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Microhabitat selection of adult female eastern diamondback rattlesnakes (Crotalus adamanteus) in the Carolinas

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MICROHABITAT SELECTION OF ADULT FEMALE EASTERN DIAMONDBACK RATTLESNAKES (*CROTALUS ADAMANTEUS*) IN THE CAROLINAS
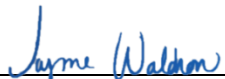
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Matthew Gacheny
Approved by
Dr. Jayme L. Waldron, Committee Chairperson
Dr. Shane M. Welch
Dr. Kyle Palmquist
Dr. Anne Axel

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Approval of Thesis

We, the faculty supervising the work of Matthew Gacheny, affirm that the thesis, *Microhabitat Selection of Adult Female Eastern Diamondback Rattlesnakes (Crotalus adamanteus) in the Carolinas*, meets the high academic standards for original scholarship and creative work established by the Department of Biological Sciences and the College of Science. The work also conforms to the requirements and formatting guidelines of Marshall University. With our signatures, we approve the manuscript for publication.

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Dedication

To my parents, Raymond and Theresa Gacheny.

Above all, I thank my family for their loving support, both from far away and in person. To my brothers, Brendan and Steven, and my sister Leah, thank you for encouraging me and always lending an ear when I struggled. To my father, I owe a lot of my interest and passion for the outdoors to you from taking me camping, boating, crabbing, and fishing at the New Jersey

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Abstract

Reptiles rely on environmental conditions to regulate body temperature using behavioral strategies to meet basic life history needs. Viviparous squamates use thermoregulatory behaviors to meet energetic requirements for vitellogenesis and gestation, which carry considerable energetic costs and must balance with maintenance and survival. Embryonic development is often optimized in a narrower temperature range than the mother's preferred temperature, creating an intergenerational conflict that should be addressed with maternal microhabitat selection. Field-based studies are needed to understand the link between the thermal biology of reptiles, their reproduction, and implications for population viability. With a use-availability logistic regression framework, I examined thermal and structural predictors of adult female gestation and overwintering microhabitat selection for the declining eastern diamondback rattlesnake (*Crotalus adamanteus*; EDB) in the Carolinas. I expected reproductive females (vitellogenic females overwinter, gravid females during gestation) would select microhabitats with cooler or more stable temperatures, denser vegetative cover, and wetter soils to alleviate energetic costs associated with reproduction in a subtropical climate. Habitat models indicated female EDBs required access to a wide range of thermal microhabitat conditions (i.e., cool surface temperatures and warm soil temperatures during gestation, warm surface temperatures and cool soil temperatures while overwintering), suggesting female EDBs employ active thermoregulation most likely to alleviate metabolic effects of opposing seasonal thermal extremes. Overwintering females selected sites with greater coarse woody debris log cover, but other microhabitat structural predictors received little or no support. This work supports EDB conservation strategies incorporating life-history and emphasizes the importance of suitable thermal microhabitats for adult females, especially in context with climate change.

1. Introduction

Ectotherms (e.g., reptiles) cannot metabolically regulate their body temperature and must therefore rely on thermal environmental conditions to meet basic life-history needs (Farallo and Miles, 2016; Liang et al., 2017; Whiles and Grubaugh, 1996). That is, basic biological functions like cellular biochemistry and tissue metabolism depend on maintenance of optimal body temperatures (Lillywhite, 1987). An animal's interaction with its abiotic environment, including temperature, may greatly affect life-history functions such as growth, survivorship, and reproduction, which in turn affect population dynamics (Huey, 1982). Thus, body temperatures experienced by ectotherms through their environment can directly or indirectly affect nearly every aspect of their ecology (Huey et al., 1989; Peterson et al., 1993).

Ectotherms employ behavioral strategies to thermoregulate in a range of environmental conditions via selection of fine-scale habitat features (hereafter microhabitats; Huey et al., 1989; Lillywhite, 1987; Reinert, 1993; Robertson and Weatherhead, 1992). Thermoregulation via microhabitat selection is especially relevant in environments where extreme temperatures fall outside optimal body temperature ranges and increase susceptibility to lethal and sublethal effects (Blouin-Demers and Weatherhead, 2001), and thermoregulatory ability should become increasingly important considering projected global temperature increases (Intergovernmental Panel on Climate Change, 2013). Avoiding extreme temperatures through microhabitat selection can maximize performance and reproduction (Dobkin, 1985; Huey and Kingsolver, 1989; Jones et al., 1987), yet few studies directly link thermoregulation to habitat use for ectotherms (Row and Blouin-Demers, 2006). Thermoregulatory behavior, and thereby thermal microhabitat use, is flexible and state dependent (e.g., reproductive state), reflecting differing thermoregulatory needs among life-history stages (Garrick, 1974; Huey et al., 1989; Peterson, 1987; Virens and Cree,

2019). Thus, basing an animal's thermal ecology and associated habitat use on a single life stage is risky, as understanding how multiple life-history stages respond to thermal environments is imperative for predicting how organisms adapt to future climatic conditions (Potter et al., 2013).

Reproduction may be the most temperature-sensitive aspect of reptile life-history (Caley and Schwarzkopf, 2004; Harvey and Weatherhead, 2010; Huey and Kingsolver, 1989; Osgood, 1978; Warner and Shine, 2008). For example, turtles commonly display temperature-dependent sex determination of developing offspring (Ewert et al., 1994; Standora and Spotila, 1985). Additionally, female viviparous vipers exhibit slower growth rates due to high energetic costs of reproductive processes like yolk provisioning (i.e., vitellogenesis; Bonnet et al., 2002) when maternal reserves are heavily invested in oocyte development (Alcobendas et al., 1992; Bonnet et at., 1994; Saint Girons, 1957; Saint Girons and Duguy, 1992). The reproductive cycle of many North American pitvipers follows type II, postnuptial secondary vitellogenesis with follicle development spanning late summer through the following spring, including winter dormancy (Aldridge and Duvall, 2002; Seigel and Ford, 1987). Female energy use during dormancy likely reduces body condition upon emergence (Brischoux et al., 2016; Costanzo et al., 2004; Muir et al., 2013), contributes to increased overwinter mortality (Zani, 2008; Zani et al., 2012), and potentially limits post emergent energy allocation to reproductive effort, e.g., gametogenesis (Derickson, 1976). Snakes may use microhabitats that minimize energy losses by selecting relatively cooler or more stable body temperatures during periods of dormancy or fasting (Burger et al., 1988; Huey et al., 1989). While feeding is suspended during winter dormancy, snakes should benefit from overwintering at subterranean depths that confer relatively lower body temperatures that reduce cumulative energy loss leading to starvation (Huey et al., 2020), and energy conservation might be especially relevant for overwintering reproductive females.

While behavioral thermoregulation may buffer thermal extremes, it can conflict with other essential processes and necessitate tradeoffs among resource requirements (Aublet et al., 2009; Cunningham et al., 2015; Edwards et al., 2015). Conflicting thermoregulatory resource requirements between gestating mothers and their embryos can force female squamates to compromise their preferred thermal optimum to benefit developing embryos (Beuchat, 1988). North American pit vipers gestate during summer (Means, 2017; Seigel and Ford, 1987), when warm temperatures place gestating females at risk of water stress (Le Galliard et al., 2012; Lourdais et al., 2004) likely exacerbated by energy loss from feeding cessation (Reinert et al., 2011; Waldron et al., 2013) and fetal oxygen demands (Blackburn, 2006). Although offspring nutrient allocation occurs during vitellogenesis, which minimizes the need for gestational nutrient provisioning (Lorioux et al., 2013), gravid vipers must provide water to developing embryos during gestation (Stewart and Thompson, 2000; Thompson and Speake, 2002; Van Dyke et al., 2014), and the combined effects of heat stress and fetal water demands may be more limiting than energy loss amid summer gestation (Le Galliard et al., 2012; Lourdais et al., 2004).

Gestation exhibits critical ties to maternal thermoregulatory behavior. Indirect maternal effects (e.g., maintenance of female body temperature via reproductive site selection) are vital to ectotherm fitness in affecting aspects of offspring phenotype, including sex (Roosenburg, 1996), morphology (Deeming and Ferguson, 2004), and behavior (Downes and Shine, 1999) that may in turn shape evolutionary trajectories (Kirkpatrick and Lande, 1989). Gestation temperature affects gestation length and exhibits varied effects on offspring fitness (Beuchat and Ellner, 1987; Qualls and Andrews, 1999), with hotter temperatures decreasing gestation length while cooler temperatures extend it (Lorioux et al., 2013). Natural selection should favor adaptations that maintain embryonic temperatures within a suitable range during gestation (Beuchat, 1988),

which vary across squamate taxa. For example, gravid females will select warmer microhabitats than non-reproductive females (e.g., garter snakes (*Thamnophis sirtalis*): Stewart, 1965; desert striped whipsnakes (*Masticophis t. taeniatus*), great basin rattlesnakes (*Crotalus viridis lutosus*), and yellow-bellied racers (*Coluber constrictor mormon*): Hirth and King, 1969; prairie rattlesnakes (*Crotalus viridis*): Charland and Gregory, 1990), although data tend to be biased toward reptiles inhabiting relatively cool climates (Shine, 2004). The opposite relationship is exhibited by viviparous lizards and pit vipers, however, with gravid females selecting cooler microhabitats than non-reproductive females potentially aimed at avoiding deleterious high body temperatures (Beuchat, 1986; Garrick, 1974; Le Galliard et al., 2003; Sanders and Jacob, 1981; Virens and Cree, 2019).

The eastern diamondback rattlesnake (*Crotalus adamanteus;* EDB) is a declining species associated with the imperiled longleaf pine ecosystem of the southeastern US (Martin and Means, 2000). The EDB is a habitat specialist that selects open-structure habitats of fire-maintained pine savannas and woodlands at multiple spatial scales (Hoss et al., 2010; Waldron et al., 2013). However, habitat studies have yet to directly link habitat structure with EDB thermal ecology. Limited to a subtropical climate, the EDB likely experiences different thermoregulatory constraints as compared to well-studied congeners in cooler, more temperate climates, e.g., timber rattlesnakes (*C. horridus*) and western rattlesnakes (*C. oreganus*). For instance, crotalids at northern latitudes or in montane habitats rarely emerge from winter dormancy (timber rattlesnakes: Nordberg, 2013; Nordberg and Cobb, 2016; great basin rattlesnakes: Cobb and Peterson, 2008; prairie rattlesnakes: Jacob and Painter, 1980), but crotalids at southern latitudes commonly bask and occasionally move to new hibernacula when winter temperatures are mild (EDBs: Kain, 1995; Stevenson, 2003, Timmerman, 1995; Timmerman and Martin, 2003;

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Waldron et al., 2013; dusky pygmy rattlesnakes (*Sistrurus miliarius barbouri*): May et al., 1996; southern timber rattlesnake populations: Andrews and Waldron, 2017). Characterizing thermal environments for more snake species throughout their ranges will enhance understanding of variation in thermal requirements that is largely lacking despite snakes' suitability for such life-history studies (Hill and Beaupre, 2008; Peterson et al., 1993). Current understanding of reptile thermal biology and its relation to female reproduction is largely based on experimental, laboratory-based research (e.g., Burger et al., 1987; Dorcas et al., 2004; Fox et al., 1961; Osgood, 1978; Rice et al., 2006; Virens and Cree, 2019), creating a need for additional field-based studies on free-ranging animals to facilitate understanding of how thermoregulatory patterns vary in nature and implications for population viability. Such information is critical for species residing in environments experiencing climate change by allowing us to link microhabitat attributes to important reptile life stages with implications for conservation and management of reptiles.

I examined the effects of reproductive condition on adult female EDB microhabitat selection to improve our understanding of how females balance tradeoffs between reproduction and survival. With a use-availability framework, I examined thermal and structural predictors of female microhabitat selection specific to gestation and overwintering sites. Based on potential energetic costs associated with vitellogenic yolk provisioning preceding and following winter brumation, I expected reproductive condition (vitellogenic versus non-reproductive) would influence winter microhabitat selection and reflect thermal and structural microhabitat conditions. Specifically, I expected vitellogenic females to conserve energetic stores and maximize post-emergence body condition by selecting winter microhabitats with cool, stable thermal conditions, dense vegetative cover, and greater access to soil moisture. Similarly, I

expected gestating females would select summer gestation microsites with cool thermal conditions to reduce gestational stress while optimizing embryonic development, dense vegetative cover to provide shade, and soil structure conferring greater water holding capacity to reduce potential gestational water loss.

2. Methods

2.1. Study Species

The EDB (Figure 1) is a crotalid rattlesnake species native to the southeastern US Coastal Plain. Eastern diamondbacks are characterized by a slow life history, including high adult survival, delayed maturation, high spatial fidelity, and high longevity (individuals surviving > 20 years; Waldron et al., 2013). Additionally, female EDBs typically exhibit triennial or longer birthing intervals, reflecting high energetic costs of female reproduction (Timmerman and Martin, 2003; Waldron et al., 2013). The EDB's range is largely congruent with the historical range of the longleaf pine (*Pinus palustris*) ecosystem reflecting the species' dependence on the open structure of high integrity southeastern pine savannas and woodlands (Martin and Means, 2000; Waldron et al., 2006, 2008).

Figure 1.

The eastern diamondback rattlesnake (*Crotalus adamanteus*).

Historically, EDBs ranged from eastern Louisiana to southeastern North Carolina and south throughout Florida (Hoss et al., 2010; Martin and Means, 2000; Waldron et al., 2006, 2008; Figure 2). Range-wide population declines have resulted from a combination of habitat loss, collection, targeted killing by humans, and a general lack of protective measures for the species (Martin and Means, 2000; Means, 2009, 2017; Dodd, 1987). Subsequently, EDBs are considered vulnerable in Florida, South Carolina, Mississippi, and Alabama, are endangered in North Carolina, and are critically imperiled in Louisiana where they are possibly extirpated (Martin and Means, 2000; Timmerman, 1995; Timmerman and Martin, 2003). The EDB was petitioned for listing under the Endangered Species Act by the US Fish and Wildlife Service (US Fish and Wildlife Service, 2012). The EDB has been detected on 28 military installations in the

southeastern US (e.g., Marine Corps Recruit Depot Parris Island, Camp Lejeune, Camp Shelby, Eglin Airforce Base; Petersen et al., 2018), highlighting the DoD's need for ecological data to help guide natural resource management practices that conserve EDB populations while ensuring continued, uninterrupted access to military training areas.
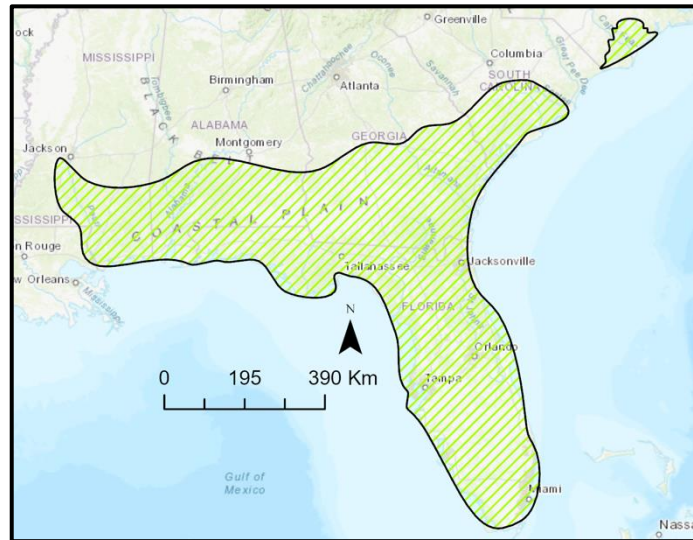
Figure 2.

Approximate range of the eastern diamondback rattlesnake (*Crotalus adamanteus*). Basemap source: Esri.

Like most viperids, female EDBs are assumed to follow type II postnuptial secondary vitellogenesis, i.e. ovarian follicles begin growing in late summer into the overwintering period, briefly halt growth in the winter, and resume growth at emergence (Aldridge and Duvall, 2002; Seigel and Ford, 1987). The onset of estrus initiates vitellogenesis in late summer, coinciding with the mating season (Aldridge and Duvall, 2002). Following spring ovulation and fertilization, females gestate from April or May (Means, 2017) through August or September (Fill et al., 2015). In South Carolina, female EDBs select gestation sites from late June to late August, and parturition occurs in late summer from mid-August to mid-September (Fill et al.,

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2015; Waldron et al., 2013). Gestation microhabitats typically include structures that provide cover and subterranean refuge, e.g., windthrown tree tip-ups/fallen logs, partially buried concrete slab debris, and burned-out stumps/root channels (Fill et al., 2015; Means, 2017; Figure 3). Parturition (i.e., birthing) sites may be identical to gestation sites for some individuals, although females can select new sites for parturition within days of giving birth (J.L. Waldron, unpublished data). Overwintering microhabitats are similar to gestation and parturition sites in that they provide cover and subterranean refuge. For example, EDBs use burrows of other reptiles and mammals (e.g., gopher tortoise (*Gopherus polyphemus)*, nine-banded armadillo (*Dasypus novemcinctus*)), rotting/burned out tree bases and stump holes, logs, and fallen tree mounds as subterranean overwintering refugia (Kain, 1995; Klauber, 1972; Means, 2017; Murphy et al., 2021; Timmerman and Martin, 2003).

Figure 3.

Typical subterranean refuge used by adult female eastern diamondback rattlesnakes (*Crotalus adamanteus*).

2.2. Study Areas

I conducted this study on two military installations – Marine Corps Base Camp Lejeune (MCBCL), Onslow County, North Carolina, and Marine Corps Recruit Depot Parris Island (MCRDPI), Beaufort County, South Carolina (Figure 4).
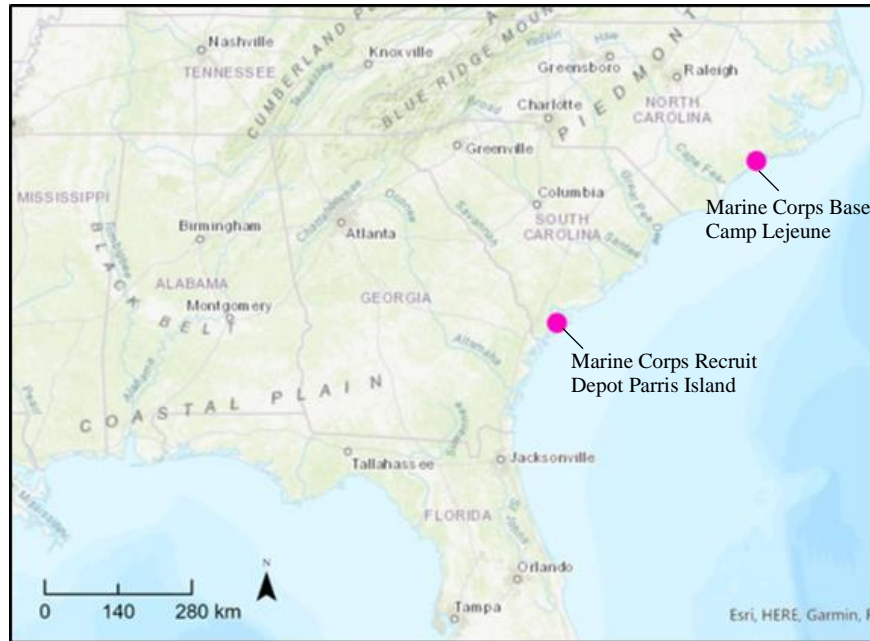
Figure 4.

Map depicting locations of Marine Corps Base Camp Lejeune, North Carolina, USA, and Marine

Corps Recruit Depot Parris Island, South Carolina, USA, within the southeastern Coastal Plain.

Basemap source: Esri.

2.2.1. MCBCL

Marine Corps Base Camp Lejeune is a coastal United States Marine Corps installation on

the Atlantic coast within the North American Coastal Plain, encompassing >56,000 ha of mixed

uplands, wetlands, and coastal habitat (Marine Corps Base Camp Lejeune, 2015). The Main Base

and Verona Loop areas of the installation (Figure 5) comprise ≈41,000 ha bisected by the New

River (≈6,600 ha) and include 10.3 nautical miles of beach, while the Greater Sandy Run Area

encompasses ≈16,600 additional ha. Upland habitat is mainly comprised of pine forests and

woodlands with a graminoid-dominated understory intermixed with undrained shallow

depressions forming upland bogs or pocosins dominated by evergreen shrubs (Bailey, 1995).

Barrier islands are separated from the installation mainland by the Atlantic Intracoastal Waterway and extensive tidal marsh. The Main Base area contains ≈2,400 ha of urbanized area including residential areas, commercial facilities, offices, and various miliary infrastructure and storage facilities. Natural, high-integrity habitats persist throughout the installation, including ≈7,200 ha of wet-mesic/wet longleaf-mixed pine savannas, ≈5,600 ha of mesic longleaf-mixed pine savannas, ≈10,000 ha of xeric/dry mesic longleaf savanna, ≈3,000 ha of maritime forest, and ≈1,400 ha of maritime dunes/swales/marshes. Pocosin wetlands of various sizes occur throughout the installation, including ≈3,500 ha of small streamhead pocosins, ≈6,800 ha of pond-pine (*Pinus serotina*) pocosins, and ≈3,100 ha of pocosin fringes. Among the area's complex topography, relatively fire-intolerant hardwood communities occur on the installation, including Cypress-Gum Swamps, Mixed Mesic Hardwoods, and Coastal Plain Small Stream Swamps (Frost, 2001). The MCBCL supports extensive air to ground military training operations, including an administrative cantonment area, air station, 96 training and maneuver areas (TAs; including 11 water training areas and two ocean training areas), drop zones, tactical landing zones, gun positions, outlying landing fields, 98 active firing ranges, and three munitions impact areas.
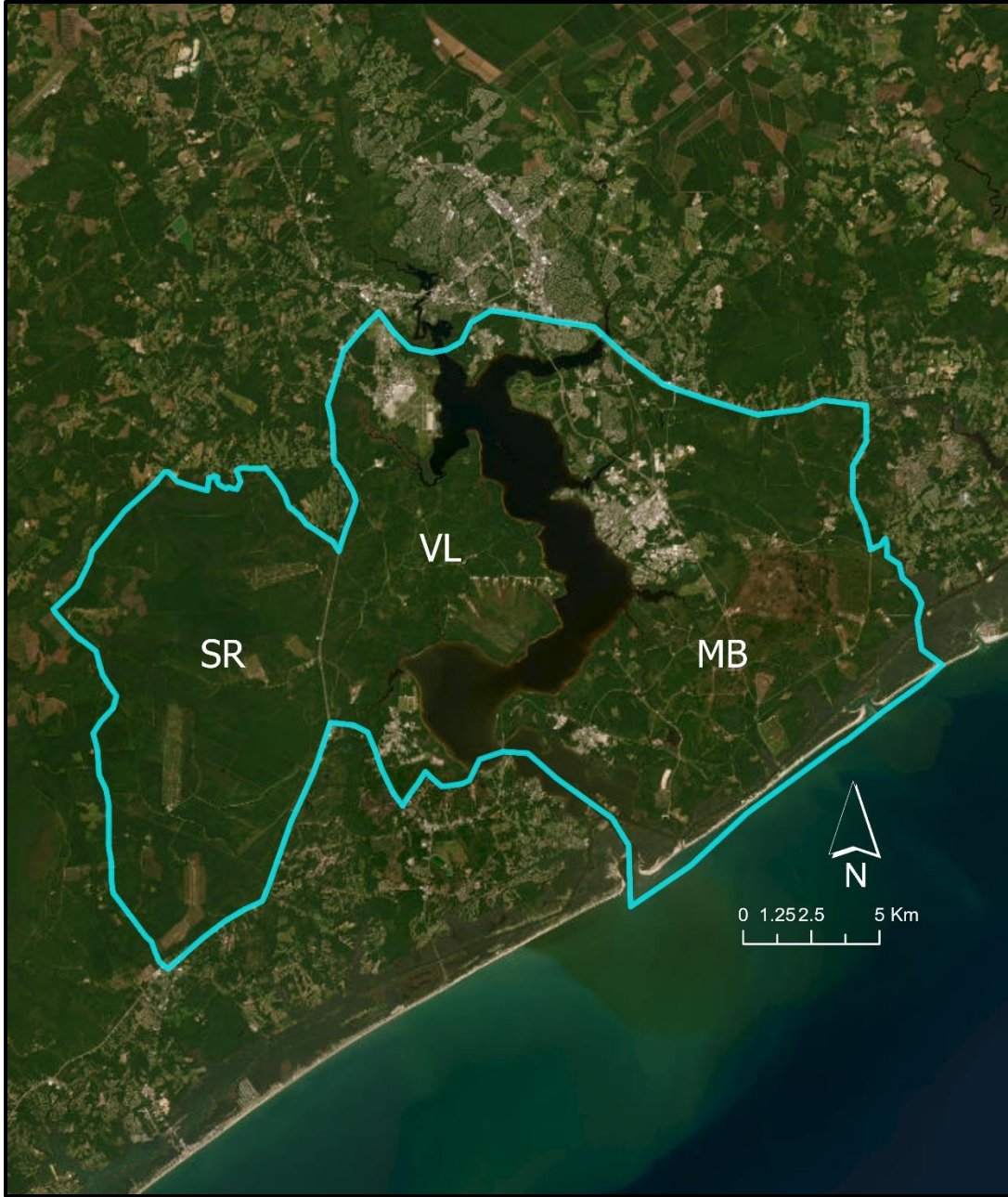
Figure 5.

Marine Corps Base Camp Lejeune (MCBCL), Onslow County, North Carolina, USA. MCBCL

property is outlined in blue. VL = verona loop area, MB = main base area, SR = sandy run area.

Basemap source: Esri.

I conducted this study in the Main Base area within ≈24,000 ha of high integrity, largely contiguous upland pine savannas and woodlands maintained by high-frequency (1-3 year) fires stemming from fire prescriptions and wildfires ignited during military training (Figure 6, Figure 7). Marine Corp Base Camp Lejeune personnel practice active conservation of ≈5,600 ha of longleaf pine savannas, flatwoods, pine-oak sandhills and adjacent ecotones. In addition to prescribed fire, stand management includes commercial timber harvest, herbicide treatment, and pre-commercial mechanical thinning. This high-integrity area provides habitat for rare fire-dependent species, including mimic glass lizards (*Ophisaurus mimicus*), Bachman's sparrows (*Peucaea aestivalis*), southern hognosed snakes (*Heterodon simus*), the state threatened Carolina gopher frog (*Rana capito capito*) and ornate chorus frog (*Pseudacris ornata*), the federally endangered red-cockaded woodpecker (*Picoides borealis*; managed on MCBCL since 1986), venus flytrap (*Dionaea muscipula*), and the federally endangered rough-leaved loosestrife (*Lysimachia asperulifolia*). Wiregrass (*Aristida stricta*) was the dominant ground cover in my study area, although additional native fire-dependent cover included toothache grass (*Ctenium aromaticum*), various Andropogon grass species, *Schizachyrium scoparium*, *Ilex glabra*, ericaeous shrubs (i.e., *Vaccinium, Gaylussacia,* and *Lyonia* species), and Asteraceae and Fabaceae forb species. The G-10 impact area (≈2,000 ha) provided core habitat for the MCBCL EDB population (Marine Corps Base Camp Lejeune, 2015). The impact area center receives almost daily heavy artillery live-fire as well as live-fire of various types from multiple firing ranges and aircraft. Although the center is largely open only consisting of herbaceous ground cover with interspersed drains and pocosins, the remaining area of the G-10 contains large, contiguous tracts of high-integrity pine savanna with interspersed turkey oak (*Quercus laevis*) ridges and pocosins maintained via almost yearly wildfires stemming from live-fire training.
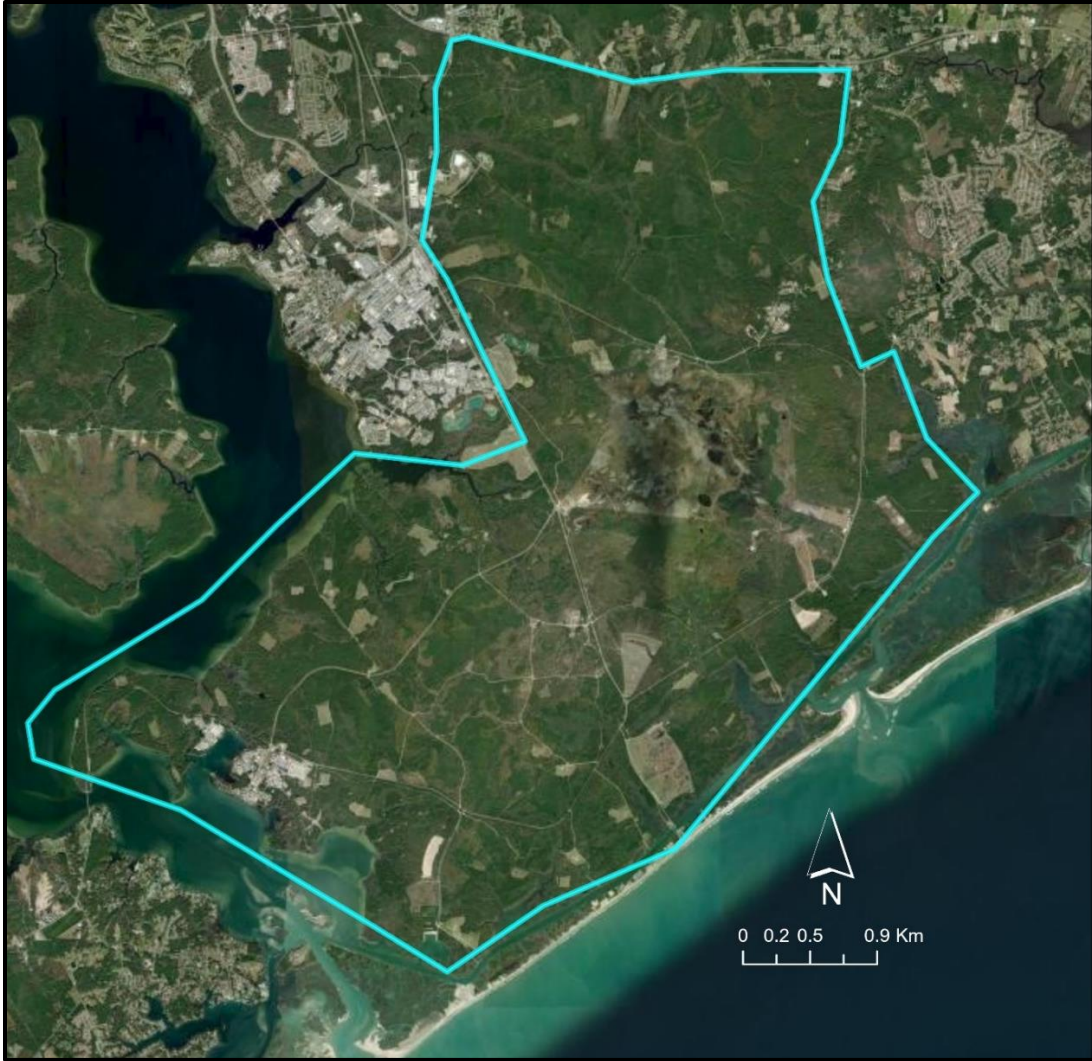
Figure 6.

Focal research area within the Main Base area on Marine Corps Base Camp Lejeune, Onslow

County, North Carolina, USA. The focal area is outlined in blue. Basemap source: Esri.

Figure 7.

Typical longleaf pine (*Pinus palustris*) upland habitat observed in my focal study area at Marine Corps Base Camp Lejeune, North Carolina, USA.

The MCBCL supports the northern limital EDB population. Despite an abundance of suitable EDB habitat, e.g., open canopy pine savannas and flatwoods (Martin and Means, 2000; Waldron et al., 2006, 2008), local wildlife authorities have detected few individuals at MCBCL. Wildlife staff at MCBCL observed a few scattered individual snakes between 1988 and the early 2000s. Starting in 2007, EDB sightings were reported on a near-annual basis from military contractors, MCBCL wildlife staff, and North Carolina Wildlife Resources Commission personnel within Main Base in the G-10 impact area and immediately surrounding TAs. At that time, EDBs were not marked, but MCBCL and NCWRC staff kept detailed records of sightings

of individual snakes identifiable by photographed head scale patterns resulting in sightings of at least 14 individual EDBs through 2021.

The MCBCL initiated an EDB monitoring program in Spring 2022, using mark-recapture and radio telemetry surveys to examine movement, habitat use, and survival. Between 2022 and 2024, monitoring efforts yielded 27 captures of nine individuals, and telemetry data were collected from seven adults. Reproduction had not been observed in NC EDB populations until MCBCL monitoring provided neonate captures (n = 2) and telemetry data from two gravid females.

2.2.2. MCRDPI

The Marine Corps Recruit Depot Parris Island (MCRDPI), Beaufort County, South Carolina is a United States Marine Corps installation located on a sea island on the Atlantic coast within the North American Coastal Plain at the confluence of the Broad and Beaufort Rivers (Figure 8). It encompasses 3,256 ha split into 1,325 ha of dry land and 1,931 ha of tidal marsh and creeks. The habitat is predominately coastal salt marsh, maritime forest, naturalized woodlands composed of pine-hardwood mixed open canopy savanna dominated by loblolly (*Pinus taeda*) and slash pine (*Pinus elliottii*) and closed canopy forest, hammock islands, and developed areas (Amspacher, 2018; Fill et al., 2015; Goodson, 2023; Mausteller, 2020; Figure 9). Soft and hard salt marsh habitats together comprise nearly 50% of the covered area, with all salt marsh types covering 56% (Goodson, 2023). Other prevalent cover types include maritime forest (the dominant forest type, 4%), pine forest (≈7% combined pine forest cover types), developed areas (≈8% mowed lawns, ≈6% infrastructure), and open water (14%). Development is most prevalent in the northern, upland portion of the island and includes recruit housing, offices, and residential areas, with additional development including a firing range, a public golf

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course, and an old airfield (Goodson, 2023). Miliary training activities are limited to basic recruit training and do not include air to ground operations, with approximately 19,000 recruits receiving annual training. Marine Corps Recruit Depot Parris Island natural resources personnel employ habitat management via prescribed burns, mechanical thinning, and herbicide treatment to maintain open forest habitat as well as salvage logging following major storms and timber production management.

Figure 8.

Marine Corps Recruit Depot Parris Island (MCRDPI), Beaufort County, South Carolina, USA.

MCRDPI property is outlined in blue. Basemap source: Esri.

Figure 9.

Typical habitat at Marine Corps Recruit Depot Parris Island, Beaufort County, South Carolina, USA, illustrating predominant salt marsh habitat type.

In 2008, the MCRDPI initiated EDB monitoring, focusing on individual-level protocols using radio telemetry and mark recapture surveys with initial adaptive goals of mitigating EDB/recruit interactions while maintaining EDB population viability. To date, the MCRDPI supports the longest-running EDB monitoring program with over 300 captures/recaptures of at least 100 adult individuals since 2008 (J.L. Waldron, unpublished data).

2.3. EDB Monitoring

I captured EDBs opportunistically through visual encounter surveys, during telemetry surveys, and on roads. I used snake hooks to capture EDBs and processed individuals restrained

in clear snake tubes. I measured snout-to-vent length (SVL, cm), total length (TL, cm), and mass (g) and subcutaneously inserted passive integrated transponder (PIT) tags to mark individual snakes. I determined sex using cloacal probes.

All females included in this study were mature adults (i.e., SVL ≥ 100 cm based on Waldron et al., 2013). I attached very high frequency (VHF) radio transmitters (Advanced Telemetry Systems, model R1640, weight=2.0g, battery life≈243 days) to adult female EDBs. I attached transmitters to the rattle following Jungen et al. (2019), using thread, nail glue, and nail top coat gel to secure transmitters. Some females at the MCRDPI population were surgically implanted with radio transmitters (Holohil Systems, model SI2, weight=11-13g, battery life≈24 mo.) using modifications from Reinert and Cundall (1982). I radio-tracked free-ranging female EDBs from June-August 2023 and December-January 2024 once to twice weekly during the gestation and overwintering periods, respectively, with a radio receiver (Telonics, TR-4, Mesa, AZ, USA) and three-element directional Yagi antenna. Tracking frequencies slightly varied by site due to limited access to live-fire training areas at the MCBCL. I recorded snake locations using a GPS unit (Trimble Juno, Sunnyvale, CA) with ≤ 5m spatial accuracy.

I used radio telemetry locations to identify subterranean microhabitat structures used in the active season by gestating EDBs. Similarly, I used telemetry locations to identify microhabitats used by vitellogenic and non-reproductive females during winter. I determined gestation status using snake behavior, i.e., gestating females move infrequently and do not forage, and by observing females with neonates at parturition sites. I determined the vitellogenic status of females from ultrasonography (Clarius L7 Vet HD$_3$) indicating the presence of developing ovarian follicles during late winter and early spring 2024.

2.4. Study Design

Through a use-availability framework, I examined the importance of structural and thermal microhabitat characteristics used by adult female EDBs for summer gestation and overwintering. Like other crotalids and viperids, EDBs suspend or reduce foraging while overwintering and gestating (Naulleau, 1983; Nordberg and Cobb, 2016; Reinert et al., 2011; Waldron et al., 2013), allowing us to examine microhabitat selection based on behaviors that were not confounded by foraging activities. I quantified thermal profiles and vegetation structure at used and random locations, sampling habitat at two random locations per used location. I used ArcGIS Pro (version 3.1.4, Environmental Systems Research, Inc., Redlands, California, USA) to randomly select 'available' sites that were located > 40 m from a used site based on 34-m daily movement distances reported in Heres et al. (2018). I generated one random site within 1 km of used sites, encompassing EDB activity ranges at both study sites. Full activity ranges estimated using 95% minimum convex polygons (MCP) averaged 45.4 ha (range: 36-52 ha) for the MCBCL population and 5.0 ha (range: 0-73 ha) using 100% MCP for the MCRDPI population (Wiles, 2022). I generated a second 'available' location per used site that was randomly located across the study area to ensure I captured sufficient spatial heterogeneity within both installations. At all random points, I randomly selected a subterranean microhabitat structure by walking outward in concentric circles from the random point while increasing the radius of each circle by ≈5-m until I detected a structure (e.g., tip-up, concrete pile) with subterranean access.

2.5. Vegetation Structure

At used and random microhabitats, I quantified above-ground vegetation structure to assess site openness and aspect. I used modified circular James and Shugart (1970) plots (≈11.3

m^2) centered on subterranean structures to quantify structure. I divided each plot into quarters with four transects radiating from the plot center along each cardinal direction (Figure 10). Starting four meters from the plot center, I used the point intercept method to characterize ground cover and canopy cover at eight points per transect, i.e., points were located every meter between 4 and 11 m along each transect, providing 32 point-intercepts per plot. At each point intercept, I recorded canopy cover using a densitometer and characterized ground cover categories of litter, coarse woody debris (CWD), woody cover, graminoid cover, and forb cover (Table 1). Coarse woody debris was considered any dead woody material (e.g., downed logs) on the ground >12 cm diameter. Within each vegetation plot, I measured CWD length (CWDL, cm; Table 1), and recorded CWD diameter of the widest portion of each CWD log (CWDW, cm; Table 1; Lutes et al. 2006). I calculated mean values per plot for the CWD metrics (CWDL, CWDW). I measured diameter at breast height (DBH) for all trees > 8 cm DBH within plots, and I used tree DBH measurements to estimate basal area/ha (Table 1). I recorded the aspect of the subterranean entrance for each used and random subterranean structure at the plot center. I collected fine-scale ground cover metrics at five 1-m^2 subplots within the circular vegetation plots. One subplot was located at the center of the circular plot and each of four subplots were located six meters from the circular plot center along transects (one subplot per transect; Figure 7). Within each subplot, I counted live and dead woody stems > 50 cm height and visually estimated ground cover to the nearest five percent for woody, graminoid, forb, and CWD cover types. I collected data from vegetation plots for the gestation analysis from 25 July-11 October 2023 and from 8 January-7 March 2024 for the overwintering analysis.
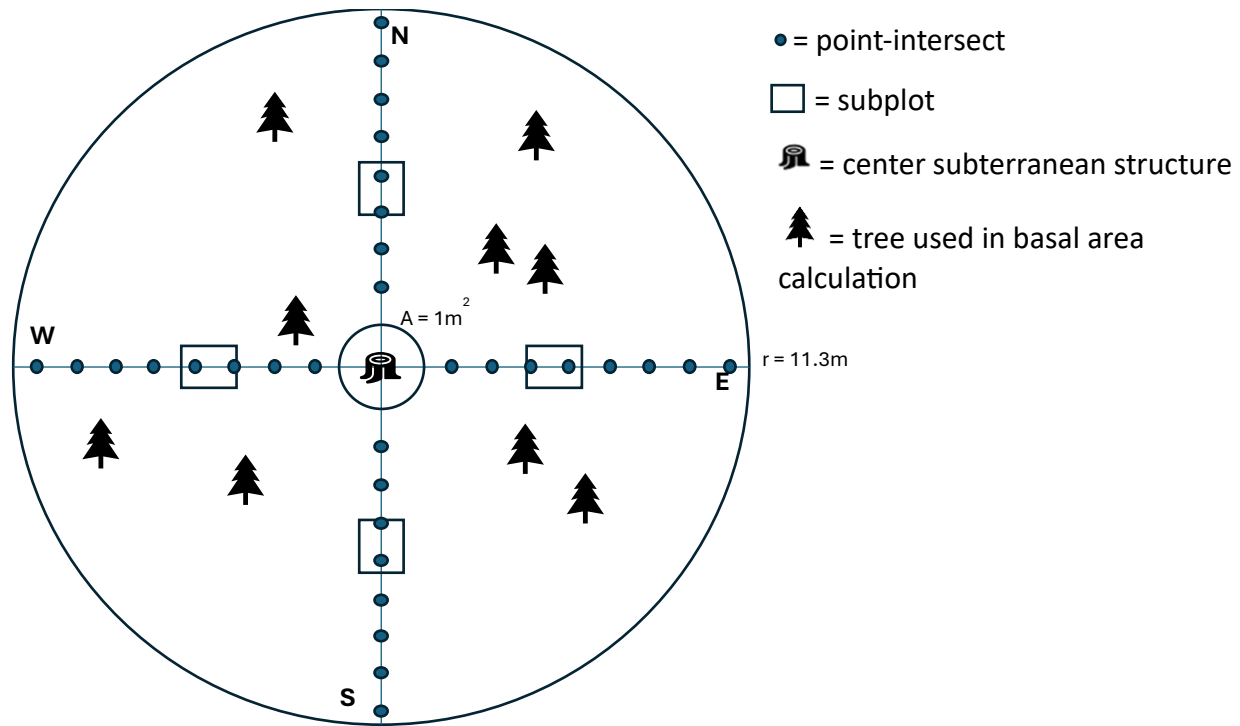
Figure 10.

Modified circular James and Shugart vegetation plots and subplots centered on subterranean structures and used to quantify above-ground vegetation structure at used and random microhabitat sites to examine adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection and overwintering site selection in North Carolina and South Carolina, USA 2023-2024. Dots represent point-intercepts along cardinal direction transects. A= area of subplots, r= radius of James and Shugart plot.

Table 1.

Vegetation structure predictors used to examine adult female eastern diamondback rattlesnake

(*Crotalus adamanteus*) gestation site selection and overwintering site selection in North Carolina

and South Carolina, USA 2023-2024.

Variable	Description
Basal Area (BA)	Cm^2/ha; Calculated using diameter-at-breast height (cm; DBH) measurements for all trees > 8cm DBH, standardized by hectare
Canopy Cover (CC)	Percent canopy closure; Calculated from canopy closure data collected using a densitometer at 32 point intercepts/$11.3m^2$ plot
Aspect (ASP)	Negative cosine transformation of circular azimuth data; raw angles were converted to radians, then cosine transformed. Negative cosine means higher/more positive values are more south-facing aspects; values bound between -1 and 1
Center subplot woody cover (CSW)	Percent live woody (i.e., perennial, containing bark) vegetation groundcover averaged across 4 quarters of central $1-m^2$ subplots
Center subplot forb cover (CSF)	Percent forb vegetation groundcover estimated to the nearest 5%, averaged across 4 quarters of central $1-m^2$ subplots
Center subplot total graminoid cover (CSG)	Percent groundcover (nearest 5%) of all combined graminoid species averaged across 4 quarters of central $1-m^2$ subplots; used maximum percentage among species
Center subplot coarse woody debris cover (CSCWD)	Percent coarse woody debris, i.e., any dead woody material (e.g., downed logs) on the ground >12 cm diameter; groundcover estimated to the nearest 5%, averaged across 4 quarters of central $1-m^2$ subplots
Center subplot stem counts (CSS)	Woody stems (live and dead, >50cm height) counted from 4 quarters of central $1-m^2$ subplots; z-standardized
Subplot woody cover 6 m from center structure (6SW)	Percent woody (i.e., perennial, containing bark) vegetation groundcover estimated to nearest 5% averaged from four $1-m^2$ subplots located 6m from central subterranean structures
Subplot forb cover 6 m from center structure (6SF)	Percent forb vegetation groundcover estimated to nearest 5% averaged from four $1-m^2$ subplots located 6m from central subterranean structure

Subplot total graminoid cover 6 m from center structure (6SG) | Percent groundcover of all combined graminoid species estimated to nearest 5% averaged from four 1-m^2 subplots located 6m from central subterranean structure; used maximum percentage among species

Subplot coarse woody debris cover 6 m from center structure (6SCWD) | Percent coarse woody debris (any dead woody material, e.g., downed logs on the ground >12 cm diameter); groundcover estimated to nearest 5% averaged from four 1-m^2 subplots located 6m from central subterranean structure

Subplot stem counts 6 m from center structure (6SS) | Woody stem (live and dead, >50cm height) counts averaged from four 1-m^2 subplots located 6m from central subterranean structure; z-standardized

Litter Cover (LC) | Percent litter groundcover; Calculated from 32 point intercepts/11.3-m^2 plot

Graminoid Cover (GC) | Percent graminoid groundcover; Calculated from 32 point intercepts/11.3-m^2 plot

Woody Cover (WC) | Percent woody vegetation (i.e., perennial, containing bark) groundcover; Calculated from 32 point intercepts/11.3-m^2 plot

Forb Cover (FC) | Percent forb groundcover; Calculated from 32 point intercepts/11.3-m^2 plot

Coarse woody debris cover (CWDC) | Percent coarse woody debris (any dead woody material, e.g., downed logs on the ground >12 cm diameter); Calculated from 32 point intercepts/11.3m^2 plot

Coarse woody debris counts (CWDCT) | Coarse woody debris counts (any dead woody material, on the ground >12 cm diameter) within 11.3m^2 plot; z-standardized

Coarse woody debris length (CWDL) | Mean length of coarse woody debris >12 cm diameter within 11.3-m^2 plot; z-standardized

Coarse woody debris width (CWDW) | Mean diameter of coarse woody debris >12 cm within 11.3-m^2 plot, measured at the widest portion of log; z-standardized

Vitellogenic Status (VS) | Binary variable used in overwintering microhabitat analyses indicating vitellogenic status of female snakes, determined using ultrasonography; V=vitellogenic, NV=nonvitellogenic)

2.6. Soil Structure

     I characterized soil properties to evaluate the importance of soil moisture retention on microhabitat selection. I collected soil samples from all sites to assess microhabitat soil texture based on percentages of silt, sand, and clay present in samples (Table 2). Within each circular 11.3-m^2 vegetation plot, I collected three soil subsamples by walking a random distance between 1-10 m along three random bearings from the plot center. Soil samples were sieved (2 mm mesh) and dried in 40°C drying ovens for 48-72 hours. I combined subsamples collected within the same plot (≈100 g of combined soil, ≈35 g representing each subsample) and sent them to Brookside Laboratories, Inc. for texture composition lab analysis (American Society of Testing Materials D422 hydrometer particle size analysis; American Society for Testing and Materials, 2000). I paired the soil texture analysis with select variables from the US Department of Agriculture's Gridded Soil Survey Geographic Database (gSSURGO) product for North and South Carolina (Soil Survey Staff, 2023). Using ArcGIS Pro (version 3.1.4, Environmental Systems Research, Inc., Redlands, California, USA), I retrieved attribute table information from gSSURGO map unit polygons that overlapped used or random snake points. From the gSSURGO database, I included three categorical variables pertaining to polygon soil types – a hydric rating variable indicating whether soils are generally considered hydric, a drainage class variable indicating how well a soil type is expected to drain water on a scale of six categories, and a soil order variable (Table 2). The gSSURGO soils data unit polygons cover larger areas of varying sizes compared to circular sample plots and thus represent coarser soils data. I collected soil subsamples from gestation and overwinter microhabitats simultaneously with vegetation plot metrics.

Table 2.

Soil structure variables used to examine adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection and overwintering site selection in North Carolina and South Carolina, USA 2023-2024.

Variable	Description
Clay	Percent clay particles in 11-m^2 plot soils, calculated from three random subsamples per plot
Silt	Percent silt particles in 11-m^2 plot soils, calculated from three random subsamples per plot
Sand	Percent sand particles in 11-m^2 plot soils, calculated from three random subsamples per plot
Hydric Rating (HR)	USDA Gridded Soil Survey Geographic Database (gSSURGO) categorical variable - a yes/no field indicating whether a map unit component was classified as "hydric soil"
Drainage Class (DC)	USDA Gridded Soil Survey Geographic Database (gSSURGO) categorical variable identifying natural soil drainage conditions referencing frequency and duration of wet periods. Six categories; 1) SPD = somewhat poorly drained, 2) PD = poorly drained, 3) VPD = very poorly drained, 4) SED = somewhat excessively drained, 5) MWD = moderately well drained, 6) ED = excessively drained
Soil Order (SO)	USDA Gridded Soil Survey Geographic Database (gSSURGO) categorical variable - The highest taxonomic level in soil taxonomy
Vitellogenic Status (VS)	Binary variable indicating vitellogenic status of female snakes determined using ultrasonography (only applicable for overwintering analyses, V=vitellogenic, NV=nonvitellogenic)

2.7. Thermal Profile

To assess the role of temperature on microhabitat selection, I characterized thermal profiles of used and random microhabitat sites using Thermochron iButton miniature temperature dataloggers (iButton®, 1921G-F5, Maxim Integrated Products, Sunnyvale, California, USA; accuracy 1°C, resolution 0.5°C). I quantified operative environmental temperatures (T_e), which provide a measure of the true environmental temperature available to a non-thermoregulating animal using thermal snake models (Bakken and Gates, 1975). I constructed hollow snake thermal models from copper tubing (30.5 cm length, 3.2 cm diameter) painted to mimic the skin reflectance of an adult EDB using gray primer spray paint (Krylon #K05513007) and flat black spray paint (Krylon #K05546007) to paint diamond patterns on models (Figure 11; Peterson et al., 1993). I suspended an ibutton on a dowel rod at the center of each model, sealing T_e model ends with cork stoppers (Nordberg and Cobb, 2017). I placed a model in full sun exposure near the subterranean structure and one under the nearest shaded cover to capture the range of available T_es at each used and random site. I calibrated the T_e model by regressing hourly temperatures recorded with an iButton suspended in a model placed in full sun exposure for approximately eight hours during the day against hourly temperatures recorded simultaneously by an iButton inside the body cavity of a recently deceased snake placed beside the model. Operative temperature model temperatures reasonably predicted those of the dead snake (n=8, r^2=0.94, intercept not significantly different from 0 – 95% CI 5.58 to -0.027. Slope 95% CI, 0.92 to 0.77, did not include but approached 1). A correlation analysis between the model and dead snake temperatures indicated temperatures were highly correlated (r=0.98, df=7, P<0.001), and thus I assumed models reasonably simulated operative temperatures available to EDBs.

Figure 11.

Operative temperature (T_e) models (left), constructed from copper tubing painted to mimic

eastern diamondback rattlesnake (*Crotalus adamanteus;* EDB) skin reflectance patterns,

equipped with an iButton® (1921G-F5, Maxim Integrated Products, Sunnyvale, California,

USA; accuracy 1°C, resolution 0.5°C) to estimate true environmental microhabitat temperatures

available to adult female EDBs in North Carolina and South Carolina, USA 2023-2024. A

photograph of the scale pattern of a live EDB (right) is provided for reference.

    I collected temperature data using iButtons to characterize soil and ambient thermal

conditions at microsites. To record soil temperature, I placed iButtons at 10 cm and 30 cm soil

depths within 1-2 m of each structure's subterranean entrance. I recorded ambient microsite air

temperature by affixing an iButton to a stake one meter aboveground and shaded iButtons using

a 55% shade rated cloth. Thus, I collected temperature data at five unique locations for every used and random site – i.e., a T_e model placed in full sun, a T_e model placed in shaded cover, shaded ambient temperature one meter aboveground, and at 10 cm and 30 cm underground (Figure 12). I programmed iButtons to simultaneously record hourly temperatures for all thermal metrics, ensuring each used location was sampled simultaneously with its two paired random locations over a 1-week period. I summarized iButton data by obtaining daily means, minimums, maximums, and standard deviations of hourly temperature recordings from each iButton. The sun exposed T_e model from one random site was stolen, and thus I fixed this site's sun exposed T_e values to the mean of the sun exposed T_e variables among other random sites for which temperatures were recorded on the same dates.

Figure 12.

Example of thermal profile data collection set up at subterranean structures including operative environmental temperature physical models, 1 m ambient shaded air temperature, and 10 and 30 cm soil temperature (not pictured).

I recorded temperatures at gestation sites between 1 August and 12 October 2023. I recorded temperatures at overwintering sites between 7 February and 12 March 2024. I standardized daily iButton temperature summary statistics with local NOAA weather station

daily temperature summary statistic data (Menne et al., 2012a – MCRDPI: station=Beaufort MCAS, USW00093831, distance=21 km; MCBCL: station=New River MCAF, USW00093727, distance=24 km). I subtracted NOAA temperature summaries from iButton temperature summaries for each day. Thus, in addition to daily standard deviations, every site contained variables on temperature differences for daily means, minimums, and maximums for each of the five unique iButton locations (Table 3). I examined the effect of female body size (SVL, cm) and body condition on microhabitat selection. I derived body condition indices (BCI) separately for females in the gestation and overwintering analyses using residuals from ordinary least squares regression of body mass on SVL, such that 0 represented individuals of average body condition, positive values indicated above average condition, and negative values indicated below average condition to assess size-related thermal dynamic influences on site selection.

Table 3.

Thermal predictors collected using iButton® dataloggers (1921G-F5, Maxim Integrated Products, Sunnyvale, California, USA; accuracy 1°C, resolution 0.5°C) to examine adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection and overwintering site selection in North Carolina and South Carolina, USA 2023-2024.

Variable	Description
Ambient temperature standard deviation (ASD)	Standard deviation of daily microhabitat iButton temperatures (°C) recorded hourly 1 m above ground shaded with 55% rated shade cloth for ≈1 week
Ambient mean temperature (AMEAN)	Mean daily microhabitat iButton temperatures (°C) recorded hourly 1 m above ground shaded with 55% rated shade cloth for ≈1 week; standardized by subtracting daily local NOAA weather station temperature means from daily iButton means

Ambient maximum temperature (AMAX)	Maximum daily microhabitat iButton temperatures (°C) recorded hourly 1 m above ground shaded with 55% rated shade cloth for ≈1 week; standardized by subtracting daily local NOAA weather station temperature maximums from daily iButton maximums
Ambient minimum temperature (AMIN)	Minimum daily microhabitat iButton temperatures (°C) recorded hourly 1 m above ground shaded with 55% rated shade cloth for ≈1 week; standardized by subtracting daily local NOAA weather station temperature minimums from daily iButton minimums
10 cm temperature standard deviation (10SD)	Standard deviation of daily microhabitat iButton soil temperatures (°C) recorded hourly 10 cm underground within 1-2 m of subterranean structures for ≈1 week
10 cm mean temperature (10MEAN)	Mean daily microhabitat iButton soil temperatures (°C) recorded hourly 10 cm underground within 1-2 m of subterranean structures for ≈1 week; standardized by subtracting daily local NOAA weather station temperature means from daily iButton means
10 cm maximum temperature (10MAX)	Maximum daily microhabitat iButton soil temperatures (°C) recorded hourly 10 cm underground within 1-2 m of subterranean structures for ≈1 week; standardized by subtracting daily local NOAA weather station temperature maximums from daily iButton maximums
10 cm minimum temperature (10MIN)	Minimum daily microhabitat iButton soil temperatures (°C) recorded hourly 10 cm underground within 1-2 m of subterranean structures for ≈1 week; standardized by subtracting daily local NOAA weather station temperature minimums from daily iButton minimums
30 cm temperature standard deviation (30SD)	Standard deviation of daily microhabitat iButton soil temperatures (°C) recorded hourly 30 cm underground within 1-2 m of subterranean structures for ≈1 week
30 cm mean temperature (30MEAN)	Mean daily microhabitat iButton soil temperatures (°C) recorded hourly 30 cm underground within 1-2 m of subterranean structures for ≈1 week; standardized by subtracting daily local NOAA weather station temperature means from daily iButton means
30 cm maximum temperature (30MAX)	Maximum daily microhabitat iButton soil temperatures (°C) recorded hourly 30 cm underground within 1-2 m of

subterranean structures for ≈1 week; standardized by subtracting daily local NOAA weather station temperature maximums from daily iButton maximums

30 cm minimum temperature (30MIN)	Minimum daily microhabitat iButton soil temperatures (°C) recorded hourly 30 cm underground within 1-2 m of subterranean structures for ≈1 week; standardized by subtracting daily local NOAA weather station temperature minimums from daily iButton minimums
Shaded T_e temperature standard deviation (SDT$_e$SD)	Standard deviation of daily microhabitat iButton temperatures (°C) recorded hourly within snake T_e (i.e., operative environmental temperature) models placed in shaded cover at microhabitats for ≈1 week
Shaded T_e mean temperature (SDT$_e$MEAN)	Mean daily microhabitat iButton temperatures (°C) recorded hourly within snake T_e (i.e., operative environmental temperature) models placed in shaded cover at microhabitats for ≈1 week; standardized by subtracting daily local NOAA weather station temperature means from daily iButton means
Shaded T_e maximum temperature (SDT$_e$MAX)	Maximum daily microhabitat iButton temperatures (°C) recorded hourly within snake T_e (i.e., operative environmental temperature) models placed in shaded cover at microhabitats for ≈1 week; standardized by subtracting daily local NOAA weather station temperature maximums from daily iButton maximums
Shaded T_e minimum temperature (SDT$_e$MIN)	Minimum daily microhabitat iButton temperatures (°C) recorded hourly within snake T_e (i.e., operative environmental temperature) models placed in shaded cover at microhabitats for ≈1 week; standardized by subtracting daily local NOAA weather station temperature minimums from daily iButton minimums
Sun exposed T_e temperature standard deviation (SET$_e$SD)	Standard deviation of daily microhabitat iButton temperatures (°C) recorded hourly within snake T_e (i.e., operative environmental temperature) models placed in full sun at microhabitats for ≈1 week
Sun exposed T_e mean temperature (SET$_e$MEAN)	Mean daily microhabitat iButton temperatures (°C) recorded hourly within snake T_e (i.e., operative environmental temperature) models placed in full sun at microhabitats for ≈1 week; standardized by subtracting

	daily local NOAA weather station temperature means from daily iButton means
Sun exposed T_e maximum temperature (SET$_e$MAX)	Maximum daily microhabitat iButton temperatures (°C) recorded hourly within snake T_e (i.e., operative environmental temperature) models placed in full sun at microhabitats for ≈1 week; standardized by subtracting daily local NOAA weather station temperature maximums from daily iButton maximums
Sun exposed T_e minimum temperature (SET$_e$MIN)	Minimum daily microhabitat iButton temperatures (°C) recorded hourly within snake T_e (i.e., operative environmental temperature) models placed in full sun at microhabitats for ≈1 week; standardized by subtracting daily local NOAA weather station temperature minimums from daily iButton minimums
Female snake snout-to-vent length (SVL)	Body size (cm), z-standardized
Female snake body condition index (BCI)	Residuals from ordinary least squares regression of body mass on SVL such that 0 represented individuals of average body condition, positive values indicated above average condition, and negative values indicated below average condition
Vitellogenic Status (VS)	Binary variable representing vitellogenic status of female snakes determined from ultrasonography (only applicable for overwinter analyses; V=vitellogenic, NV=nonvitellogenic)

2.8. Data Analysis

I used standard logistic regression to examine the influence of vegetation structure, soil structure, and thermal profile environmental factors on microhabitat selection in separate analyses of gestation site selection and overwintering site selection (Figure 13). The gestation site selection analysis was limited to data collected at used and random locations for gestating female EDBs (i.e., the analysis did not include data from non-reproductive females). The overwintering site selection analysis included data collected from reproductive (i.e., vitellogenic)

and non-reproductive (non-vitellogenic) females. I quantified winter microhabitat selection for reproductive and non-reproductive females to examine the effect of reproductive condition on site selection. All females remained at selected microhabitat structures for prolonged periods during the overwintering period (mean ≈81 days), allowing me to assess winter microhabitat selection among reproductive and non-reproductive females. However, I only quantified microhabitat selection by reproductive females during the gestation period (May-Aug) when non-reproductive females were foraging and not constrained to subterranean structures. Due to differences in habitat types between MCBCL and MCRDPI, I did not make direct comparisons of microhabitat use between the populations of these two installations.

In the gestation analysis, I used MCRDPI gestation sites from summer 2022 (no telemetered females on MCRDPI were gravid during the 2023 active season). Physical site conditions at MCRDPI did not differ between 2022 and 2023 (M. Gacheny, personal observation), and comparisons of daily weather station temperatures for each year to 20-year monthly temperature normals (Menne et al., 2012b) indicated summer temperatures at MCRDPI were not anomalous for either year on data collection dates. Because the gestation site selection analyses included data from two study areas, I compared fit of intercept-only (null) fixed and mixed-effect models. The mixed effect model included study area as a random effect to account for variation stemming from a lack of independence among observations from the same study site. I used AIC scores to evaluate null model fit, retaining model structure with the lowest AIC score for analysis. I z-transformed all continuous vegetation metrics and standardized all percentage values between 0 and 1 (e.g., 0=0% cover, 1=100% cover).
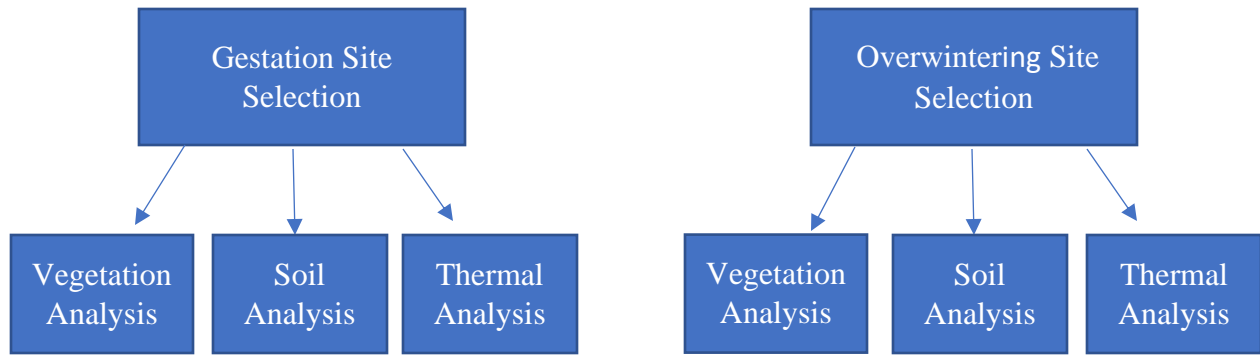
Figure 13.

Outline of gestation site and overwintering site selection analyses for eastern diamondback

rattlesnakes (*Crotalus adamanteus*) in North and South Carolina, USA.

The gestation site analysis included 21 vegetation structure predictors (Table 1), six soil

predictors (Table 2), and 22 thermal metrics (Table 3). Six vegetation predictors were measures

of CWD, and I expected selection would be positively associated with CWD by providing

protective cover and shelter from summer heat. Sixteen vegetation predictors were indicators of

above-ground cover, and I expected selection would be positively associated with above-ground

cover that provided shade and refuge from summer heat. I standardized aspect using a negative-

cosine transformation such that positive values represented more southerly aspects (Huertas and

Díaz, 2001; Table 1). I expected females would be more likely select gestation sites with

orientations that minimized sun exposure and reduced subterranean temperatures.

Six soil predictors measured water retention properties, and I expected microsite selection

would be positively associated with soils that exhibited greater water retention properties,

reducing water loss and minimizing hydration stress during gestation. I predicted a greater

percentage of clay within soil texture parameters would infer greater moister retention properties.

I expected gSSURGO soil categorical variables indicating presence of hydric soils, poorer soil

drainage class, and poorly drained soil orders would also infer greater moisture retention properties.

Twelve thermal predictors measured the above-ground thermal profile, and I expected gestation site selection would be negatively associated with surface temperature to ameliorate gestation-associated metabolic costs concerning heat stress, water loss, and reduced ventilation capacity. Eight thermal predictors measured the below-ground thermal profile, and I expected gestation site selection would be negatively associated with soil temperature to ameliorate gestation-associated metabolic costs concerning heat stress, water loss, and reduced ventilation capacity. Two 'thermal' metrics described gestating female EDB body size and condition, as I expected snake body size and condition would affect thermal dynamics and thus influence site selection.

The overwintering site selection analysis included 21 vegetation structure predictors (Table 1), six soil predictors (Table 2), and 22 thermal metrics (Table 3). Six vegetation predictors were measures of CWD, and I expected overwintering site selection would be positively associated with CWD to maximize concealment. Sixteen vegetation predictors were indicators of above-ground cover, and I expected vitellogenic females would be more likely to select overwintering sites with more cover to reduce or stabilize temperatures compared to non-reproductive females. I expected vitellogenic females would be more likely to avoid sun exposure to reduce or stabilize subterranean temperatures compared to non-reproductive females. Six soil predictors measured water retention properties, and I expected vitellogenic females would be more likely to select overwintering sites with hydric, clayey soils, assuming water availability would be limiting compared to non-reproductive females. Twelve thermal predictors measured the above-ground thermal profile, and I expected vitellogenic female overwintering

site selection would be negatively associated with surface temperature, allowing vitellogenic

females to maintain low body temperatures during winter brumation to conserve energy reserves

for completion of vitellogenesis after spring emergence compared to non-vitellogenic females.

Eight thermal predictors measured the below-ground thermal profile, and I expected vitellogenic

females would select overwintering sites with cool temperatures to conserve energy for

completing vitellogenesis following spring emergence compared to non-reproductive females.

Two 'thermal' metrics described overwintering female EDB body size and condition. I expected

snake body size and condition would influence thermal dynamics and thus influence site

selection. Each of the overwintering site selection environmental factor analyses contained a

categorical variable of vitellogenic status (Table 1, Table 2, Table 3), allowing me to evaluate

effects of reproductive state on adult female overwinter site selection.

I examined Pearson correlation (r) among all continuous variables in each dataset. When

predictors were correlated ($|r| > 0.7$), I retained the more biologically informative covariate for

analysis. I used global models to examine fit and multicollinearity. I used the R package

'performance' (v.0.11.0; Lüdecke et al., 2021) to examine collinearity of global model

predictors, which uses variance inflation factors (VIF) to assess collinearity of each variable. I

removed covariates with high VIF (>10) and assessed goodness of fit of the reduced global

model using diagnostic plots in conjunction with a Kolmogorov-Smirnov test, dispersion test

(non-parametric comparison of the variance of simulated to observed residuals), outlier test, and

combined-adjusted quantile test of residual versus fitted values via R package 'DHARMa'

(v.0.4.6; Hartig, 2022).

I used generalized linear models (GLM) with a binomial distribution to conduct logistic

regression analysis in program R (version 4.3.1; R Core Team, 2023), including 'use' as the

binary response (1 = used, 0 = random). I evaluated *a priori* candidate models in separate analyses for each environmental factor (i.e., vegetation structure, soil structure, and thermal profile; Table 1, Table 2, Table 3). Due to mild overdispersion, I used Quasi-Akaike's Information Criterion adjusted for small sample size ($QAIC_c$; Burnham and Anderson, 2002) to rank candidate models using R package 'MuMIn' (Bartoń, 2023). I used candidate models within $\Delta QAIC_c \leq 2.0$ for inference (Burnham and Anderson, 2002). I used Wald Chi-Squared tests (R package 'car', v.3.1.2; Fox, 2019) to assess covariate significance in supported models and used model specific beta (β) coefficients to evaluate covariate effects. I further examined significance by calculating weighted-average parameter estimates across supported models. Model averaging of supported ($\Delta QAIC_c \leq 2.0$) models was performed using R package 'MuMIn' (v.1.47.5; Bartoń 2023). For significant covariate effects I report covariate means with standard errors and odds ratios with 95% confidence intervals.

3. Results

3.1. Gestation Site Selection

I collected microhabitat data for seven gestating female EDBs, yielding eight (MCRDPI, n = 6; MCBCL, n = 2) used and 16 random gestation sites. The dataset included two gestation sites used by one female at MCBCL during summer 2023. In addition to movement behavior indicative of gestation, I determined this female was gravid and gave birth based on a considerable, sudden loss of mass postpartum fall of 2023 despite not visually observing the clutch (mass loss = 508 g over ≈2 mo.). The MCBCL female gestated at microsites that were ≈140 m apart and both contained downed CWD with subterranean root channels adjacent to shallow depression ponds within xeric longleaf sandhills habitat containing shaded understory shrub cover provided by sweetgum (*Liquidambar styraciflua*) tree saplings. The gestation site

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dataset included one gestation site each for six gravid females from the MCRDPI population that were visually confirmed to have given birth. Gestation sites used by MCRDPI females generally consisted of a subterranean structure (e.g., tree tip up with open root channels, partially buried concrete structures) associated with CWD cover and dense ground cover (e.g., *Ilex vomitoria, Morella cerifera*).

3.1.1. Vegetation Structure

I removed 11 vegetation covariates due to multicollinearity (CSF, CSG, 6SW, 6SF, 6SG, LC, GC, CSS, BA, CWDW, 6SCWD; Table 1). The resulting global model contained 10 vegetation covariates (ASP, CSW, CSCWD, 6SS, CC, WC, FC, CWDC, CWDCT, CWDL; Table 1). Fixed effect model structure provided the best fit for gestation site vegetation data and thus I used fixed effect model structure for analyzing gestation site vegetation data. The global model was over-dispersed but provided adequate model fit (c-hat=1.32, Kolmogorov-Smirnov test p=0.55, dispersion test p=0.7, outlier test p=1). I evaluated 18 candidate models (Table 4), five of which received support (i.e., ΔQAICc $\leq$ 2.0). All but one supported model included CWD variables, and the top-ranking model was a univariate model of CWD counts (CWDCT). I failed to detect a significant effect of CWDCT ($\chi^2 = 3.72$, $p = 0.054$) in the top-ranking model but detected a significant CWDCT effect ($\chi^2 = 3.88$, $p < 0.05$) in the supported additive model (use=CWDL + CWDCT). Coarse woody debris counts were positively associated with gestation site selection (Figure 14); however, wide confidence intervals from 95% CIs of beta coefficients limited my ability to make inferences about CWD effects (Table 5). Model averaging indicated CWDCT was the most important predictor of gestation site selection, accounting for 49% of model weights and suggesting that the probability of a female EDB using a gestation site tended to increase as CWDCT increased at the microhabitat scale.
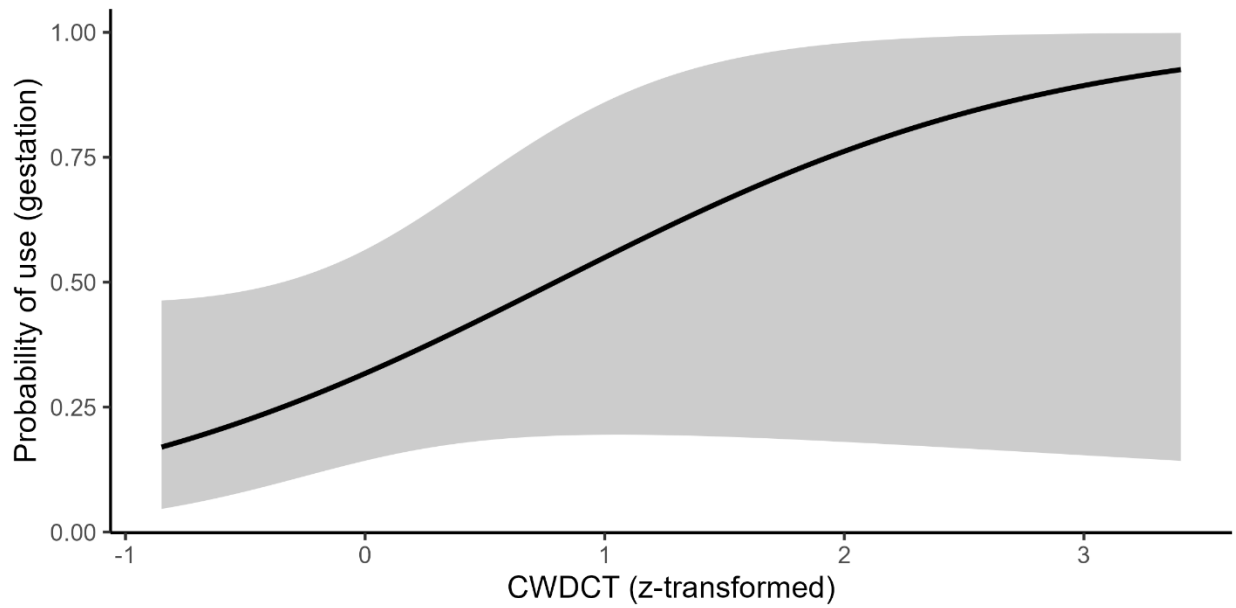
Figure 14.

Predicted relationship between coarse woody debris counts (CWDCT; z-transformed) and

female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection in

North Carolina and South Carolina, USA 2022-2023, based on output from the top-ranking

logistic regression model {Use = CWDCT + mean coarse woody debris length}.

Table 4.

Vegetation structure candidate logistic regression models ranked in order of support using QAIC$_c$ model selection to examine adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection in North Carolina and South Carolina, USA 2022-2023. K = number of parameters, QAIC$_c$ = quasi-Akaike's information criterion for small sample size, ΔQAIC$_c$ = the difference between the model with the lowest QAIC$_c$ score and the current model, w = model weights, Log Likelihood = log of model likelihood. CWDCT = coarse woody debris counts; CWDC = coarse woody debris percent cover; ASP = aspect of plot center structure; CWDL = mean coarse woody debris length of plot; CSCWD = center subplot coarse woody debris percent cover; FC = forb percent cover; WC = woody plant percent cover; CC = canopy percent cover; CSW = center subplot woody plant percent cover; CSS = center subplot stem counts.

Rank	Model	K	QAIC$_c$	ΔQAIC$_c$	w	Log Likelihood
1	CWDCT[a]	2	27.5	0.00	0.196	-13.417
2	CWDC[a]	2	28.7	1.16	0.110	-14.181
3	ASP[a]	2	28.8	1.29	0.103	-14.268
4	CWDL + CWDCT[a]	3	29.6	2.03	0.071	-12.840
5	CWDL[a]	2	29.6	2.07	0.070	-14.781
6	CSCWD	2	29.7	2.13	0.068	-14.821

7	FC	2	29.7	2.19	0.066	-14.864
8	WC	2	29.9	2.40	0.059	-14.999
9	CC	2	30.2	2.66	0.052	-15.169
10	CSW	2	30.2	2.67	0.052	-15.178
11	CSS	2	30.4	2.81	0.048	-15.272
12	ASP + CC	3	31.2	3.68	0.031	-13.929
13	CSW + CSCWD	3	32.3	4.78	0.018	-14.651
14	CSW + WC	3	32.8	5.30	0.014	-14.995
15	6SS + WC	3	32.8	5.30	0.014	-14.996
16	CSCWD + CWDC + CWDCT	4	32.9	5.35	0.013	-12.903
17	CSW + 6SS	3	33.1	5.57	0.012	-15.177
18	CC + CSW + WC	4	35.7	8.20	0.003	-14.780

[a] =Model was used for inference ($\Delta QAIC_c \leq 2.0$)

Table 5.

Coefficients from supported (i.e., ΔQAICc ≤ 2.0) logistic regression vegetation structure models examining adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection in North Carolina and South Carolina, USA 2022-2023. CWDCT = coarse woody debris counts; CWDC = coarse woody debris percent cover; ASP = aspect of plot center structure; CWDL = mean coarse woody debris length of plot.

Model	Parameter	β(SE)	95% CI	p
CWDCT	Intercept	-0.718(0.472)	-1.71, 0.19	0.128
	CWDCT	0.936(0.575)	-0.014, 2.366	0.104
CWDC	Intercept	-1.094(0.539)	-2.264, -0.103	<0.05
	CWDC	16.40(11.608)	-5.299, 42.537	0.158
ASP	Intercept	-0.647(0.452)	-1.592, 0.219	0.153
	ASP	0.855(0.621)	-0.32, 2.179	0.168
CWDL + CWDCT	Intercept	-0.766(0.494)	-1.828, 0.173	0.121
	CWDL	0.530(0.503)	-0.441, 1.617	0.291
	CWDCT	0.965(0.588)	0.004, 2.464	0.101
CWDL	Intercept	-0.722(0.446)	-1.662, 0.119	0.105
	CWDL	0.466(0.478)	-0.451, 1.502	0.330

3.1.2. Soil Structure

Fixed effect model structure provided the best fit for the gestation soil structure data and was used for the gestation soil structure analysis. I removed three soil structure covariates due to multicollinearity (Silt, DC, SO; Table 2). The reduced global model contained three soil covariates (Sand, Clay, HR; Table 2). The global model was over-dispersed but provided adequate model fit (c-hat=1.40, Kolmogorov-Smirnov test p=0.57, dispersion test p=0.7, outlier test p=1). Three out of six candidate models received support (Table 6). Supported models included percent clay, percent sand, and hydric soil rating. The top model included Sand as the

sole predictor, but I failed to detect a significant covariate effect ($\chi^2 = 2.419$, $p = 0.12$; Table 7).
Model averaging indicated Sand was the most important predictor of gestation site selection,
accounting for 53% of model weights.

Table 6.

Candidate soil structure logistic regression models ranked in order of support using QAIC$_c$
model selection to examine adult female eastern diamondback rattlesnake (*Crotalus adamanteus*)
gestation site selection in North Carolina and South Carolina, USA 2022-2023. K = number of
parameters, QAIC$_c$ = quasi-Akaike's information criterion for small sample size, ΔQAIC$_c$ = the
difference between the model with the lowest QAIC$_c$ score and the current model, w = model
weights, Log Likelihood = log of model likelihood. Sand = percent sand particles; Clay = percent
clay particles; HR = soil hydric rating.

Rank	Model	K	QAIC$_c$	ΔQAIC$_c$	w	Log Likelihood
1	Sand[a]	2	27.2	0.00	0.441	-14.067
2	Clay[a]	2	28.8	1.57	0.201	-15.172
3	HR[a]	2	29.0	1.72	0.186	-15.276
4	Clay + Sand	3	30.1	2.89	0.104	-14.055
5	Clay + HR	3	31.7	4.48	0.047	-15.172
6	Clay + Sand + HR	4	33.3	6.10	0.021	-14.04

[a] =Model was used for inference (ΔQAIC$_c \leq 2.0$)

Table 7.

Coefficients from supported (i.e., ΔQAICc ≤ 2.0) logistic regression soil structure models examining female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection in North Carolina and South Carolina, USA 2022-2023. Sand = percent sand particles; Clay = percent clay particles; HR = soil hydric rating.

Model	Parameter	β(SE)	95% CI	p
Sand	Intercept	-8.767(5.77)	-21.791, 1.232	0.129
	Sand	9.426(6.65)	-2.234, 24.343	0.156
Clay	Intercept	-0.194(1.173)	-2.572, 2.201	0.868
	Clay	-8.131(17.998)	-46.989, 26.579	0.651
HR	Intercept	$-6.931e^{-1}(8.66e^{-1})$	-2.669, 0.941	0.423
	HR	$-1.11e^{-16}(1.00)$	-1.922, 2.166	1.00

3.1.3. Thermal Profile

I removed 10 cm soil temperature because it was correlated with 30 cm soil temperature. I removed an additional six thermal covariates due to multicollinearity (AMAX, 30MAX, 30MIN, SDT$_e$MAX, SET$_e$SD, SET$_e$MEAN, BCI; Table 3). The reduced global model contained 10 thermal covariates (ASD, AMEAN, AMIN, 30SD, 30MEAN, SDT$_e$MEAN, SDT$_e$SD, SET$_e$MAX, SET$_e$MIN, SVL; Table 3). Fixed effect model structure provided the best fit for the gestation site thermal data and was used for the analysis of thermal data. The global model was over-dispersed but provided adequate model fit (c-hat=1.22, Kolmogorov-Smirnov test p=0.74, dispersion test p=0.76, outlier test p=1). Two out of 24 candidate models received support (Table 8). The top-performing model included additive effects of mean shaded T$_e$ (SDT$_e$MEAN) and mean 30 cm soil temperature (30MEAN) as predictors. Output from the top model indicated a significant ($\chi^2 = 8.159$, $p < 0.01$) effect of mean shaded T$_e$, which was negatively associated with gestation site selection (β= -0.509, SE= ± 0.189, Z=2.7, $p < 0.01$; Table 9, Figure 15A). I

detected a significant effect of mean 30 cm soil temperature from the top model ($\chi^2 = 10.215$, $p < 0.01$), which contrasted with effects of mean shaded T_e in displaying a positive associated with gestation site selection (β=0.278, SE= $\pm$ 0.09, Z=3.09, $p < 0.01$; Table 9, Figure 15B). Model averaging of supported models indicated a negative effect of mean shaded T_e (β= -0.512, 95% CI= -0.885, -0.138) and a positive effect of mean 30 cm soil temperature (β= 0.275, 95% CI= 0.087, 0.462; Table 9) on gestation site selection. Odds ratios indicated that for every unit increase in the difference of mean 30 cm soil temperature from regional ambient mean temperature, gravid female EDBs were 1.32 times more likely to use a gestation site (mean difference of used site 30 cm soil and regional temperature means = 0.08 ± 0.34 SE °C; 95% OR CI= 1.09, 1.59). Odds ratios indicated that for every unit increase in the difference of mean shaded T_e from regional ambient mean temperature, gravid female EDBs were 1.67 times less likely to use a gestation site (mean difference of used site shaded T_e and regional temperature means = -1.45 ± 0.14 SE °C; 95% inverse OR CI=1.15, 2.42). Model averaging indicated both mean shaded T_e and mean 30 cm soil temperature each accounted for 100% of model weights.
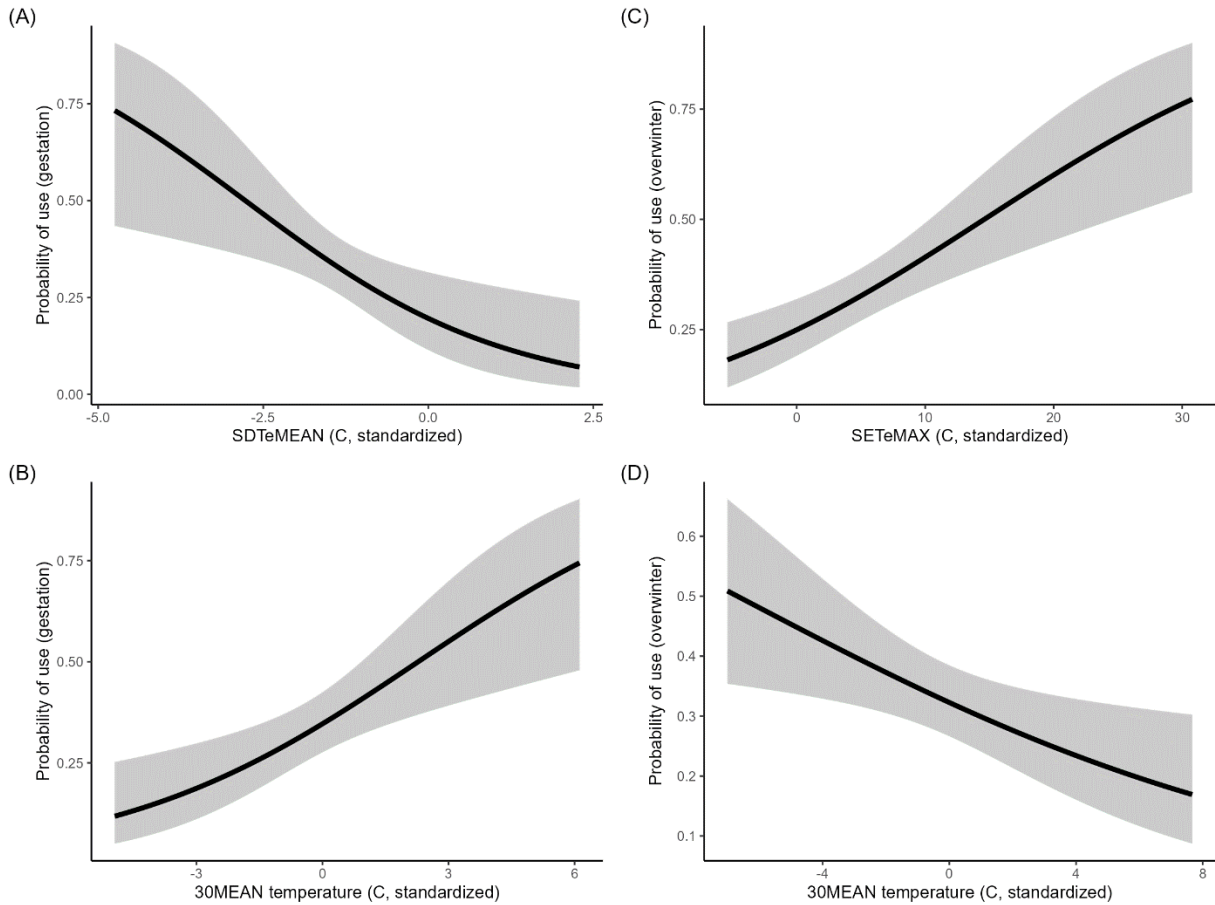
Figure 15.

Predicted relationships between thermal profile covariates (SDT$_e$MEAN - mean shaded operative environmental temperature; 30MEAN - mean 30 cm soil temperature; SET$_e$MAX - maximum sun exposed operative environmental temperature; °C standardized with NOAA weather station data) and eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection in North Carolina and South Carolina, USA 2022-2023 (A and B), and overwintering site selection in South Carolina, USA 2023-2024 (C and D), based on output from the top-ranking logistic regression models.

Table 8.

Candidate thermal profile logistic regression models ranked in order of support using $QAIC_c$ model selection to examine adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection in North Carolina and South Carolina, USA 2022-2023. K = number of parameters, $QAIC_c$ = quasi-Akaike's information criterion for small sample size, $\Delta QAIC_c$ = the difference between the model with the lowest $QAIC_c$ score and the current model, w = model weights, Log Likelihood = log of model likelihood. SDT_eMEAN = mean shaded operative environmental temperature; 30MEAN = mean 30 cm soil temperature; SDT_eSD = standard deviation of shaded operative environmental temperature; ASD = standard deviation of ambient air temperature; 30SD = standard deviation of 30 cm soil temperature; SET_eMAX = maximum sun exposed operative environmental temperature; SVL = snake snout-to-vent length; AMEAN = mean ambient air temperature; AMIN = minimum ambient air temperature.

Rank	Model	K	$QAIC_c$	$\Delta QAIC_c$	w	Log Likelihood
1	SDT_eMEAN + 30MEAN[a]	3	180.5	0.00	0.469	-105.08
2	SDT_eMEAN + 30MEAN + SDT_eMEAN*30MEAN[a]	4	182.5	2.07	0.167	-105.047
3	30MEAN	2	185.1	4.59	0.047	-109.159
4	SDT_eSD + ASD	3	185.1	4.63	0.046	-107.904
5	SDT_eSD	2	185.4	4.97	0.039	-109.391
6	30SD	2	186.1	5.65	0.028	-109.806
7	30SD + 30MEAN	3	186.3	5.85	0.025	-108.65

8	SDT$_e$MEAN	2	186.8	6.28	0.020	-110.187
9	30MEAN + SET$_e$MAX	3	187.1	6.67	0.017	-109.148
10	AMIN	2	187.3	6.80	0.016	-110.507
11	ASD	2	187.4	6.97	0.014	-110.608
12	SDT$_e$MEAN + SDT$_e$SD	3	187.5	7.02	0.014	-109.364
13	AMEAN	2	187.7	7.17	0.013	-110.736
14	SET$_e$MAX	2	187.7	7.20	0.013	-110.751
15	SVL	2	187.7	7.20	0.013	-110.753
16	ASD + 30SD	3	188.0	7.55	0.011	-109.687
17	30MEAN + AMEAN + ASD	4	188.3	7.79	0.010	-108.539
18	AMEAN + SDT$_e$MEAN	3	188.4	7.90	0.009	-109.899
19	SDT$_e$MEAN + SET$_e$MAX	3	188.7	8.23	0.008	-110.104
20	SVL + 30MEAN + SET$_e$MAX	4	189.1	8.64	0.006	-109.058
21	AMEAN + SET$_e$MAX	3	189.7	9.27	0.005	-110.734
22	SVL + AMEAN	3	189.7	9.27	0.005	-110.736
23	SVL + 30SD + ASD	4	189.8	9.33	0.004	-109.478

| 24 | ASD + AMEAN + AMIN | | 4 | 191.1 | 10.66 | 0.002 | -110.29 |

Table 9.

Coefficients from supported (i.e., ΔQAICc ≤ 2.0) logistic regression thermal profile models examining adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) gestation site selection in North Carolina and South Carolina, USA 2022-2023. SDT$_e$MEAN = mean shaded operative environmental temperature; 30MEAN = mean 30 cm soil temperature.

Model	Parameter	*β*(SE)	95% CI	*P*
SDT$_e$MEAN + 30MEAN	Intercept	-1.302(0.30)	-1.928, -0.742	<0.001
	SDT$_e$MEAN	-0.509(0.189)	-0.899, -0.155	<0.01
	30MEAN	0.278(0.09)	0.106, 0.461	<0.01
SDT$_e$MEAN + 30MEAN + SDT$_e$MEAN*30MEAN	Intercept	-1.279(0.314)	-1.931, -0.694	<0.001
	SDT$_e$MEAN	-0.518(0.191)	-0.913, -0.157	<0.01
	30MEAN	0.264(0.107)	0.058, 0.479	<0.05
	SDT$_e$MEAN*30MEAN	-0.017(0.066)	-0.149, 0.111	0.799

3.2. Overwintering Site Selection

The overwintering site selection dataset included microhabitat selection data for 17 adult female EDBs from the MCRDPI population. Overwintering sites used by adult females at the MCRDPI were located adjacent to marsh edges in maritime forest or mixed pine forest. Used microhabitats included subterranean structures of either tree tip-up root channels and CWD or partially buried concrete slabs. Vegetative ground cover varied but was typically sparse and consisted of marsh graminoids (e.g., *Juncus roemarianus, Spartina alterniflora*) and a low shrubby understory.

3.2.1. Vegetation Structure

I collected vegetation data at overwintering sites used by 17 adult females and at 34 paired random sites at MCRDPI. Two females dropped their transmitters at ingress, and one female died before her vitellogenic status could be determined. Thus, I excluded microhabitat data collected from used and random overwintering sites corresponding to these three females, leaving 14 adult female used sites and 28 paired random sites for analysis (vitellogenic = 10; non-reproductive = 4).

I removed six vegetation covariates due to multicollinearity (6SW, 6SF, LC, GC, CWDW, 6SG; Table 1). The resulting global model contained 16 vegetation covariates (VS, ASP, BA, CSW, CSF, CSG, CSCWD, CSS, 6SCWD, 6SS, CC, WC, FC, CWDC, CWDCT, CWDL; Table 1). The global model was over-dispersed but provided adequate model fit (c-hat=1.20, Kolmogorov-Smirnov test p=0.75, dispersion test p=0.71, outlier test p=1). Two out of 35 candidate models received support (Table 10). The top model included CWD counts (CWDCT) as the sole predictor, indicating a significant positive effect of CWD counts ($\chi^2 = 6.8489$, $p < 0.01$) on overwintering site selection (β= 0.946, SE= ± 0.407, Z=2.324, $p < 0.05$;

Figure 16, Table 11). Model averaging of supported models also indicated a positive effect of coarse woody debris counts (β= 0.947, 95% CI= 0.123, 1.77) on site selection. Odds ratios indicated that for every unit increase in coarse woody debris counts, female EDBs were 2.58 times more likely to use an overwinter site (mean used site coarse woody debris counts = 5.93 $\pm$ 0.85 SE; 95% OR=1.13, 5.87). Model averaging indicated coarse woody debris counts accounted for 71% of model weights. The second-ranked supported model included center subplot stem counts (CSS) as the sole predictor and indicated a significant effect on overwintering site selection (χ^2 = 4.742, $p < 0.05$); however, wide 95% CIs precluded our ability to make inferences about the relationship (Table 11, Figure 17).
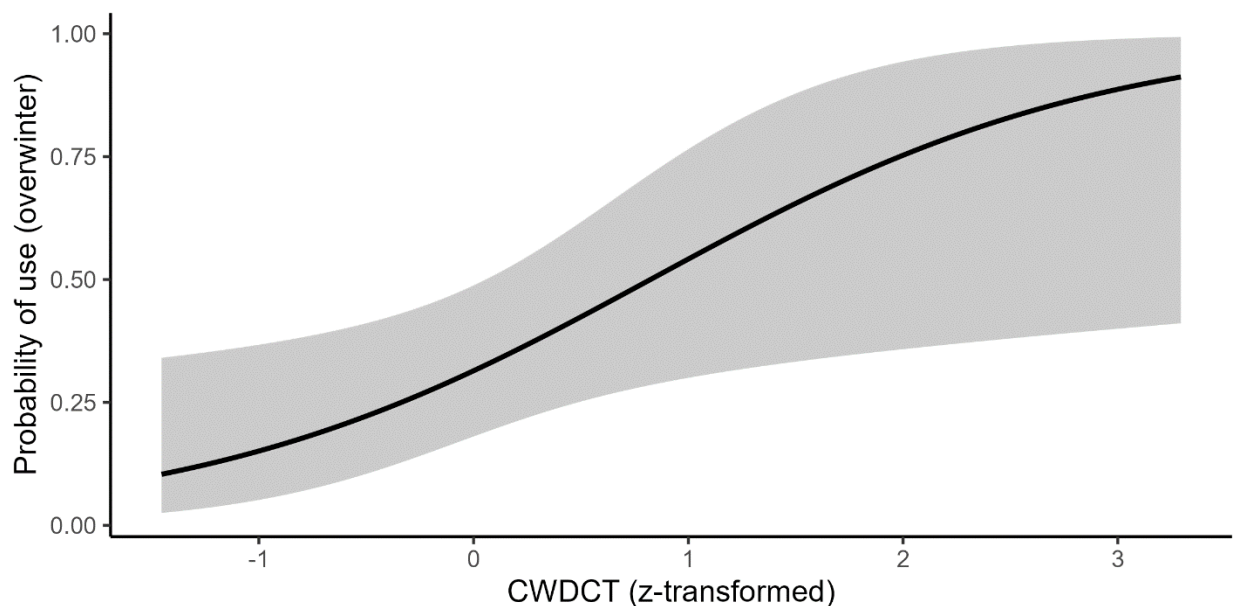
Figure 16.

Predicted relationship between coarse woody debris counts (CWDCT; z-transformed) and adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) overwintering site selection in South Carolina, USA 2023-2024, based on output from the top-ranking logistic regression model {Use = CWDCT}.
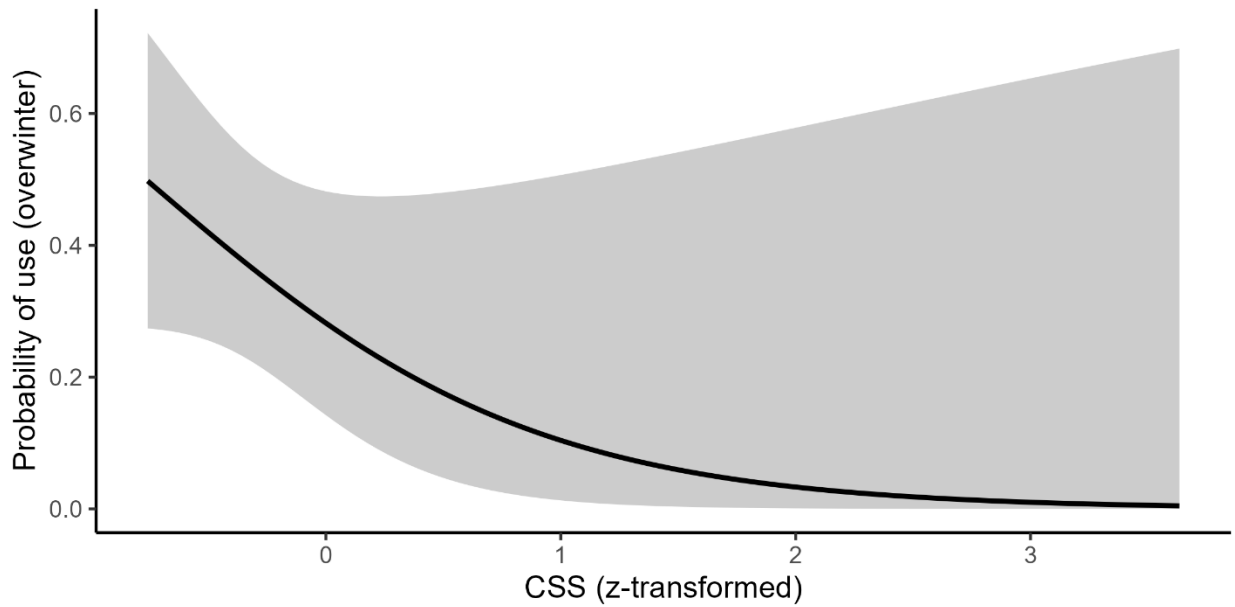
Figure 17.

Predicted relationship between center subplot stem counts (CSS; z-transformed) and adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) overwintering site selection in South Carolina, USA 2023-2024, based on output from a supported logistic regression model {Use = CSS}.

Table 10.

Candidate vegetation structure logistic regression models ranked in order of support using QAIC$_c$ model selection to examine adult

female eastern diamondback rattlesnake (*Crotalus adamanteus*) overwinter site selection in South Carolina, USA 2023-2024. *K* =

number of parameters, QAIC$_c$ = quasi-Akaike's information criterion for small sample size, ΔQAIC$_c$ = the difference between the

model with the lowest QAIC$_c$ score and the current model, *w* = model weights, Log Likelihood = log of model likelihood. CWDCT =

coarse woody debris counts; CWDC = coarse woody debris percent cover; ASP = aspect of plot center structure; CWDL = mean

coarse woody debris length of plot; CSCWD = center subplot coarse woody debris percent cover; FC = forb percent cover; WC =

woody plant percent cover; CC = canopy percent cover; CSW = center subplot woody plant percent cover; CSS = center subplot stem

counts; 6SS = woody plant percent cover at subplot 6 m from center structure; CSF = center subplot forb percent cover; BA = basal

area; CSG = center subplot graminoid percent cover; VS = vitellogenic status; 6SCWD = coarse woody debris percent cover at

subplot 6 m from center structure.

Rank	Model	K	QAIC$_c$	ΔQAIC$_c$	*w*	Log Likelihood
1	CWDCT[a]	2	45.4	0.00	0.246	-23.309
2	CSS[a]	2	47.2	1.75	0.102	-24.363
3	CWDCT + CWDL	3	47.8	2.38	0.075	-23.27
4	CSW	2	47.9	2.50	0.070	-24.814
5	CSW + CSCWD	3	48.0	2.61	0.066	-23.408

6	CSW + CSS	3	49.2	3.80	0.037	-24.12
7	CSCWD	2	49.4	4.03	0.033	-25.733
8	CSS + 6SS	3	49.6	4.18	0.030	-24.349
9	6SS	2	50.0	4.59	0.025	-26.069
10	CSF	2	50.1	4.70	0.023	-26.133
11	FC	2	50.1	4.70	0.023	-26.136
12	BA + CSW	3	50.3	4.85	0.022	-24.754
13	CSW + WC	3	50.3	4.93	0.021	-24.803
14	VS + CSW	3	50.4	4.95	0.021	-24.813
15	6SCWD	2	50.5	5.12	0.019	-26.387
16	WC	2	50.7	5.33	0.017	-26.512
17	CSW + CSS + CSW*CSS	4	50.8	5.38	0.017	-23.517
18	BA	2	51.1	5.65	0.015	-26.708
19	CC	2	51.1	5.67	0.014	-26.715
20	CSG	2	51.1	5.67	0.014	-26.715
21	CWDL	2	51.1	5.69	0.014	-26.728
22	CWDC	2	51.1	5.69	0.014	-26.729

23	VS	2	51.1	5.70	0.014	-26.734
24	CSG + CSCWD	3	51.7	6.31	0.010	-25.63
25	CSS + 6SS + WC	4	51.9	6.54	0.009	-24.211
26	CSF + FC	3	52.1	6.73	0.008	-25.882
27	CSCWD + 6SCWD + CWDC + CWDCT	5	52.4	7.04	0.007	-22.871
28	BA + CSW + WC	4	52.8	7.43	0.006	-24.748
29	CC + CSW + WC	4	52.9	7.48	0.006	-24.781
30	BA + CSG	3	53.4	8.04	0.004	-26.669
31	CC + CSG	3	53.5	8.11	0.004	-26.71
32	VS + CSG	3	53.5	8.11	0.004	-26.714
33	VS + CC	3	53.5	8.11	0.004	-26.714
34	BA + CC + BA*CC	4	55.8	10.43	0.001	-26.55
35	CSW + CSS + CSW*CSS	4	56.1	10.69	0.001	-26.709

[a] =Model was used for inference ($\Delta QAIC_c \leq 2.0$)

Table 11.

Coefficients from supported (i.e., ΔQAICc ≤ 2.0) logistic regression vegetation structure models examining adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) overwintering site selection in South Carolina, USA 2023-2024. CWDCT = coarse woody debris counts; CSS = center subplot stem counts.

Model	Parameter	β(SE)	95% CI	p
CWDCT	Intercept	-0.780(0.362)	-1.535, -0.097	<0.05
	CWDCT	0.946(0.407)	0.222, 1.846	<0.05
CSS	Intercept	-0.935(0.426)	-1.917, -0.189	<0.05
	CSS	-1.219(0.77)	-3.062, -0.09	0.113

3.2.2. Soil Structure

I analyzed soil samples for 12 (vitellogenic = 8; non-reproductive = 4) used adult female EDB overwinter sites and 24 paired random sites. Samples from one random site were lost during transport, and therefore I made the percent clay, silt, and sand values for this site equal their respective means in the dataset. I removed two soil structure covariates due to multicollinearity (Silt, DC; Table 2) and the reduced global model contained five soil covariates (Clay, Sand, HR, SO, VS; Table 2). The global model was over-dispersed but provided adequate model fit (c-hat=1.42, Kolmogorov-Smirnov test p=0.72, dispersion test p=0.93, outlier test p=1). Five of 12 candidate models received support (Table 12). The top model included Sand as the sole predictor but failed to indicate a significant effect ($\chi^2 = 0.178$, $p = 0.673$; Table 13). Model averaging indicated Sand was the most important predictor of overwinter site selection, accounting for 22.6% of model weights.

Table 12.

Candidate soil structure logistic regression models ranked in order of support using $QAIC_c$ model selection to examine adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) overwinter site selection in South Carolina, USA 2023-2024. K = number of parameters, $QAIC_c$ = quasi-Akaike's information criterion for small sample size, $\Delta QAIC_c$ = the difference between the model with the lowest $QAIC_c$ score and the current model, w = model weights, Log Likelihood = log of model likelihood. Sand = percent sand particles; Clay = percent clay particles; HR = soil hydric rating; VS = vitellogenic status.

Rank	Model	K	$QAIC_c$	$\Delta QAIC_c$	w	Log Likelihood
1	Sand[a]	2	39.0	0.00	0.169	-22.825
2	HR[a]	2	39.1	0.08	0.162	-22.884
3	Clay[a]	2	39.1	0.09	0.162	-22.888
4	VS[a]	2	39.1	0.13	0.159	-22.915
5	SO[a]	2	40.1	1.13	0.096	-21.826
6	Clay + Sand	3	41.5	2.54	0.048	-22.824
7	VS + Sand	3	41.5	2.54	0.048	-22.825
8	Clay + HR	3	41.6	2.60	0.046	-22.868
9	VS + HR	3	41.6	2.62	0.046	-22.883
10	VS + Clay	3	41.6	2.63	0.045	-22.888
11	Clay + Sand + HR	4	44.2	5.25	0.012	-22.822
12	Clay + Sand + SO	4	45.6	6.57	0.006	-21.706

[a] =Model was used for inference ($\Delta QAIC_c \leq 2.0$)

Table 13.

Coefficients from supported (i.e., ΔQAICc ≤ 2.0) logistic regression soil structure models examining adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) overwintering site selection in South Carolina, USA 2023-2024. Sand = percent sand particles; Clay = percent clay particles; HR = soil hydric rating; VS = vitellogenic status; SO = soil order.

Model	Parameter	β(SE)	95% CI	p
Sand	Intercept	1.342(4.808)	-8.697, 11.161	0.78
	Sand	-2.405(5.674)	-14.018, 9.345	0.672
HR	Intercept	-0.811(0.60)	-2.117, 0.311	0.177
	HR	0.182(0.744)	-1.254, 1.715	0.806
Clay	Intercept	-0.843(0.746)	-2.409, 0.576	0.258
	Clay	3.569(15.504)	-27.354, 34.677	0.818
VS	Intercept	$-6.93e^{-1}(6.12e^{-1})$	-2.014, 0.462	0.258
	VS	$2.49e^{-16}(7.5e^{-1})$	-1.454, 1.541	1.00
SO	Intercept	-0.693(0.612)	-2.014, 0.462	0.258
	SO (Inceptisols)	-0.811(0.993)	-2.975, 1.079	0.414
	SO (Ultisols)	0.539(0.827)	-1.071, 2.226	0.515

3.2.3. Thermal Profile

I collected iButton temperature data for 12 (vitellogenic = 8; non-reproductive = 4) used adult female EDB overwinter sites and 24 paired random sites. I removed all 10 cm soil temperature variables because these were highly correlated with 30 cm soil temperature variables. I removed nine additional thermal covariates due to multicollinearity (AMIN, 30MAX, SDT_eSD, SDT_eMEAN, SDT_eMIN, SET_eSD, SET_eMEAN, SET_eMIN, 30MIN; Table 3). The resulting global model contained nine thermal covariates (AMEAN, ASD, 30MEAN, 30SD, SDT_eMAX, SET_eMAX, VS, BCI, SVL; Table 3). The global model was over dispersed but provided adequate model fit (c-hat=1.21, Kolmogorov-Smirnov test *p*=0.30, dispersion test

p=0.88, outlier test p=1). Two of 28 candidate models received support (Table 14). The top performing model included additive effects of maximum sun exposed T_e (SET$_e$MAX) and mean 30 cm soil temperature (30MEAN) as predictors. Output from the top model indicated a significant (χ^2 = 16.922, p < 0.001) positive effect of maximum sun exposed T_e on overwintering site selection (β= 0.075, SE= ± 0.019, Z=3.968, p < 0.001; Table 15, Figure 15C). Output from the top model indicated a significant (χ^2 = 6.568, p < 0.05) negative effect of mean 30 cm soil temperature on overwintering site selection (β= -0.111, SE= ± 0.044, Z= -2.497, p <0.05; Table 15, Figure 15D), contrasting with the positive relationship observed with maximum sun exposed T_e. Model averaging of supported models indicated a negative effect of soil temperature (β= -0.115, 95% CI= -0.205, -0.026) and positive effect of maximum sun exposed T_e (β= 0.074, 95% CI= 0.035, 0.112) on overwintering site selection (Table 15). Odds ratios indicated that for every unit increase in the difference of mean 30 cm soil temperature from regional ambient mean temperature, female EDBs were 1.12 times less likely to use an overwintering site (mean difference of used site 30 cm and regional temperature means = -0.39 ± 0.36 SE °C; 95% inverse OR CI=1.02, 1.23). For every unit increase in the difference of maximum sun exposed T_e at winter microsites from regional ambient maximum temperature, female EDBs were 1.08 times more likely to use an overwinter site (mean difference of used site sun exposed T_e and regional temperature maximums = 7.15 ± 0.95 SE °C; 95% OR CI=1.036, 1.12). Model averaging indicated both mean 30 cm soil temperature and maximum sun exposed T_e each accounted for 100% of model weights.

Table 14.

Candidate thermal profile logistic regression models ranked in order of support using QAIC$_c$ model selection to examine adult female eastern diamondback rattlesnake (*Crotalus adamanteus*) overwinter site selection in South Carolina, USA 2023-2024. K = number of parameters, QAIC$_c$ = quasi-Akaike's information criterion for small sample size, ΔQAIC$_c$ = the difference between the model with the lowest QAIC$_c$ score and the current model, w = model weights, Log Likelihood = log of model likelihood. SET$_e$MAX = maximum sun exposed operative environmental temperature; 30MEAN = mean 30 cm soil temperature; SDT$_e$MAX = maximum shaded operative environmental temperature; ASD = standard deviation of ambient air temperature; 30SD = standard deviation of 30 cm soil temperature; SVL = snake snout-to-vent length; BCI = snake body condition index; AMEAN = mean ambient air temperature; VS = vitellogenic state.

Rank	Model	K	QAIC$_c$	ΔQAIC$_c$	w	Log Likelihood
1	30MEAN + SET$_e$MAX[a]	3	266.8	0.00	0.371	-156.896
2	30MEAN + SET$_e$MAX + SDT$_e$MAX[a]	4	268.0	1.19	0.205	-156.357
3	30MEAN + SET$_e$MAX + 30MEAN*SET$_e$MAX[a]	4	268.8	2.06	0.132	-156.887
4	SET$_e$MAX	2	270.1	3.35	0.070	-160.181
5	30MEAN + AMEAN	3	271.7	4.91	0.032	-159.875
6	BCI + SET$_e$MAX	3	271.8	5.06	0.030	-159.966
7	SVL + SET$_e$MAX	3	271.9	5.15	0.028	-160.02

8	AMEAN + SET$_e$MAX	3	272.0	5.22	0.027	-160.066
9	SDT$_e$MAX + SET$_e$MAX	3	272.1	5.31	0.026	-160.12
10	VS + SET$_e$MAX	3	272.2	5.41	0.025	-160.177
11	SVL + AMEAN + 30MEAN	4	273.7	6.89	0.012	-159.814
12	BCI + AMEAN + 30MEAN	4	273.7	6.94	0.012	-159.844
13	VS + AMEAN + 30MEAN	4	273.7	6.98	0.011	-159.871
14	SDT$_e$MAX + SET$_e$MAX + AMEAN	4	274.0	7.27	0.010	-160.048
15	AMEAN	2	277.0	10.21	0.002	-164.344
16	SDT$_e$MAX	2	277.9	11.11	0.001	-164.89
17	30MEAN	2	278.6	11.88	0.001	-165.357
18	AMEAN + ASD	3	279.0	12.24	0.001	-164.324
19	ASD	2	279.5	12.74	0.001	-165.876
20	30SD	2	279.8	13.00	0.001	-166.035
21	BCI	2	279.9	13.16	0.001	-166.13
22	SVL	2	279.9	13.16	0.001	-166.13
23	VS	2	279.9	13.16	0.001	-166.13
24	30MEAN + 30SD	3	280.6	13.81	0.00	-165.273

25	30SD + ASD	3	281.4	14.68	0.00	-165.8
26	BC + SVL	3	282.0	15.22	0.00	-166.13
27	VS + SVL	3	282.0	15.22	0.00	-166.13
28	VS + SVL + VS*SVL	4	284.1	17.30	0.00	-166.13

[a] =Model was used for inference ($\Delta QAIC_c \leq 2.0$)

Table 15.

Coefficients from supported (i.e., ΔQAICc ≤ 2.0) logistic regression thermal profile models examining adult female eastern

diamondback rattlesnake (*Crotalus adamanteus*) overwintering site selection in South Carolina, USA 2023-2024. SET_eMAX =

maximum sun exposed operative environmental temperature; 30MEAN = mean 30 cm soil temperature; SDT_eMAX = maximum

shaded operative environmental temperature.

Model	Parameter	β(SE)	95% CI	p
30MEAN + SET$_e$MAX	Intercept	-1.104(0.175)	-1.458, -0.771	<0.001
	30MEAN	-0.111(0.044)	-0.2, -0.026	<0.05
	SET$_e$MAX	0.075(0.019)	0.039, 0.114	<0.001
30MEAN + SET$_e$MAX + SDT$_e$MAX	Intercept	-0.904(0.258)	-1.42, -0.404	<0.001
	30MEAN	-0.123(0.046)	-0.217, -0.035	<0.01
	SET$_e$MAX	0.070(0.02)	0.032, 0.11	<0.001
	SDT$_e$MAX	0.057(0.055)	-0.051, 0.168	0.30
30MEAN + SET$_e$MAX + 30MEAN*SET$_e$MAX	Intercept	-1.10(0.176)	-1.458, -0.765	<0.001
	30MEAN	-0.107(0.052)	-0.212, -0.007	<0.05
	SET$_e$MAX	0.076(0.02)	0.038, 0.116	<0.001
	30MEAN*SET$_e$MAX	-0.0007(0.005)	-0.011, 0.01	0.89

4. Discussion

Habitat models indicated female EDBs require access to a wide range of thermal microhabitat conditions for overwintering and gestation. Similarly, wide thermal gradients may be important for EDBs in providing thermoregulatory opportunities at the macrohabitat scale (Hoss et al., 2010), and gravid female ectotherms may take advantage of thermal heterogeneity in retreat sites to thermoregulate (Dial, 1978; Huey et al., 1989; Kearney and Predavec, 2000; Pike et al., 2012). Thermoregulation is one of the most important drivers of habitat use for terrestrial squamates (Grant, 1990; Reinert, 1993), and thermal habitat conditions appear to be important for female EDB microhabitat selection, regardless of their reproductive state. Habitat models detected significant thermal influences on overwintering- and gestation-site selection, while vegetation and soil covariates received limited or no support. I failed to detect soil structure effects on microhabitat selection regardless of season, and vegetation relationships (CWDCT) were only apparent for overwintering site selection.

The relationship between surface and subsurface temperatures on EDB microhabitat selection differed seasonally, indicating temporal shifts in thermal strategies while maximizing thermoregulatory opportunities. During summer, gestating EDBs selected microsites with cool surface temperatures and warm soil temperatures, while reproductive and non-reproductive females selected overwintering sites with warmer surface temperatures and cooler soil temperatures. These relationships suggest female EDBs employ active thermoregulation, as opposed to thermoconformity (i.e., passive thermophilic behavior; Lutterschmidt, 1991), to maintain optimum body temperatures. Although snakes exhibit some degree of thermoconformity with their environments (e.g., thermoregulatory ability of non-gravid individuals constrained by their physical environment: Lutterschmidt, 1991; Peterson, 1987),

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thermoconformity is disadvantageous in thermally challenging environments that decrease performance (e.g., digestion, mate searching, predator avoidance; Row and Blouin-Demers, 2006). For instance, eastern milksnakes (*Lampropeltis triangulum*) thermoregulate more actively when thermal quality is lower (Row and Blouin-Demers, 2006). In the southeastern Coastal Plain, I would likewise expect low thermal quality during summer when surface temperatures are high, while winter thermal quality may be low at microsites that are either too cool, or too warm amid mild winter temperatures here.

Overwintering is considered a period of inactivity for ectotherms, when metabolic rates slow in response to decreasing photoperiod and temperature (Gregory, 1982; McNab, 2002; Zuffi et al., 1999). Female EDBs do not appear to exhibit inactivity during the winter season, however. Overwintering sites with cool subsurface and warm surface temperatures likely provide a range of temperatures that allow EDBs to fine-tune and accommodate active winter thermoregulation. As such, female EDBs appear to be Panglossian in their selection of overwintering sites, i.e., preferring structures that allow movements between thermal extremes to optimize metabolic needs (Huey et al., 2020). Hibernacula with insufficient access to warm temperatures place snakes at higher winter mortality risk (Charland, 1989; Gregory, 1982; MacCartney, 1985; Shine and Mason, 2004), and access to warm winter temperatures should allow snakes to boost metabolic activity to prepare for emergence and feeding. Contrary to my expectations, warm surface temperatures were important for female overwinter sites. In Florida, EDBs occasionally feed midwinter, where warm surface temperatures at overwintering sites allow snakes to raise body temperatures to facilitate digestion (Means, 2017). Conversely, overwintering sites that lack access to cool temperatures might strain the ability to maintain low

body temperatures necessary for slowing metabolism and conserving energy at emergence to compensate for the inability to gain energy from food (Huey et al., 2020).

Voluntary hypothermy, i.e., selection of cooler body temperatures, is well documented in squamates (Scott, 1978; Spellerberg, 1972) and can be influenced by reproductive activity. For example, preovulatory lizards exhibit lower body temperatures than post-parturient females, possibly due to an inhibitory estrogen effect during vitellogenesis (Garrick, 1974). Because I expected mild winter temperatures typical of the southeastern Coastal Plain would be conducive for the continuance of vitellogenesis without interruption from winter diapause (Aldridge and Duvall, 2002), I expected reproductive, pre-ovulatory female EDBs would be more likely to select cooler winter microhabitats to conserve energy for emergence when high metabolic activity and large body reserves are necessary for completing vitellogenesis (Alcobendas et al., 1992; Bonnet et al., 1994; Bonnet et al., 2011; Bonnet and Naulleau, 1995; Saint Girons, 1957). Contrary to my expectations, I failed to detect an effect of reproductive condition on overwintering site selection. Low winter metabolism may negate potential benefits of surface-level temperature variations, particularly at higher latitudes (Cobb and Peterson, 2008). For example, great basin rattlesnakes in Idaho maintain similar body temperatures during winter, regardless of body size and reproductive state (Cobb and Peterson, 2008). Eastern diamondbacks in Florida apparently overwinter at about 12.8°C (Means, 2017), although the winter body temperatures of EDBs toward the northern portion of their range might be slightly lower. Thus, similar to the reasoning presented for great basin rattlesnakes, it is possible the depressed metabolic state of EDBs at my study site in South Carolina associated with winter temperatures negates any advantage vitellogenic females might gain from selecting slightly cooler microhabitats over non-reproductive individuals. Relationships between reproduction and

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overwintering ecology warrant further investigation in EDBs, as overwintering in the

southeastern Coastal Plain may require that females exhibit greater control over balancing

reproduction, maintenance, and survival. Additionally, given the long birthing intervals of

crotalids, female EDBs are ideal for studying variables affecting reproduction (e.g.,

vitellogenesis) since in any given year, both reproductive and non-reproductive females should

be available for comparison (Ladyman et al., 2003).

Similar to overwintering sites, EDB gestation sites provided a wide range of temperature

conditions that likely facilitated thermoregulation. Gestation sites were characterized by cool

surface temperatures and warm subsurface temperatures offering females greater control of their

metabolic needs during the hottest part of the active season. Observed relationships between

temperature (surface and subsurface) and gestation site selection challenge the notion that gravid

females solely select warmer gestation sites to raise body temperatures (e.g., Charland and

Gregory, 1990; Gardner-Santana and Beaupre, 2009; Gier et al., 1989; Graves and Duvall, 1993;

Reinert, 1984). Kain (1995) suggested EDB gestation sites provided basking opportunities

necessary for maintaining high body temperatures to facilitate predator avoidance when

locomotor capacity is compromised (Brodie, 1989; Cooper et al., 1990; Seigel and Ford, 1987;

Shine, 1980). Although gestation site selection was positively associated with soil temperature in

my study, it is unlikely that gestating females need diurnal access to warm soil temperatures to

raise their body temperature. Rather, warm subsurface temperatures more likely serve as a buffer

from cooler nocturnal temperatures (Kain, 1995), while cool surface temperatures reduce risks of

diurnal heat stress. If surface temperatures quickly cool off as night approaches, gravid females

might take advantage of subsurface habitat with higher thermal inertia, i.e., if heat is retained for

longer periods underground through soil insulation. However, amid intense summer heat, EDB

thermoregulatory behavior likely prioritizes keeping cool (Means, 2017), which I expect is of highest concern diurnally. In Florida, where summer has been deemed the "most thermally dangerous" time within EDB life-history, EDBs avoid high summer body temperatures above 35°C (Means, 2017). Gravid EDBs, specifically, have been observed using subterranean burrows more frequently as gestation progresses, seldom moving a few meters from such structures (Kain, 1995). Similarly, gravid eastern copperheads (*Agkistrodon contortrix*) in the southern US reduce basking during summer gestation to avoid high diurnal temperatures in Tennessee (Sanders and Jacob, 1981).

Cool gestational microhabitats facilitate maintenance of lower body temperature, the benefits of which include reduced metabolic costs of gestation, maximized embryonic development, and reduced water stress. Gestating females are vulnerable to hydric stress due to high energetic water demands (Dupoué et al., 2015, 2016, 2018; Lorioux et al., 2013; Rozen-Rechels et al., 2020; Shine, 1979), and ectotherms may undergo thermal depression (i.e., lowered thermal preference) under harsh hot or dry conditions to reduce dehydration risk (Ladyman and Bradshaw, 2003). Gravid lizards are more susceptible to overheating and more tolerant of cold temperatures as compared to males and non-reproductive, post-parturient females (Beuchat, 1986; Garrick, 1974; Le Galliard et al., 2003). Further, gravid lizards are more likely than males to select cooler microhabitats and reduce basking to lower body temperatures, reducing water loss during pregnancy (Rozen-Rechels et al., 2020). Females also alter water balance to protect embryos from hydric stress (Dupoué et al., 2016, 2018; Shine, 1979), and embryonic water demands are more pronounced in late gestation when development may be optimized by cooler temperatures (Rozen-Rechels et al., 2020; aspic viper: Lourdais et al., 2015). Although I did not directly measure water balance of adult females, gravid EDB's selection of cooler surface

temperatures supports the avoidance of high diurnal summer gestation temperatures that would likely exacerbate water loss through increased metabolic rate. Soil moisture properties were not indicative of female EDB site use contrary to my expectations, although this may be unsurprising considering EDB's use of multiple habitat types within open canopy systems.

Gravid squamates may intentionally lower body temperature to ameliorate high oxygen demands indicative of increased metabolism during gestation (Virens and Cree, 2019). Increased oxygen demands of pregnant squamates is due to the physical burden of embryos, as well as the combined oxygen demand of developing embryonic tissues with that of the mother's tissues (Virens and Cree, 2019). In skinks, pregnant females experience a threefold increase in energetic costs of breathing due to lung compression from pregnancy (Munns, 2013; Munns and Daniels, 2007). Anoxic effects are also evidenced by increased heart rate, hyperventilation, and reduced maternal blood-oxygen affinity of pregnant female squamates (Birchard et al., 1984; Holland et al., 1990; Ingermann et al., 1991; Munns, 2013; Ragsdale and Ingermann, 1991). Fetal oxygen needs increase with development (DeMarco, 1993), and high temperatures may elevate metabolic rates of embryos beyond the optimal rate and exhibit negative costs (Monasterio et al., 2013).

High body temperatures have numerous effects on gestating females and their developing embryos, including metabolism, development, and survival. Thermal conditions of EDB gestation sites should allow females to alter body temperatures to optimize embryonic development. The positive association between body temperatures and standard metabolic rate has significant implications at high temperatures, increasing energy demands that can only be offset by foraging (Dorcas et al., 2004), which is suspended during gestation. High metabolic rates can become critical during gestation, leading to embryonic mortality and accelerated energy

loss (Ladyman et al., 2003). Extreme gestation temperatures (cold or hot), especially during early

development (Osgood, 1978; Lorioux et al., 2013), exhibit direct effects on developmental

instability of viviparous squamates, e.g., scale pattern malformities and split scales (Lorioux et

al., 2013), altered number of vertebrae (Fox et al., 1961; Osgood, 1978), and reduced offspring

survival (Burger et al., 1987; Fox et al., 1961; Lourdais et al., 2004; Monasterio et al., 2013).

Gestation length can be affected two-fold with gestation temperature differences as small as 2°C

(Beuchat, 1988), and altered gestation length as a function of temperature can exhibit a negative

relationship with neonate body size, mass, body condition, and size-specific growth rate

(Monasterio et al., 2013; Qualls and Andrews, 1999). As such, gestation site selection should be

considered a consequential maternal effect on reptile fitness. Microhabitats with narrow thermal

ranges likely limit thermoregulatory control, challenging a female's ability to maintain optimum

gestational temperatures. Gestating EDBs likely selected microsites with broad thermal gradients

to maximize thermoregulatory opportunities and avoid deleterious developmental and metabolic

effects of extreme high temperatures.

Optimal temperatures for embryonic development can conflict with the mother's thermal

needs, creating an inter-generational conflict between mother and offspring (Schwarzkopf and

Andrews, 2012). Consequences of altering body temperature during pregnancy include

compromised metabolic rates and water capacity, but data on physiological effects of gestation

temperature on adult females are scant for ectotherms. Nonetheless, live-bearing reptiles carry

significant reproductive costs that are likely exacerbated when gestating females must accept

thermal conditions that deviate from their own optimal body temperature for an extended time

(Beuchat 1986; Beuchat and Ellner 1987; Pike et al., 2012; Webb et al., 2006). Embryonic

development is lengthy and usually constrained by a narrow thermal tolerance that is more

restrictive than the mother's (Andrews et al., 1997; Bursell, 1974; Deeming, 2004; Hanna and Cobb, 2006; Li and Jackson 1996; Shine 1987). For example, female children's pythons (*Antaresia childreni*) suffer reduced immune function when compromising their preferred body temperature to benefit embryonic development (Lourdais et al., 2008). Thermal intergenerational conflicts, if relevant to EDBs, should affect EDB gestation site selection. Thermal attributes of EDB gestation sites appear to be important drivers of selection, but, beyond the scope of this study, it remains unclear if gravid females select gestation sites based on thermal attributes most suited to their own preferred optimum body temperature, that of their developing embryos, or some combination thereof.

Microhabitat thermal conditions logically depend on vegetation structure, making it difficult to decipher the relative importance of structural and thermal metrics at the microhabitat scale. By using separate analyses of microhabitat environmental factors, I detected biologically relevant, but a limited number of vegetation predictors of overwintering site selection. Female EDBs selected winter microhabitats with higher counts of coarse woody debris that likely provided protective cover while providing shade. Vegetation structure is considered an important factor for habitat selection in reptiles (e.g., turtles: Meseth and Sexton, 1963; Sexton, 1958; lizards: Heatwole, 1966; Kiester et al., 1975), and hibernacula can be associated with greater vegetative cover. For example, northern pine snakes (*Pituophis melanoleucus*) select hibernacula with more vegetative and leaf cover over the burrow entrance (Burger et al., 1988). However, the EDB's association with open-canopied habitats at multiple spatial scales may limit reliance on dense cover. Open habitats likely support warm surface temperatures, which are favored by female EDBs in the winter. Warm surface temperatures should allow snakes to reach high body temperatures quickly, increasing locomotor capacity and reducing the need for dense, protective

cover. Further, warm southeastern summer temperatures likely prioritize thermoregulatory behaviors in gravid EDBs to avoid extreme heat over other needs (Blouin-Demers and Weatherhead, 2001). This is evident for other crotalids as well; vegetative cover is not a consistent predictor of overwintering sites for northern pacific rattlesnakes (*C. oreganus;* Gienger and Beck, 2011).

Studies on habitat selection and its thermal associations are crucial for gaining insight into whole-organism energy dynamics for EDBs (Dorcas et al., 2004). Thermoregulatory behaviors are closely tied to local conditions, with local conditions largely influencing reproductive activity and life-history adaptations (Bronikowski and Arnold 1999; Shine 2005). Thus, identification of habitats essential for thermoregulation is important for understanding EDB life-history traits and is needed for the species' conservation (Dorcas et al., 2004). Reptiles are vulnerable to habitat loss and fragmentation (Gibbons et al., 2000), with reptiles in the southeastern US especially affected due to the rapid loss of sensitive native habitats like the longleaf pine ecosystem (Noss, 1989). The EDB's range is shrinking due to agriculture, silviculture, urbanization, and wide-scale post-industrial fire exclusion leading to contemporary landscapes in alternate steady states, with this being rampant at the species' northern fringe in North Carolina (Means, 2017). Furthermore, the removal of stumps from occupied areas is thought to negatively affect local EDB populations, and the types and locations of subterranean refugia is a possible limiting factor for the species (Means, 2017). Stump removal may be especially harmful at the northern portion of the EDBs range studied herein considering the lack of gopher tortoise or armadillo burrows that are important refugia in core populations (Murphy et al., 2021).

Habitats that support reproduction are critical for maintaining viable rattlesnake populations. Adult female survival is critical for timber rattlesnake population stability (Brown et al., 2007; Martin et al., 2008), and management efforts aimed at adult EDBs are predicted to have the greatest impact on population growth (Stohlgren, 2013). Access to high-quality gestation habitats should enhance fitness of female snakes (Burger et al., 1988), and knowledge of intraspecific variation in habitat use by females is vital from a basic science perspective, but also useful for informing detection surveys, habitat assessments, and management for snakes (Christensen et al., 2022). The quality and availability of adult female gestation microhabitats should affect recruitment, reflecting the sensitivity of embryogenesis to environmental temperature (Lourdais et al., 2004). Thus, adult female EDBs would benefit from reduced disturbances, e.g., large woody debris or other potential gestation refugia removal (Fill et al., 2015).

Habitat selection studies that include thermal metrics are vital toward increasing our understanding of reptiles' vulnerability to climate change. This study illustrated the importance of fine-scale thermal heterogeneity at critical life-history phases for female EDBs. Climate change is predicted to increase global temperatures (Intergovernmental Panel on Climate Change, 2013), with average annual temperature changes projected to increase by 2.2-4.4 °C across the southeastern United States throughout the 21st century and exceeding 1.5 °C within the next six years (Marvel et al., 2023). As the globe progressively heats, changes to species and population distributions and demographics are predicted to primarily be driven by physiological tolerance limits, i.e., environmental temperatures exceeding animals' upper thermal maxima (Deutsch et al., 2008; Hoffmann et al., 2013; Sunday et al., 2011; Williams et al., 2008). Ectotherms are especially vulnerable to projected climate shifts due to their reliance on ambient

temperature for essential life-history processes like reproduction (Deutsch et al., 2008; Kingsolver et al., 2013; Paaijmans et al., 2013), and widespread temperature shifts can interfere with females' ability to manipulate offspring phenotype and fitness via maternal behaviors (Huang and Pike, 2011). Shifting climates can disproportionately affect northern peripheral ectotherm populations largely due to constraints on reproduction with sub-optimal range edge thermal regimes (Martin, 2002; Peterson et al., 1993). For viviparous mothers particularly, it is expected that the ability to buffer developing embryos against adverse ambient temperatures should be more important at the periphery of a species' range (Lourdais et al., 2004). Although the mechanisms of temperature as a barrier to species distributions are poorly understood for reptiles (Harvey and Weatherhead, 2010), northern EDB populations might be more vulnerable due to environmental limits on reproduction, which could be exacerbated by limited availability of suitable thermal microhabitats (Means, 2017).

Habitat selection relative to thermoregulatory needs of reproductive female EDBs should be examined at larger scales. Macrohabitat selection studies are needed to compliment knowledge linking habitats to thermoregulatory needs at the microhabitat scale (Johnson, 1980; Reinert, 1993), and landscape composition may have a greater impact on EDB populations at larger scales (Stohlgren, 2013). Studies evaluating how true body temperatures influence the relative physiological and ecological performance of female EDBs are needed (Huey, 1991), as different species have unique thermal optima and sensitivities that may drive behavior (Bennett, 1980). Relevant thermal indices of performance and organismal fitness specific to female EDBs and their embryos from more laboratory and field investigations involving direct body temperature measurements are needed to truly assess the thermal quality of their habitats (Hailey and Davies 1988; Huey 1982, 1983; Huey and Stevenson 1979) and understand how adult

females interact with their environment to address potential maternal-offspring conflicts in thermal requirements (Garrick, 1974). Further, quantitative data on basking frequencies of female EDBs while overwintering and gestating are needed to give context surrounding the energetics of this vital population demographic.

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Appendix A: IRB Approval Letter

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Office of Research Integrity

October 16, 2024

Matthew Gacheny
Department of Biological Sciences, Rm 352
Marshall University

Dear Matthew,

This letter is in response to the submitted thesis abstract entitled *"Microhabitat Selection of Adult Female Eastern Diamondback Rattlesnakes (Crotalus adamanteus) in the Carolinas."* After assessing the abstract, it has been deemed not to be human subject research and therefore exempt from oversight by the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocol #776. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract, you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Brett M. Williams, CIC
Director
Office of Research Integrity

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