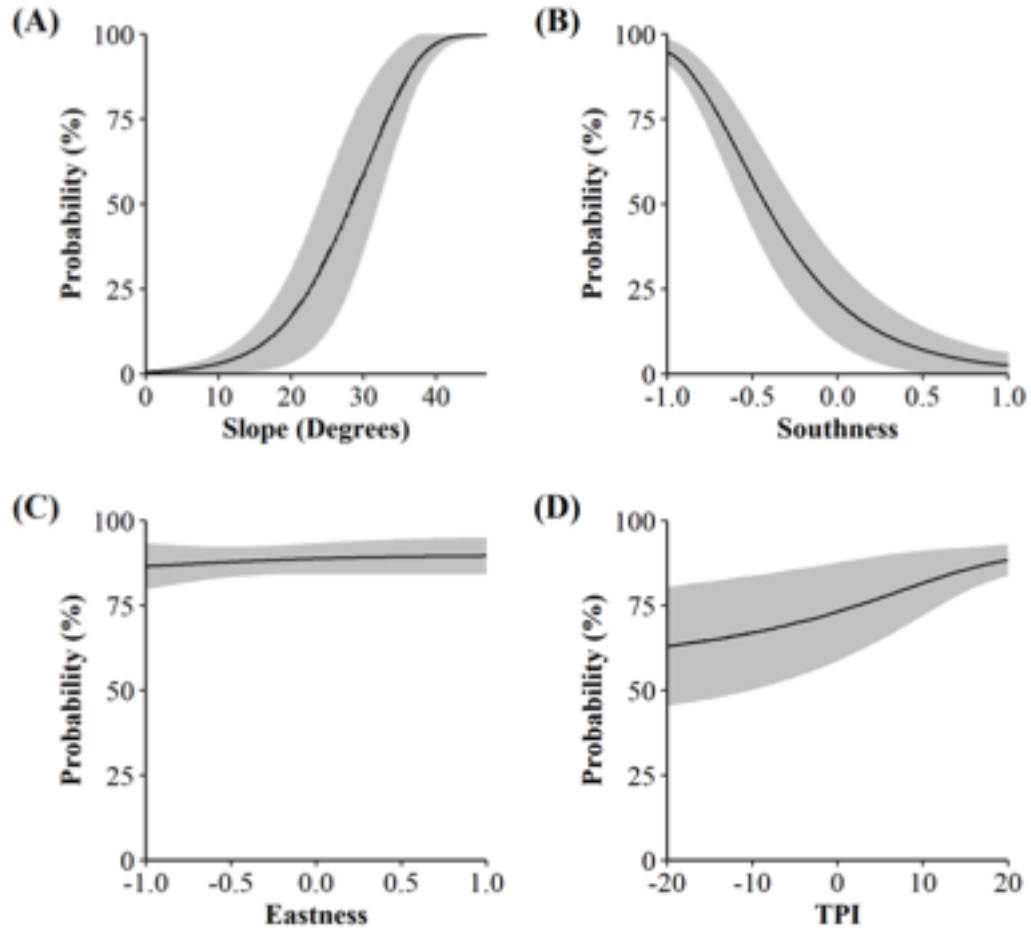


Figure A.3 Marginal response plots for the first Maxent model iteration (round one) in 2019, depicting the predicted probability of suitable refugia habitat for each predictor variable while holding all other predictor variables at their mean values. Each plot shows the mean response of 100 bootstrapped replicate runs (black lines) $\pm$ one standard deviation (grey ribbons).

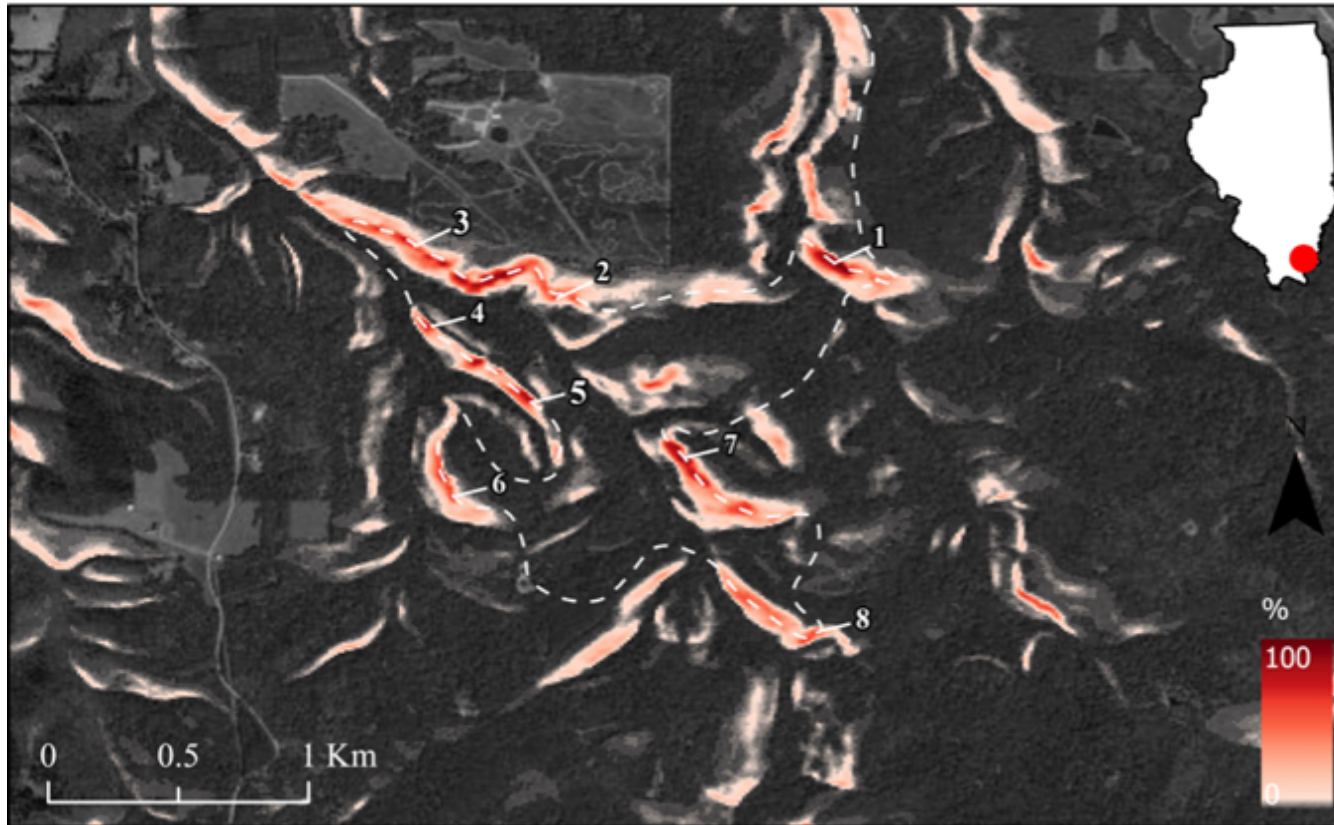
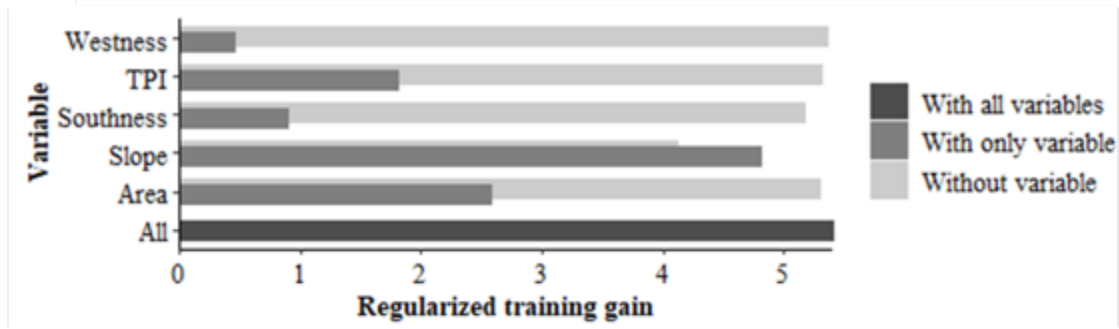


Figure A.4 The model-guided survey route (white dashed line) during the second model iteration (round two) in spring 2023 at northern Pope County, Il. I have overlain the probability raster (Clog-log output; Probability values of 0% made transparent) used during surveys to aid in map interpretation. Searches for *C. horridus* and refugia occurred along the survey route (white-dashed line) in all habitats with probability > 75%. Number labels correspond to the locations of photos in Figure A.6, taken during the survey period.



Figure A.5 Photos of surveyed habitat in Pope County identified as "highly suitable" ($P > 75\%$) by the second Maxent model iteration. Each photo number corresponds to labeled locations detailed in Figure A.4. I found two new refugia during the model-guided surveys.

(A)



(B)

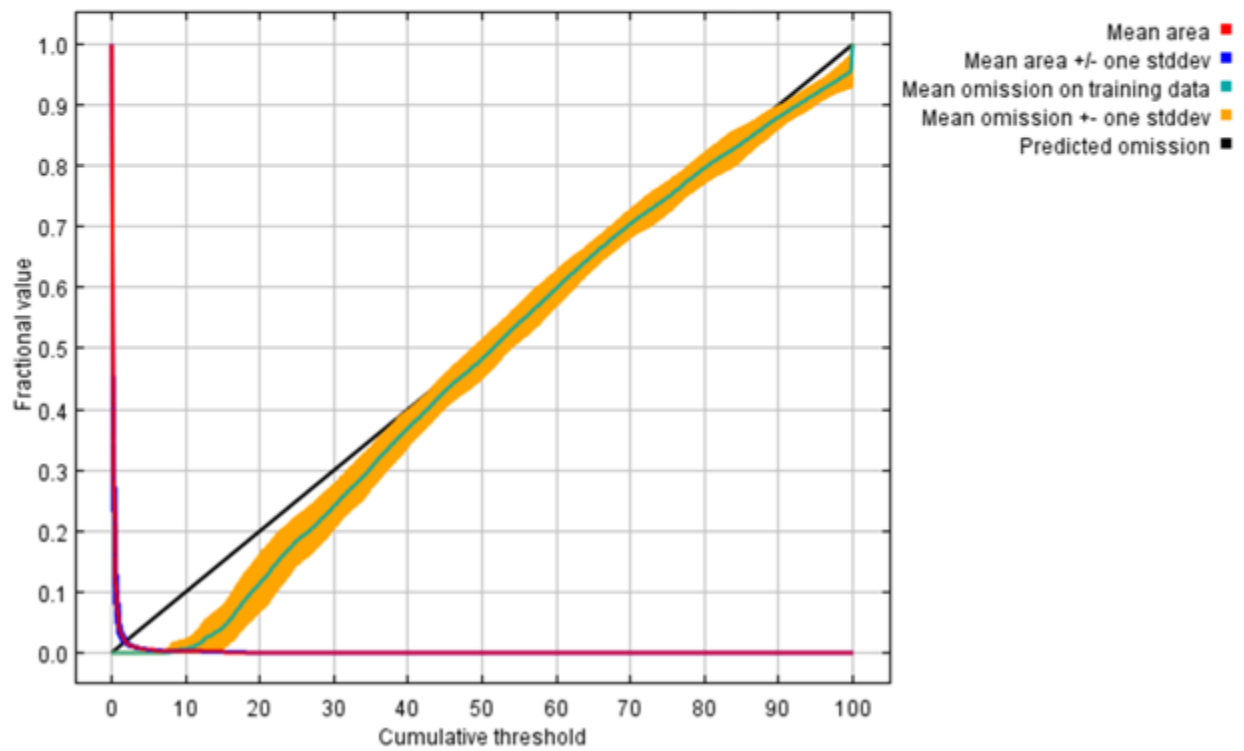


Figure A.6 Model performance metrics for the second Maxent model iteration (Round two) in 2023, averaged over 100 bootstrapped replicate runs: A) Jackknife plot of variable importance; and B) the omission rate and predicted area as a function of the cumulative threshold.

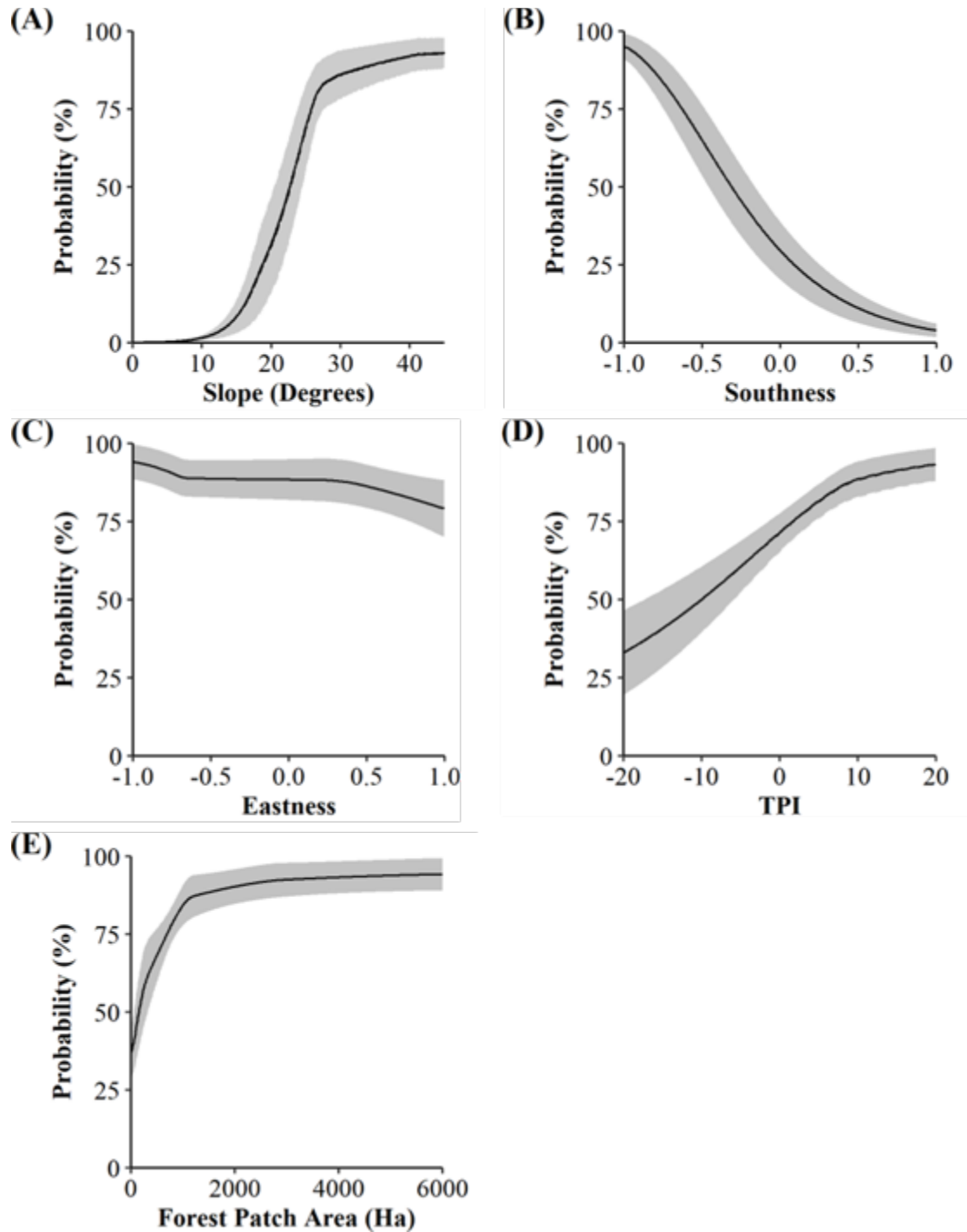


Figure A.7 Marginal response plots for the second Maxent model iteration (round two) in 2023, depicting the predicted probability of suitable refugia habitat for each predictor variable while holding all other predictor variables at their mean values. Each plot shows the mean response of 100 bootstrapped replicate runs (black lines) $\pm$ one standard deviation (grey ribbons).

APPENDIX B: SUPPLEMENTARY MATERIALS FOR CHAPTER THREE

Table B.1 Candidate model set examining the effects of environmental variables on the daily surface presence of Timber Rattlesnakes (*Crotalus horridus*) during spring emergence at two sites in Illinois, USA. I created 32 Generalized Logistic Mixed Effects Models (GLMMs) to examine the environmental drivers of daily surface presence of *C. horridus* at refugia in Jo Daviess (northern Illinois; $n = 3$) and Jersey (west-central Illinois; $n = 3$) counties during late winter and spring of 2018–2020. I chose the best model from the set for further analysis using Akaike's Information Criterion corrected for small sample sizes (AICc). Descriptions of predictor variables are found in Table 3.1.

Model	Parameters
1	Intercept only
2	Global
3	Latitude + ADD ² + Min. Temp ²
4	Latitude * Min. Temp ² + ADD ²
5	Latitude * ADD ² + Min. Temp ²
6	Latitude * Min. Temp ²
7	Latitude + Min. Temp ²
8	Latitude + ADD ² + Max. Temp ²
9	Latitude * Max. Temp ² + ADD ²
10	Latitude * ADD ² + Max. Temp ²
11	Latitude * Max. Temp ²
12	Latitude + Max. Temp ²
13	Latitude + ADD ² + Mean Temp ²
14	Latitude * Mean. Temp ² + ADD ²
15	Latitude * ADD ² + Mean Temp ²
16	Latitude * Mean Temp ²
17	Latitude + Mean Temp ²
18	Latitude + ADD ² + Min ₅ . Temp ²
19	Latitude * Min ₅ . Temp ² + ADD ²
20	Latitude * ADD ² + Min ₅ . Temp ²
21	Latitude * Min ₅ . Temp ²
22	Latitude + Min ₅ . Temp ²
23	Latitude + ADD ² + Max ₅ . Temp ²
24	Latitude * Max ₅ . Temp ² + ADD ²
25	Latitude * ADD ² + Max ₅ . Temp ²
26	Latitude * Max ₅ . Temp ²
27	Latitude + Max ₅ . Temp ²
28	Latitude * Day of Year ²
29	Latitude * Day of Year ² + Max. Temp ²
30	Latitude * Day of Year ² + Min. Temp ²
31	Latitude * Day of Year ² + Min ₅ . Temp ²
32	Latitude * ADD ²

Table B.2 The day of year and date when the predicted probability of surface presence for Timber Rattlesnakes (*Crotalus horridus*) typically exceeded a given threshold in Illinois during my study (2018–2020). I determined day of year (day 1 = 1-Jan) and corresponding date thresholds (TH) for each latitude (in 1° increments) using 14-day moving averages of predicted probabilities averaged across all years, allowing examination of general phenological trends (Figure 3.6). I do not report probability threshold values > 60% as 14 day moving averages did not typically exceed this value, and do not report probability threshold values of 0% as such occurrences were rare (i.e., there is always some probability of surface presence). Selection of probability thresholds should be determined by the appropriate authorities depending on the specific application (see "Conservation Implications" in the discussion) and should be treated as hypotheses until verified with empirical data.

TH	Latitude (°)					
	37	38	39	40	41	42
5%	54 (23-Feb)	59 (28-Feb)	69 (10-Mar)	87 (28-Mar)	90 (31-Mar)	95 (5-Apr)
10%	56 (25-Feb)	62 (3-Mar)	84 (25-Mar)	93 (3-Apr)	94 (4-Apr)	110 (20-Apr)
15%	65 (6-Mar)	83 (24-Mar)	85 (26-Mar)	94 (4-Apr)	95 (5-Apr)	112 (22-Apr)
20%	80 (21-Mar)	84 (25-Mar)	87 (28-Mar)	95 (5-Apr)	96 (6-Apr)	114 (24-Apr)
25%	82 (23-Mar)	85 (26-Mar)	90 (31-Mar)	96 (6-Apr)	96 (6-Apr)	119 (29-Apr)
30%	85 (26-Mar)	86 (27-Mar)	94 (4-Apr)	97 (7-Apr)	99 (9-Apr)	120 (30-Apr)
35%	86 (27-Mar)	88 (29-Mar)	95 (5-Apr)	98 (8-Apr)	111 (21-Apr)	121 (1-May)
40%	90 (31-Mar)	90 (31-Mar)	95 (5-Apr)	99 (9-Apr)	112 (22-Apr)	122 (2-May)
45%	91 (1-Apr)	95 (5-Apr)	96 (6-Apr)	100 (10-Apr)	113 (23-Apr)	122 (2-May)
50%	92 (2-Apr)	95 (5-Apr)	98 (8-Apr)	111 (21-Apr)	116 (26-Apr)	124 (4-May)
55%	95 (5-Apr)	96 (6-Apr)	98 (8-Apr)	112 (22-Apr)	119 (29-Apr)	134 (14-May)
60%	96 (6-Apr)	99 (9-Apr)	99 (9-Apr)	114 (24-Apr)	121 (1-May)	135 (15-May)

APPENDIX C: SUPPLEMENTARY MATERIALS FOR CHAPTER FOUR



Figure C.1 Photos of a funnel trap and enclosure system used to capture emerging Timber Rattlesnakes (*Crotalus horridus*) at refugia in Jersey County, Illinois, during spring egress 2021. A team of researchers visited dens daily to retrieve snakes which had emerged from overwintering refugia, moved through the funnel traps, and become trapped in enclosures.

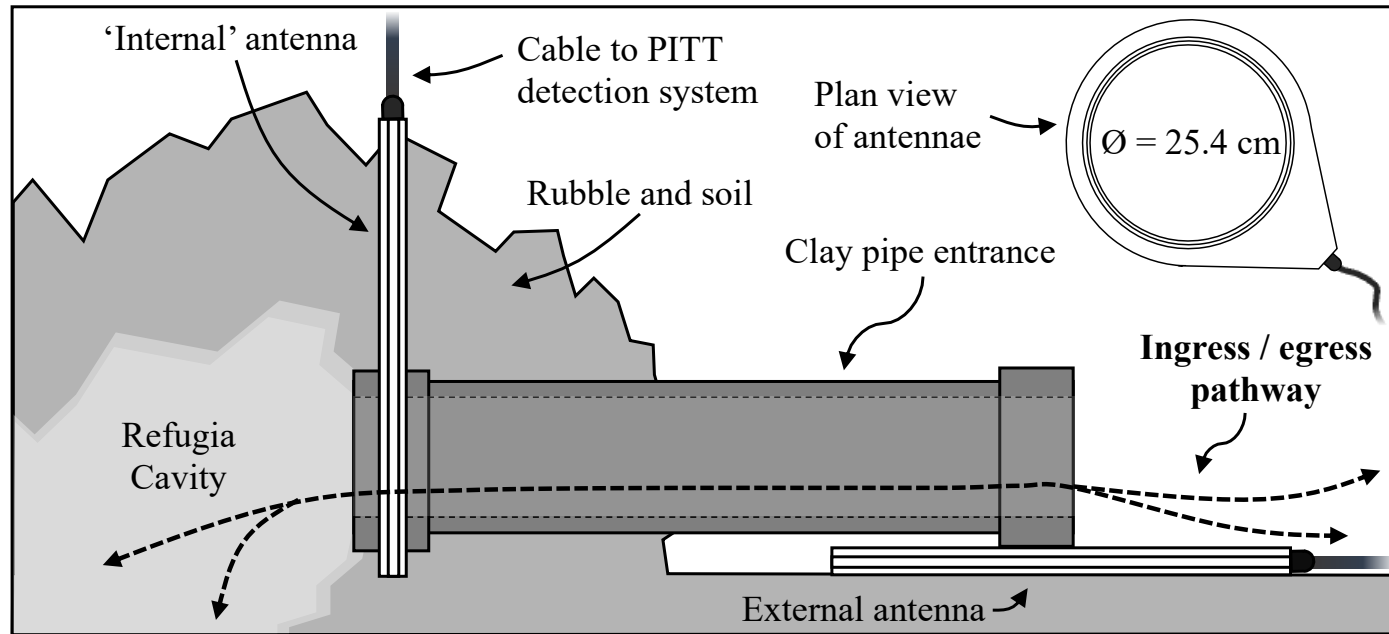


Figure C.2 Side-view diagram (not to scale) of a modified Timber Rattlesnake (*Crotalus horridus*) refugium entrance fitted with a small-scale PIT tag detection system. A PIT detection system was comprised of a BioMark IS1001-12V PIT tag reader and circular antennae (25.4 cm internal diameter; 15 m cable length). We deployed three PIT tag systems at three refugia in Jersey County IL, with two antennae arranged at known entrances which we carefully modified using a clay pipe and rubble/soil to ensure successful detection of snakes.

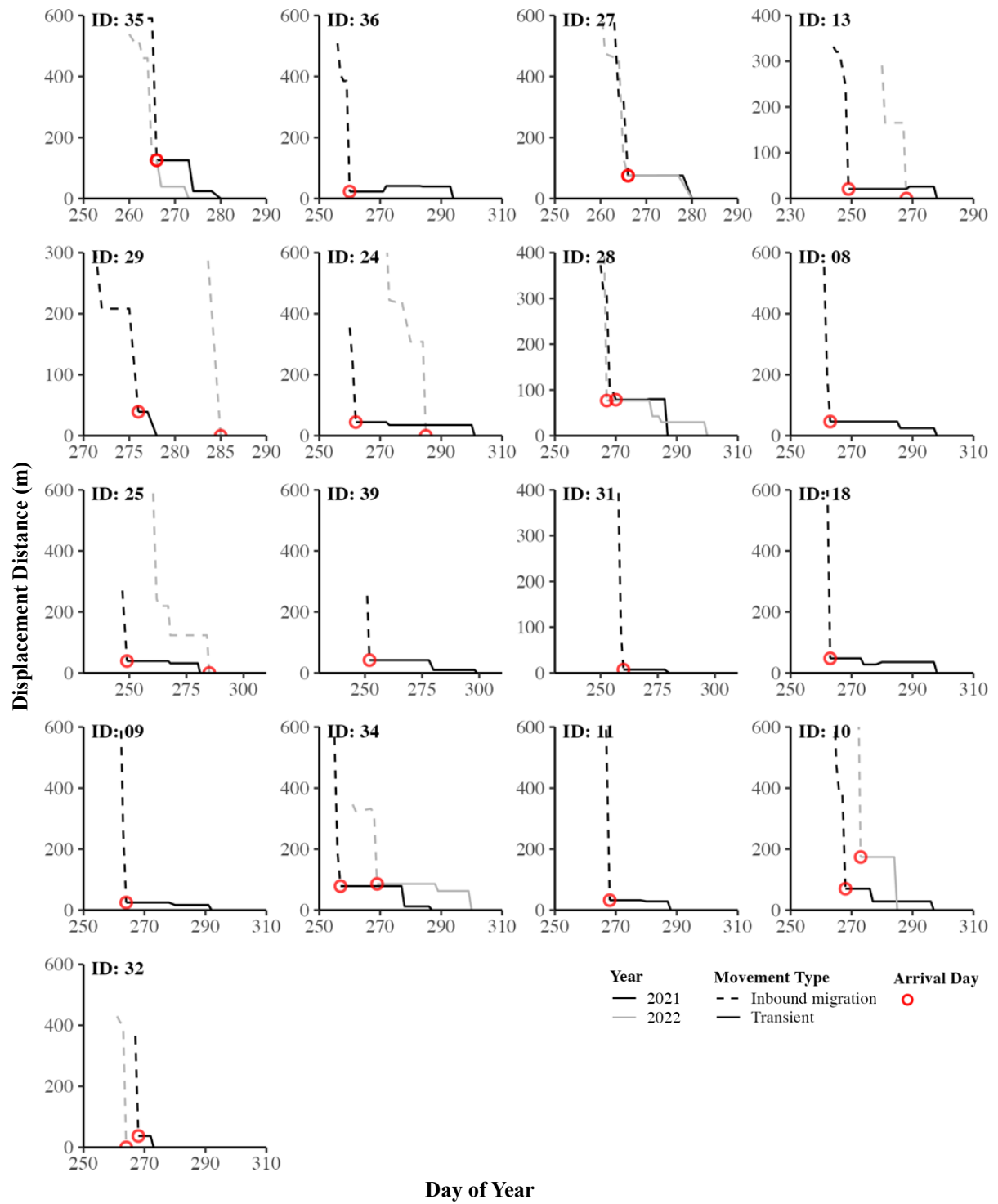


Figure C.3 Maximum Euclidean distance from overwintering refugia ("displacement") by 17 Timber Rattlesnakes (*Crotalus horridus*) during the fall of 2021 and 2022 prior to entry into refugia (27 snake-year combinations).

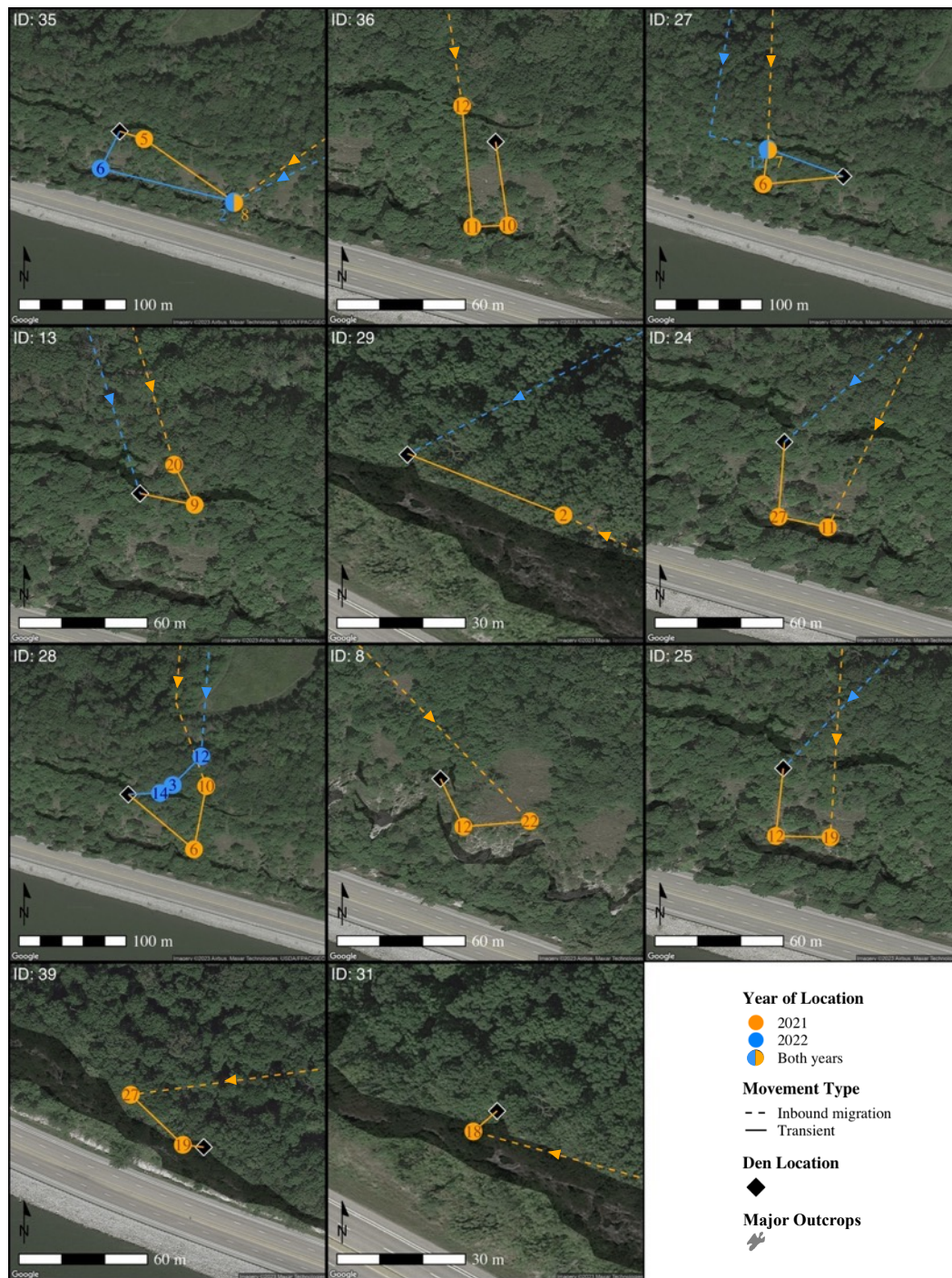


Figure C.4 Transient movements of 17 Timber Rattlesnakes (*Crotalus horridus*) between arrival from summer habitats and ingress into overwintering refugia during the fall of 2021 and 2022 (27 snake-year combinations). Numbers within each circle represent the duration (in days) snakes spent at a given location. Lines connect sequential movements of each snake with arrows indicating the direction of movement. Major outcrops (black polygons) are shown as reference.

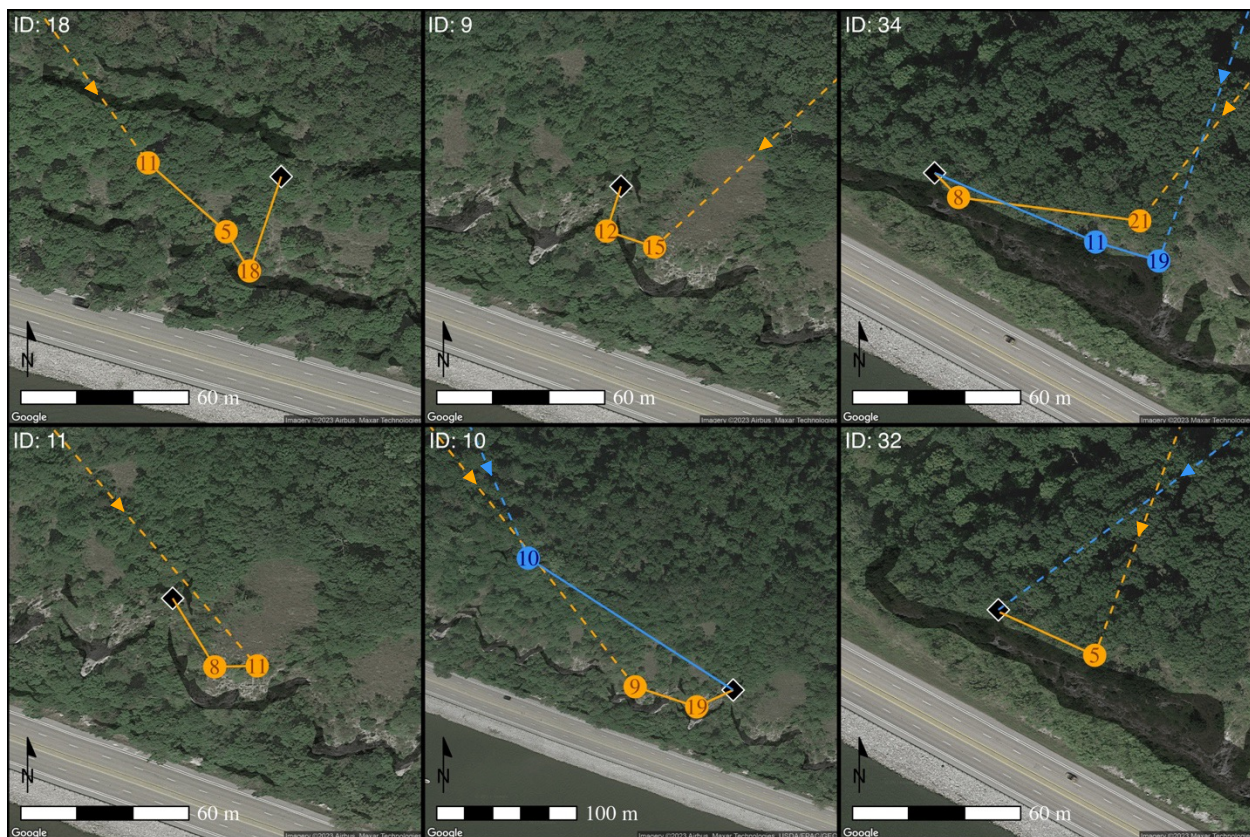


Figure C.4 (cont.)

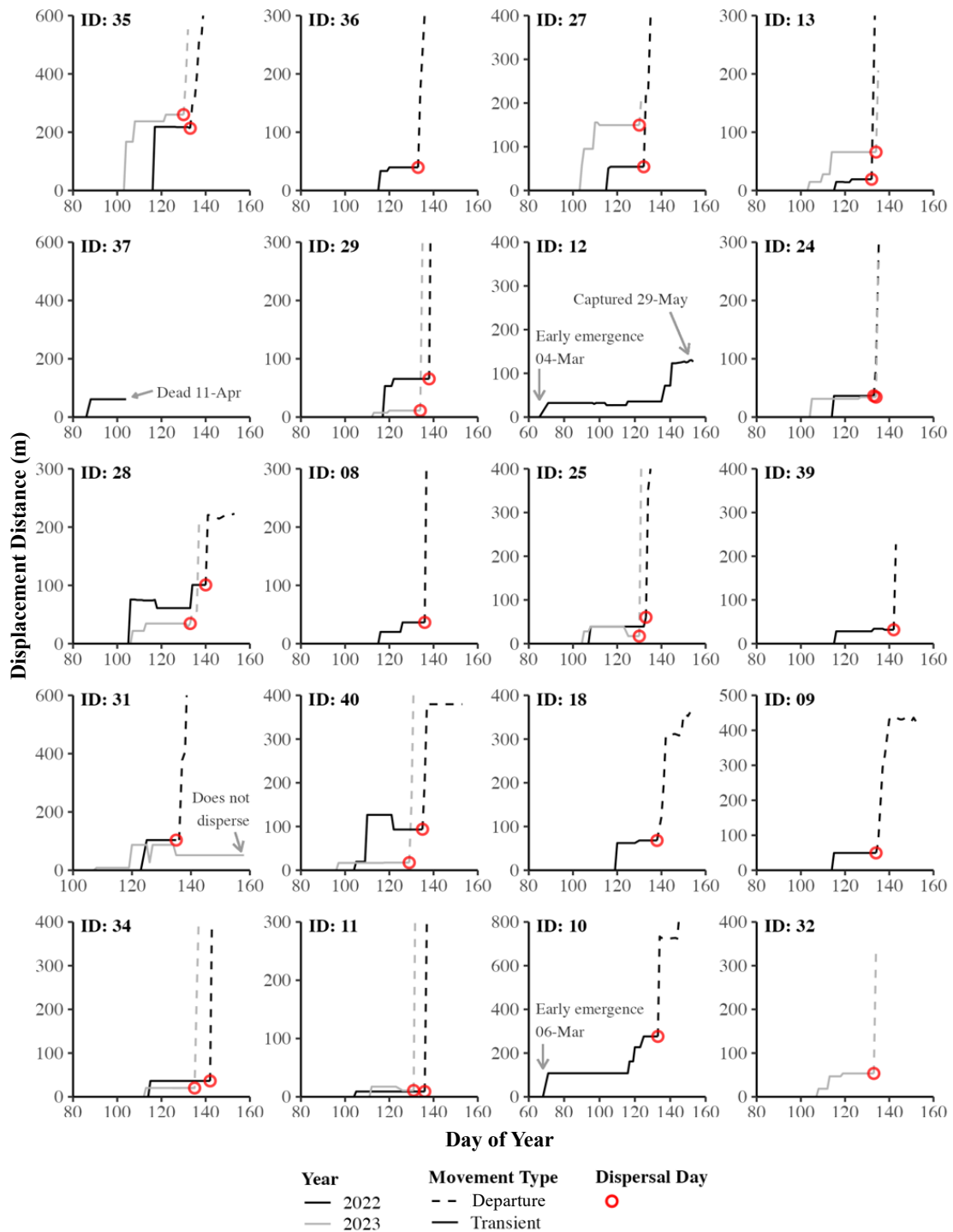


Figure C.5 Maximum Euclidean distance from overwintering refugia ("displacement") by 20 post-emergent Timber Rattlesnakes (*Crotalus horridus*) during the spring of 2022 and 2023 (31 snake-year combinations).

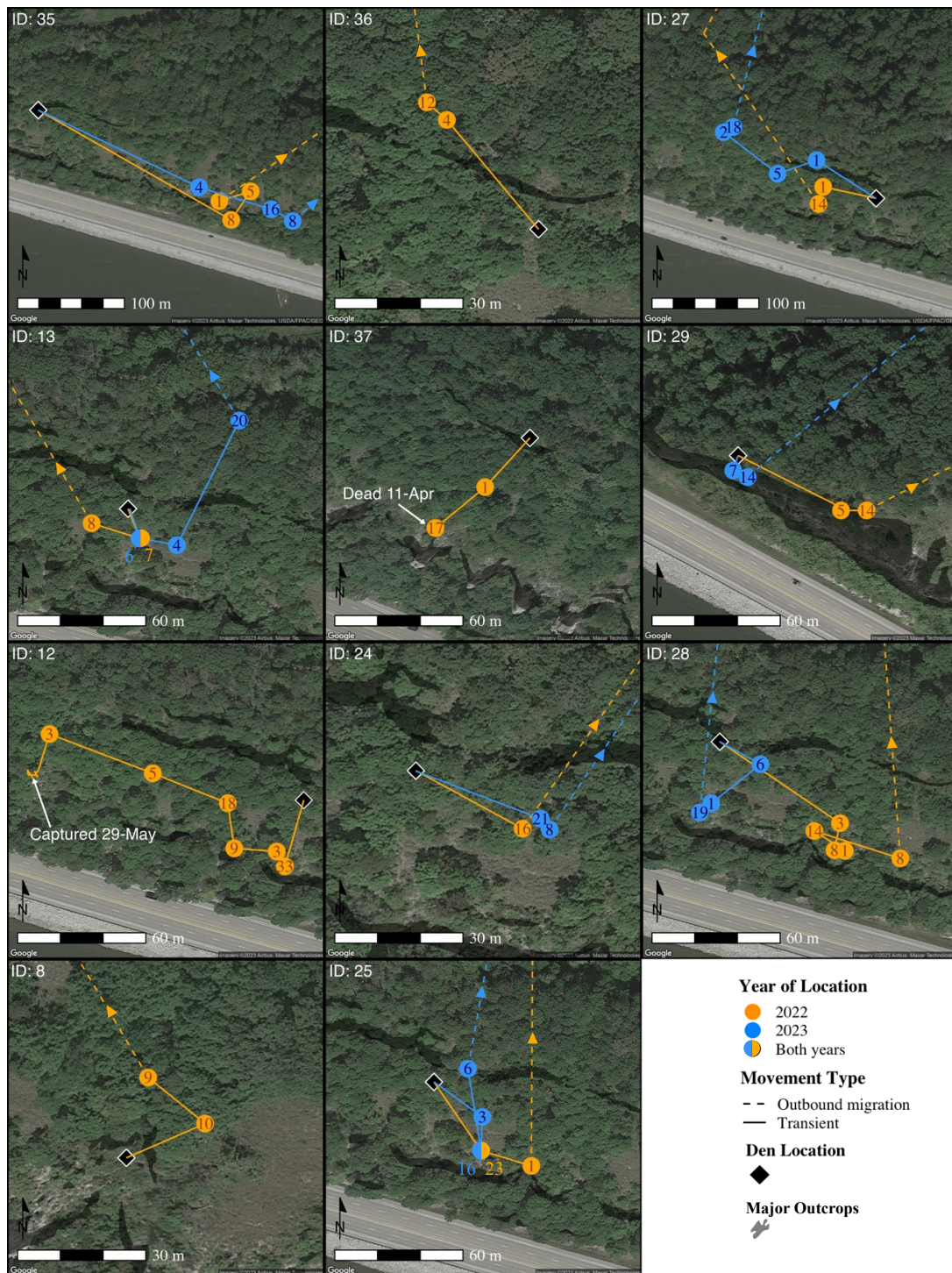


Figure C.6 Post-egress "transient" movements from 20 Timber Rattlesnakes (*Crotalus horridus*) from emergence from refugia to departure to summer habitats in the spring of 2022 and 2023 (31 snake-year combinations). Individual snakes are color coded with numbers representing the duration (in days) each snake spent at a given location. Lines connect sequential movements of each snake with arrows indicating the direction of the first "long-distance" departure / outbound migratory movement. Major outcrops (black polygons) are shown as reference.

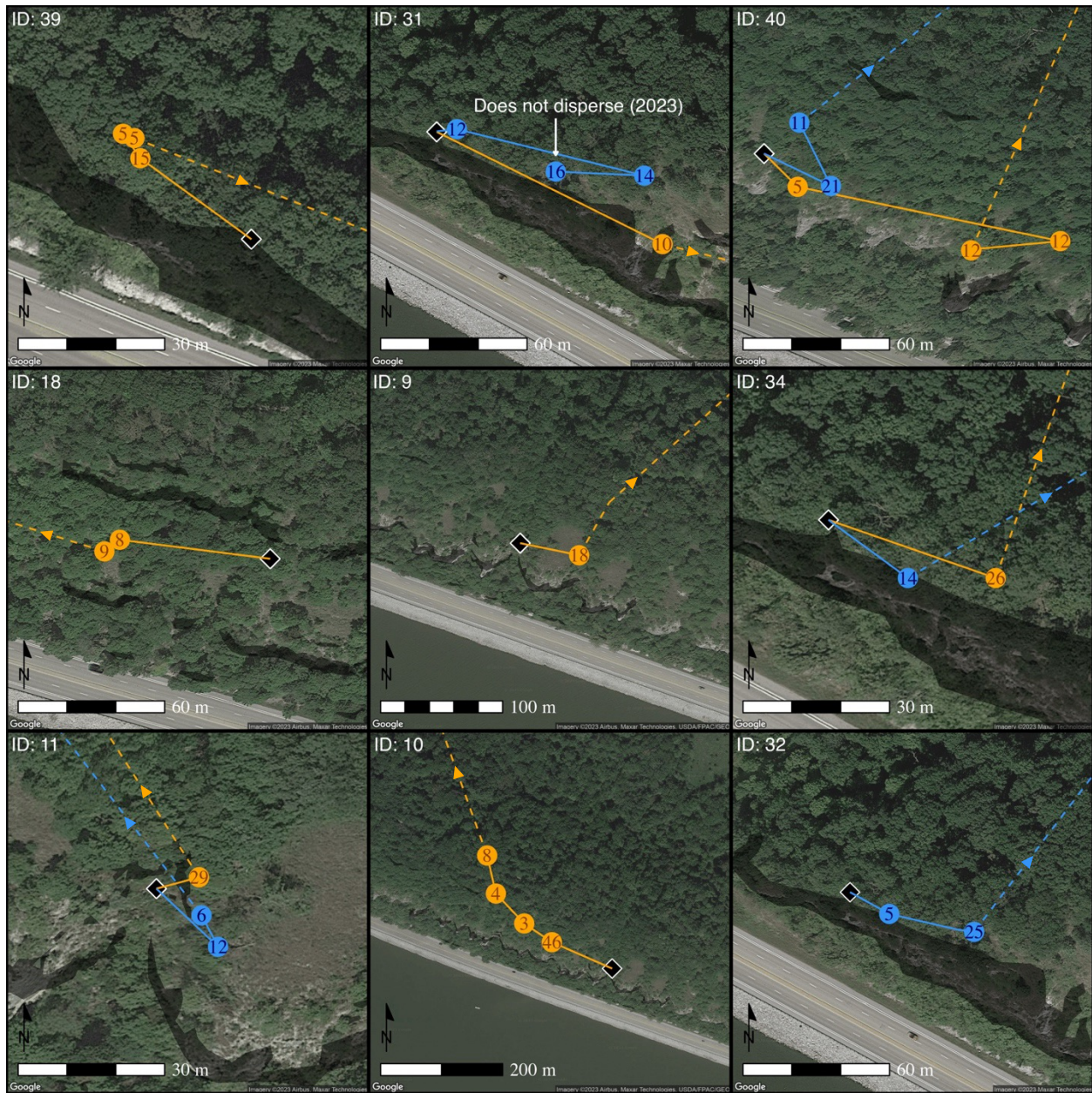


Figure C.6 (cont.)