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Mortality Risk Associated With Urban Land Use for Adult Eastern Diamondback Rattlesnakes (Crotalus adamanteus)

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MORTALITY RISK ASSOCIATED WITH URBAN LAND USE FOR ADULT EASTERN DIAMONDBACK RATTLESNAKES (*CROTALUS ADAMANTEUS*)

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Master of Science
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by
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Approved by
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APPROVAL OF THESIS

We, the faculty supervising the work of Mya Wiles, affirm that the thesis, *Mortality Risk Associated with Urban Land Use for Adult Eastern Diamondback Rattlesnakes (Crotalus adamanteus)*, meets the high academic standards for original scholarship and creative work established by the Department of Biological Sciences and the College of Science. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

Jayme Waldron 14 November 2022

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ABSTRACT

Snakes have been a traditionally under-represented organism in urban ecology, but they face similar, if not greater challenges in the face of growing urban sprawl. Eastern diamondback rattlesnakes (*Crotalus adamanteus*, EDBs) are under consideration for listing under the Endangered Species Act due to population declines resulting from historical human persecution and habitat loss. This study used radio-telemetry data from a long-term monitoring project of adult EDBs on a developed sea island in South Carolina, USA. I reclassified a National Land Cover Dataset to reflect relative mortality risk for snakes attempting to move through the landscape. High-risk cover types included all developed areas such as roads, parking lots, buildings, and golf courses. Low-risk cover types included forested/naturally vegetated areas where human activity was typically low. To assess when and why snakes may choose to cross high-risk areas, I examined the frequency and probability of high-risk crossings as a function of demographic, spatial, and temporal predictors in mixed effect models that included individuals as a random effect. Reduced activity and movement associated with cool winter temperatures reduced the number of overall crossings, but there was no detectable difference between sexes, even within seasons. Larger home ranges and those with a proportionately greater amount of high-risk cover within the home range were positively correlated with number of high-risk crossings. Considerations for conservation in urban landscapes include assessing the potential impact of urban development on neighboring EDB populations, as well as for managing wildlife-human conflicts.

INTRODUCTION

Urban wildlife ecology has become an increasingly important field, highlighting the importance of ecological research within the novel anthropogenic landscape (Collins et al. 2021). Despite the increasing number of publications within this field, knowledge gaps remain for understudied taxa like herpetofauna (Collins et al. 2021). Most research for urban reptiles, for example, have focused on management of nuisance species, particularly snakes (Perry et al. 2020). Snakes have a long history of persecution by humans due to perceived risk from venomous species (e.g., Alves et al. 2012) and current public views regarding snakes is complex, but still generally negative (Bateman et al. 2021).

Navigating the urban landscape is particularly dangerous for snakes (e.g., Row et al. 2007; Brehme et al. 2018; Winton et al. 2020). A large volume of literature indicates that road strikes are a significant source of snake mortality, and that a complex array of variables influence the likelihood of snake road crossings. For example, snake behavior near or along roads is influenced by life history strategy, sex, and body size (Bonnet et al. 1999; Andrews and Gibbons 2005, 2008; Jochimsen et al. 2014; Mccardle and Fontenot 2016; Rincon-Aranguri et al. 2019), and the likelihood of snakes encountering roadways is influenced by composition of land cover of surrounding landscape (Roe et al. 2006; Meek 2015).

Snake road mortality observations often vary by season, but timing and patterns vary by species and region (Bonnet et al. 1999; Row et al. 2007; Jochimsen et al. 2014; Meek 2015). Across temperate climate regions where snakes reduce surface activity during colder months, crossings and mortality observations are more common during the active season (Bonnet et al. 1999; Row et al. 2007; Meek 2015). Temporal peaks in roadkill observations are often attributed to long-distance movements or dispersal events associated with reproductive status or age-class (Bonnet et al. 1999; Andrews and Gibbons 2005, 2008; Mccardle and Fontenot 2016), but not all studies detected significant effects of season and interactions with age or sex (Roe et al. 2006; Row et al. 2007). Surface activity levels of snakes in temperate climates are particularly pronounced between the active and inactive seasons, but patterns of activity within the active season likely vary by species (Bonnet et al. 1999; Row et al. 2007; Jochimsen et al. 2014; Mccardle and Fontenot 2016).

Some snake species are more likely to cross through high-risk areas (e.g., roads), than others based on general life history strategies such as foraging mode or mobility. Snakes with active foraging modes tend to be observed more frequently during roadkill surveys than fossorial or ambush foraging species (Roe et al. 2006; Row et al. 2007; Jochimsen et al. 2014; Rincon-Aranguri et al. 2019). Differences in observations based on foraging mode are likely reflective of the difference in home-range size between active foragers and ambush foragers (Secor and Nagy 1994; Todd and Nowakowski 2021), as well as physical limitations to mobility in long, slender species versus long, heavy species and short, slender species (Andrews and Gibbons 2005). Slower species may be more vulnerable to road mortality than faster species because slow-moving species spend more time in roadway, increasing the chances of encountering a vehicle and being hit (Andrews and Gibbons 2005). Additionally, ambush foragers tend to immobilize while vehicles pass and for minutes afterward, further increasing time spent in roads (Andrews and Gibbons 2005).

Within a species, differences in male and female movement ecology likely influences risky crossing frequency but has only been assessed for roadways. Male mate-searching behavior is often associated with longer relatively linear movements (e.g., Duvall and Schuett 1997), potentially placing males at increased risk of road mortality; however, the relationship between reproductive condition and road mortality is inconclusive based on contradictory observations in the literature (Bonnet et al. 1999; Row et al. 2007; Andrews and Gibbons 2008; Jochimsen et al. 2014; Mccardle and Fontenot 2016). For example, radio-telemetered male and female black rat snakes did not differ significantly in their propensity to cross roads, regardless of reproductive condition (Row et al. 2007). Roadkill surveys detected a significant difference in road mortality between males and females where a greater number of males were found dead on the roadways (Andrews and Gibbons 2008; Jochimsen et al. 2014; Mccardle and Fontenot 2016). Surveys of roadkill alone however are unable to detect crossing attempts where individuals survived a crossing, assuming that the rate of mortality is equal between males and females while crossing.

Body size (snout-to-vent length or mass) may be correlated with intraspecific differences in road crossing observations, but this trend is not clear as conflicting evidence suggests a positive association for some species (Andrews and Gibbons 2008; Mccardle and Fontenot 2016) and a negative association for others (Andrews and Gibbons 2005). Body size is associated with relative age-class and can be sexually dimorphic in many species (e.g., Gibbons 1972; Shine 1991; Taylor and Denardo 2005; Rothe-Groleau et al. 2018). Observational differences in body size of road-killed snakes may be more associated with differences in behaviors typical of different age or reproductive classes (Bonnet et al. 1999). The association of body-size with sex and age make it a useful predictor in spatial ecology models where age or reproductive status are unknown or difficult to ascertain.

Roadways are not the only risky land cover type for snakes attempting to navigate the landscape. The effects of habitat and landscape characteristics on snake road crossings has been mixed, likely varying by species, region, and the spatial scale being considered. Suitable habitat surrounding roads was positively associated with crossings of some species (Jochimsen et al. 2014; Meek 2015; Wagner et al. 2021), but other studies did not detect any association of crossings and surrounding habitats (Roe et al. 2006; Row et al. 2007; Rincon-Aranguri et al. 2019). Other risky land use types include urban and agricultural areas, where increased mortality has been documented in multiple species (e.g., Whitaker and Shine 2000; Kapfer et al. 2010; Pomara et al. 2014). Most snakes tend to avoid urban and agricultural areas or must adopt different behaviors in order to minimize mortality risk in fragmented landscapes (e.g., Kapfer et al. 2010; Hyslop et al. 2013; Bauder et al. 2018; Maddalena 2018; Carrasco-Harris 2020), but with increasing fragmentation of the landscape, individuals will increasingly need to cross high-risk areas. This study will define a risk landscape that incorporates all potentially risky land use types, rather than roads or agriculture alone.

Snake habitat use is scale dependent (Bauder et al. 2018; Maddalena 2018; Bauder et al. 2021) and tends to be associated with structural characteristics (e.g., canopy closure and ground cover density) rather than floral species composition (Garden et al. 2007). Therefore, the habitat and land use categories being considered need to be relevant for the species being studied and at the spatial scale at which is likely to influence crossings. In particular, the relative amount of certain land use types within an individual's home range may alter movement (i.e. home-range size) as well as the frequency of road crossings (Kapfer et al. 2010; Breininger et al. 2011; Hyslop et al. 2013; Jochimsen et al. 2014; Meek 2015; Carrasco-Harris 2020).

The eastern diamondback rattlesnake (*Crotalus adamanteus*, EDB; Fig. 1) is the largest pit-viper in North America and has experienced population declines across their range (see Waldron et al. 2008; Means 2009; Waldron et al. 2013a). The species is particularly vulnerable to habitat loss as EDBs are remnants of southeastern longleaf pine (*Pinus palustris*) savannas and woodlands (Waldron et al. 2008), which have declined an estimated 97% due to fire exclusion, industrial forestry, and fragmentation (Frost 1993). As a result, the species is being considered for listing under the Endangered Species Act (USFWS 2012). Sources of EDB mortality include historic and ongoing human persecution, particularly exemplified by local rattlesnake round-ups (Means 2009). A common result of human-snake encounters is the subsequent death of the snake, but is particularly true for venomous species who have been vilified by local communities (Alves et al. 2012; Teixeira et al. 2015; Liordos et al. 2018; Batemen et al. 2021). Eastern diamondback rattlesnakes exhibit numerous traits that make them particularly vulnerable to mortality within developed landscapes where conflict with humans represents a direct source of mortality in addition to other indirect pressures associated with habitat loss and climate change. Further, EDBs exhibit a slow life history where females reach reproductive maturity after seven years, only reproducing every two to three years thereafter, low neonate survival, and high adult survival (Waldron et al. 2013a), increasing the potential effect of additional adult mortality on population viability. EDBs are also ambush foragers and tend to move slowly as a result of their heavy body type, thus likely prolonging time spent crossing roads and other open human-used spaces. Intentional strikes of snakes in roadways has been previously documented (Langley et al. 1989; Ashley et al. 2007; Jochimsen et al. 2014), and EDBs make for large, slow-moving targets that some drivers may hit intentionally. This indicates a need for careful management of EDBs where they closely occur with humans and highlights the importance of public education regarding snakes in order to reduce negative encounters. Eastern diamondback rattlesnakes thus make an ideal study species to investigate the spatial ecology of urban snakes across a fragmented landscape.

Figure 1. Adult Eastern Diamondback Rattlesnake from the Study Site at the Marine Corp Recruit Depot Parris Island, South Carolina.
Image of a defensive adult male eastern diamondback rattlesnake after being released at his site of capture.

In this study, we used radio-telemetry data from a long-term EDB monitoring project on a southeastern military installation and reclassified land cover data to reflect relative mortality risk associated with anthropogenic land use to examine the effects of predictors on the frequency of high-risk crossings for adult EDBs. This long-term project provided ample data points from a large number of individuals that is uncommon for studies of snake ecology. Land use types associated with high human activity were considered "high-risk" for adult EDBs. This differs from most literature on snake ecology that has previously focused on the effects of roads on movement or survival. Expanding the analysis beyond just road crossings to include urban land use types and recreational open areas should be more informative about how EDBs perceive relative mortality risk across the landscape and how they respond while navigating across their ranges. We modeled the number of high-risk crossings observed from adult EDBs with spatial and demographic variables in order to determine which factors impact the frequency of risky crossings. We hypothesized that the number of high-risk crossings would be greatest during the active season and increase with larger activity ranges, greater proportion of high-risk area within each range, and with larger body lengths. Males were expected to have a greater number of crossings than females overall, but gravid females were expected to have the lowest number of crossings.

METHODS

Study Site

The Marine Corp Recruit Depot Parris Island (MCRDPI), South Carolina is an active military training installation and the location of the longest ongoing monitoring project of any EDB population. The island is located at the confluence of the Broad and Beaufort Rivers on the Atlantic coast and is primarily composed of salt marsh (Appendix C-AA). The northern section of the island is heavily developed with buildings, roads, and residential plots for military staff who work on the island (Appendix C-A). The northwestern side of the island has large firearm ranges that are used daily (Appendix C-A). Much of the island is used for training recruits and includes an old airfield on the eastern side, but many training areas remain forested (Appendix C-A). The southern point of the island is a public golf course surrounded by marsh and maritime forest (Appendix C-A). Roads fragment the island, but most have a speed limit ≤ 40kph in developed areas. The road that leads to the golf course has a higher speed limit of 56 kph and is primarily used by visitors and maintenance staff. Undeveloped areas are composed of forested habitat, with some areas managed for pine (*Pinus elliottii* & *P. taeda*), and others composed of species typical of maritime forests (i.e. *Quercus virginiana*, *Juniperus virginiana*, & *Sabal palmetto*). Dominant ground cover in forested habitat includes *Ilex vomitoria* and other assorted native shrubs. Salt marshes consist mostly of *Sporobolus alterniflorus* with other species colonizing forest-marsh edges, such as *Juncus roemerianus*, *Borrichia frutescens*, and *Morella cerifera*. Hummocks are interspersed throughout marsh habitats and are used by EDBs at the study site (Mausteller, 2020).

Data Collection

Adult EDBs have been monitored using mark-recapture and radio-telemetry surveys at the MCRDPI since 2008. We captured adult snakes during opportunistic visual encounter surveys and measured SVL (cm) and mass (g), and determined sex using cloacal probes. We used passive integrated transponders (PIT; Biomark) and ventral scale cautery (Winne et al. 2006) to mark individuals. We gave a subset of adults radio-transmitters either internally via surgical implantation (SI-2, 11–13 g, Holohil Systems, Carp. ON; Reinert and Cundall 1982) or externally via attachment to the rattle (Model R1640; Advanced Telemetry Systems, Isanti, MN; Jungen et al. 2019). Snakes given transmitters were released at the site of capture. Data collected from 2016 through 2020 were cleaned to remove any snakes included in a previous study assessing the effects of translocation on EDB movement ecology and survival (see Waldron and Welch 2020; Kelley et al. 2022). Remaining individual points were grouped by year and season and filtered to remove individuals with fewer than five points during any particular year and season to ensure each had sufficient points to estimate an activity range polygon. Individuals with less than five points were excluded because the adehabitatHR function ‘MCP’ requires more than 5 points to estimate the home range polygon.

We used GPS units (Trimble Juno 3B; accuracy 5m) to record coordinates of each radio relocation. We radio-located snakes at least twice weekly during the active season, i.e., from emergence in March to ingress in November, and once weekly during the dormant season from December to February. During the active season, movement frequency and distance is greater and more variable than the inactive season, so a greater sampling effort is needed to ensure a suitable spatial resolution to understand the movement patterns observed for each individual snake. Telemetry relocations occurred less frequently during the inactive season while maintaining a similar quality of spatial resolution because snakes exhibit reduced movement frequency and distance during the inactive season. Season delineations followed Waldron et al. (2013a) with the year divided into two seasons based on phenology of surface activities, which may potentially be associated with the number of high-risk crossings. The dormant season (December to February) was defined by a decline in surface activity, movement distance, foraging, and breeding behaviors. We delineated the active season to reflect foraging and breeding season activities based on observations recorded during previous monitoring efforts at the study site. The foraging season (March to July) began with egress in late February and encompassed an increase in foraging activity and dispersal movements as well as occasional observations of copulation events. Gravid females usually limit movement to a small area around birthing sites beginning as early as June (Fill et al. 2015a). A majority of copulation and courtship observations occurred during the breeding season (August to November) where breeding males engaged in mate-searching behaviors and traveled longer distances than females. Gravid females gave birth beginning in late August and into September at the study site (Fill et al. 2015a). We assumed the majority of breeding activity ended before ingress, even though EDBs occasionally breed through March (Fill et al. 2015a; Palis et al. 2012).

Spatial Data Analysis

The USGS's Multi-Resolution Land Characteristics (MRLC) Consortium generates a national map of land cover based on Landsat satellite imagery categorized using the Anderson Land Cover Classification system called the National Land Cover Dataset (NLCD). We used the

2019 version of the NLCD to classify the landscape based on anthropogenic mortality risk to adult EDBs at a 30-m pixel resolution (Appendix C-A). We mapped the landscape, categorizing land-use types as either 'low' or 'high' risk based on level of human activity (Appendix B; Appendix C-B) allowing us to assess the number of EDB movements across the risk landscape. Categories of land use identified as “high” risk included roads, buildings, parking lots, residential lawns, and recreational lawns (Appendix B). Any naturally vegetated habitat cover type was considered to have a “low” risk of human-sourced mortality (Appendix B). We used the sf (v. 1.0-2: Pebesma 2018), and raster packages (v 3.3-13; Hijmans 2020) in R (v.3.6.3, R Core Team 2020) to conduct all spatial analyses.

We cropped the NLCD to the study area and reprojected the NCLD from NAD83/Albers North American to NAD83/South Carolina (EPSG: 32133). The data were reclassified to reflect the ‘high’ and ‘low’ risk categories (Appendix B) so that high-risk cells had a value of 10 and low-risk cells had a value of 1. We downloaded a road shapefile from the South Carolina Department of Transportation’s (SCDOT) spatial data website (http://info2.scdot.org/GISMapping/Pages/ GIS.aspx) and cropped it to the extent of the study area before converting the road shapefile to a raster of the same resolution as the NLCD (30m X 30m). We reclassified the road raster to reflect cells that contained a road (7) and those that did not contain a road (0). The two reclassified rasters were added together to ensure all roadways were adequately defined in the final NLCD raster. We reclassified the joined raster to reflect the same high (10) and low (1) risk categories as defined previously (Appendix C-B).

We used the final risk raster to calculate the frequency (counts) individual snakes crossed through high-risk areas. We used the *as.ltraj* function from the adehabitatLT package (v. 0.3.25; Calenge 2006) to convert GPS points collected from 2016 through 2020 to lines by connecting the points ordered by date. Each individual snake had one line per year and season. We converted lines to sf 'MULTILINESTRING' objects before using the *extract* function from the raster package to extract an ordered list of pixels each line passed over (Appendix C-C). We summarized the list object using the *rle* function in base R to compute lengths and runs of each pixel category of the risk raster (1 for low-risk and 10 for high-risk cover categories). The number of runs through the high-risk pixels were summarized to get number of high-risk crossings per season and year for individual snakes.

We estimated activity-range areas (ha) for each individual using 100% minimum convex polygons (MCPs) calculated using the adehabitatHR package (v.0.4.19; Calenge 2006) to approximate the area occupied during a given year and season. We used 100% MCPs to estimate a conservative area over which an individual snake likely moved within during a particular year and season to reflect relative movement activity. We estimated the activity ranges over the same sampling period as the number of crossings to more clearly assess a potential relationship between the size of an individual's activity-range and the number of high-risk crossings. We used the *extract* function to sum the number of risk raster cells covered by each MCP polygon and to calculate the proportion of high-risk land cover within each activity range.

Mixed Model Analysis

The final dataset included number of high-risk crossings (count), snake ID, sex (M/GF/NG), season ("Active" or "Inactive"), year (2016 through 2020), SVL (cm; z-standardized), activity-range size (ha), and proportion of high-risk cover (Table 1). Each year began with the beginning of the active season in March so that inactive season points and crossings would not get split between two calendar years. Sex differentiated between gravid females (GF) and non-gravid females (NG) for the specific year and season the individual was tracked. We assessed collinearity of fixed effects using the performance package (v. 0.8.0; Lüdecke et al. 2021) in R where a variance inflation factor (VIF) greater than 5 was considered moderately correlated and VIF greater than 10 was considered highly correlated. We used a mixed effect Poisson regression model to predict the number of high-risk crossings using the *glmer* function from the lme4 package (v. 1.1-23; Bates et al. 2015), and accommodated an uneven sampling effort across seasons. We modeled sex, season, activity-range size, proportion of high-risk cover, and SVL as fixed effects. Snake ID was defined as a random effect to account for autocorrelation of crossings by individual snakes across seasons and years. This random variation also accounted for unmeasured factors, such as daily/weekly body condition (hunger motivation), predator encounter history, and learned responses to disturbance. Model coefficients were transformed back into the response scale as incident rate ratios (IRRs) that were interpreted as the per-unit factor by which each fixed effect corresponded with the response variable such that, "The number of high-risk crossings during the inactive season decreased by a factor of 0.15 compared to the active season". We assessed model fit using ĉ estimation to determine if overdispersion was an issue (where $\hat{c} > 1.0$), and estimation of marginal and conditional R^2 values using the trigamma method as recommended for logarithmic link functions by the MuMIn package's *r.squaredGLMM* function (v. 1.43.17; Bartoń 2020). Estimated R^2 values represented the proportion of variance explained by the fixed effects alone (marginal) and all of the effects including the random effect (conditional). Output tables were visualized using the package sjPlot (v. 2.8.9; Lüdecke 2021).

Table 1. Variables Used to Calculate and Model High-Risk Crossings
Variables used to model number of high-risk crossings by adult eastern diamondback rattlesnakes on a South Carolina sea island between 2016 and 2020.

Variable	Units/Levels	Description
SnakeID	*i.e*. "ALSN" / "ABEL" / "CNCH" / "COSW"	Four letter ID for all 80 snakes included in the analysis.
Sex	"M" / "GF" / "NG"	Indicates the sex of an individual where "M" indicates male, "GF" indicates a gravid female, and "NG" indicates non-gravid female.
Season	"Active" or "Inactive"	Indicates the season each individual was tracked. "Active" season delineated by egress in March and ingress at the end of November. "Inactive" season delineated as December through February.
SVL	[centimeters]	Average Snout-to-Vent Length (SVL) of an individual snake for each year they were tracked. Z-standardized for inclusion in the model.
MCP	[hectares]	Individual activity-range sizes as estimated by 100% MCPs for each year and season.
Prop	[proportion]	Proportion of high-risk cover within each individual's activity range.

RESULTS

We used data from 80 EDBs (males = 31, females = 49) in the final analysis. Most EDBs were tracked during at least one active season (91.7% individuals) and most during at least one inactive season (70.2% individuals). Average time tracked was 232 days (SD = 203 days, range = 24 to 901 days). Seasonal activity ranges averaged 5.0 ha (SD = 10, range: 0.00 ha – 73 ha; Fig. 2A), and the proportion of high-risk cover within activity ranges averaged 0.151 (SD = 0.231, range: 0.00 – 1.00; Fig. 2B). The analysis of collinearity resulted in VIF values less than 2 across all variables, so all were included in the final model. Male SVL averaged 121 cm (SD = 14.1cm) and females averaged 118cm (SD = 9.02 cm). Snakes averaged 2.5 crossings per season (SD = 3.9, median = 1.0, range: 0.0-23). The active season averaged 3.7 crossings (SD = 4.5; Fig. 3) and the inactive season averaged less than one crossing (0.42, SD = 0.74; Fig. 3).

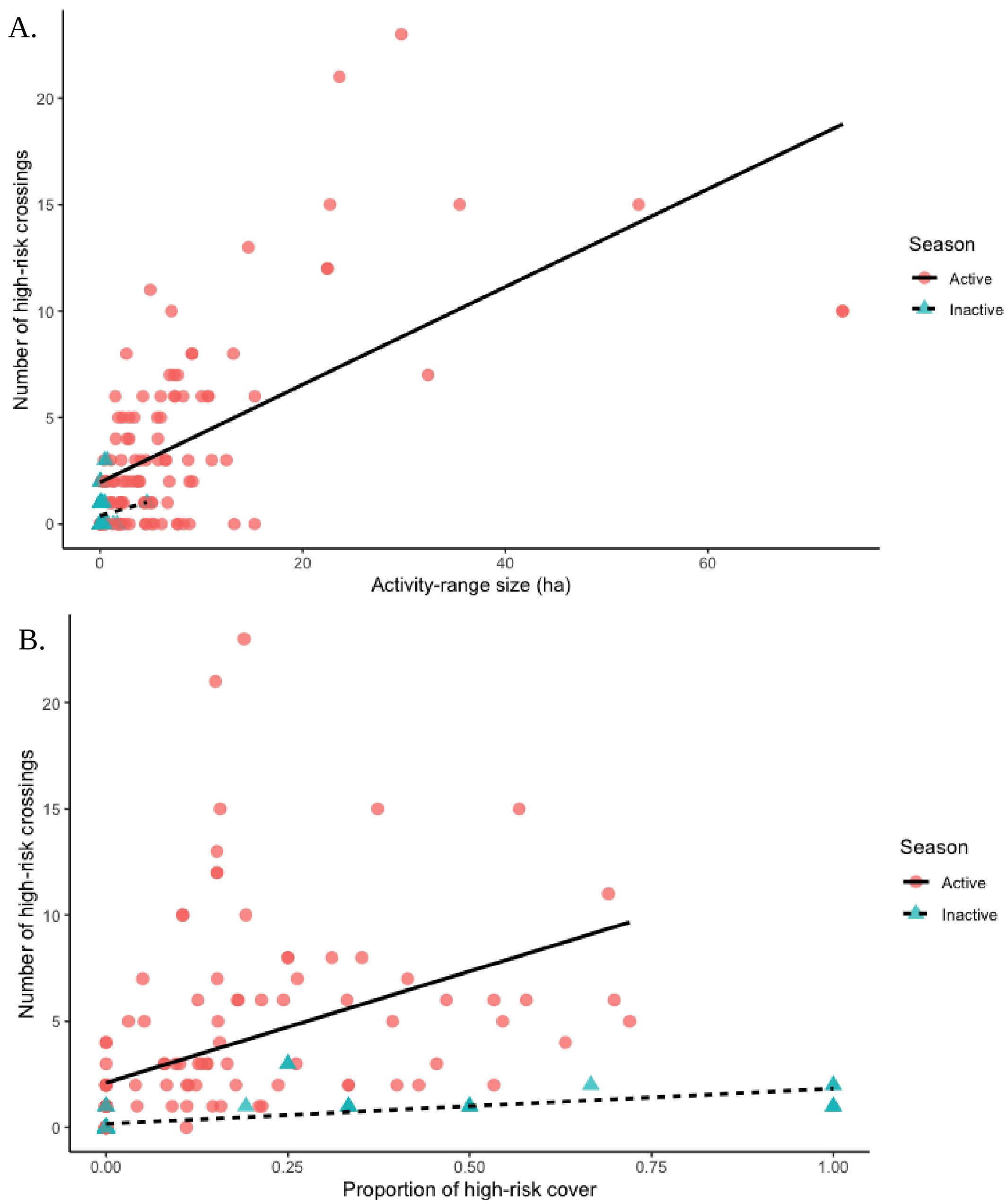

Figure 2. Observed Number of High-Risk Crossings by Activity-Range Size, Proportion of High-Risk Cover, and Season

Observed number of high-risk crossings for adult eastern diamondback rattlesnakes on a South Carolina sea island by A) activity-range size and B) proportion of high-risk cover with points and linear trend lines grouped by season (Active = red circles with solid line; Inactive = blue triangles with dashed line).

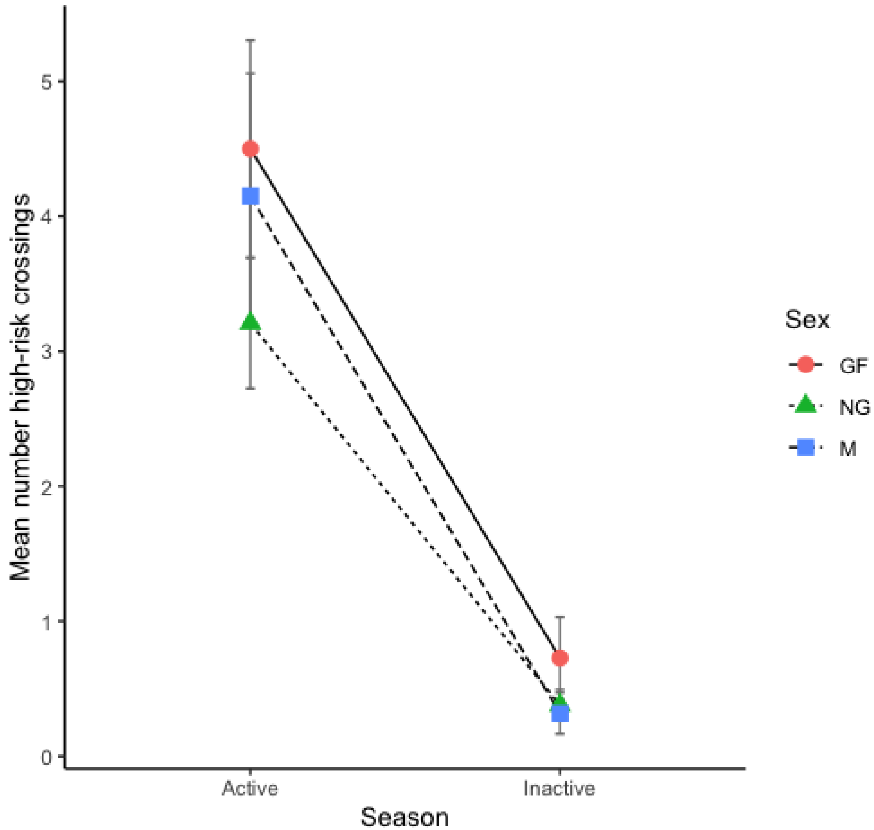

Figure 3. Mean Number of High-Risk Crossings by Sex and Season
Mean number of high-risk crossings by sex and season for adult eastern diamondback rattlesnakes on a coastal sea island in South Carolina, USA from 2016 to 2020. Males (M) blue squares with the dashed line, gravid females (GF) red circles with solid line, and non-gravid females (NG) green triangle with dotted line.

The model was underdispersed ($\hat{c} = 0.662$), but we did not detect other convergence issues or singularity. Sex did not account for a significant proportion of the observed variation ($\chi^2 = 4.424$, df = 2, $p = 0.109$). Post-hoc pairwise comparison of each sex indicated no significant differences due to large variation around each mean (Fig. 4B). Season accounted for a significant portion of the observed variation ($\chi^2 = 59.898$, df = 1, $p < 0.001$). The number of high-risk crossings decreased by a factor of 0.16 (SE = 0.04) during the inactive season (95% CI = 0.10 – 0.25, $p <$

0.001; Table 2). The predicted number of high-risk crossings during the inactive season was less than 0.5 and increased to 1.6 crossings during the active season (Fig. 4A). Activity-range size accounted for a significant portion of the observed variation (χ^2 = 63.523, df = 1, p < 0.001). For each hectare increase in activity-range size, the expected number of high-risk crossings increased by a factor of 1.06 (SE = 0.01, 95% CI = 1.04 – 1.07, p < 0.001; Table 2). Predicted number of crossings increased from 0 crossings at very small activity ranges up to 25 crossings at 60 ha activity ranges with an increasing confidence interval range at greater activity-range sizes (Fig.6A). Proportion of high-risk cover within the activity range accounted for a significant portion of the observed variation (χ^2 = 38.162, df = 1, p < 0.001). For each unit increase in proportion of high-risk cover within the activity range, the expected number of high-risk crossings increased by a factor of 9.65 (SE = 3.52, 95% CI = 4.72 – 19.73, p < 0.001; Table 2). Predicted number of crossings increased from 0 crossings within ranges that had 0% high-risk cover up to 6 crossings in ranges with 100% high-risk cover (Fig. 5B). Length (SVL) did not account for a significant portion of the observed variation (χ^2 = 0.599, df = 1, p = 0.438), and the model coefficient lacked significance (Table 2). Predicted number of crossings was slightly greater for larger individuals and the tightest confidence interval range was around the average SVL length where there was the greatest number of individual points (Fig. 6).

The inclusion of snake ID as a random effect comprised 50% of the variation observed (τ_{00} = 0.57, Total variance = 1.14; Table 2). The R^2 value of the model increased from 0.66 to 0.84 with the inclusion of the random effect. Between-group variance thus accounted for half of the observed variance.

Table 2. Poisson Mixed Effect Model Output Table Predicting Number of High-Risk Crossings

Poisson mixed effect model output predicting the number of high-risk crossings observed during radio-telemetry monitoring of adult eastern diamondback rattlesnakes on a coastal South Carolina sea island (2016-2020). Fixed effects are given on the response scale as incidence rate ratios.

	Fixed Effects			
Predictors	*Incidence Rate Ratios*	*std. Error*	*CI*	*p*
(Intercept)	1.34	0.34	0.81 – 2.22	0.249
Sex [Non-Gravid]	0.71	0.17	0.45 – 1.13	0.146
Sex [M]	0.54	0.16	0.30 – 0.97	**0.038**
Season [Inactive]	0.15	0.04	0.09 – 0.24	**<0.001**
Activity-Range size	1.06	0.01	1.04 – 1.08	**<0.001**
High-Risk Proportion	10.51	4.00	4.98 – 22.16	**<0.001**
SVL	1.10	0.13	0.87 – 1.38	0.439
Random Effects				
σ^2	0.56			
$\tau_{00\ \mathrm{Name}}$	0.56			
ICC	0.50			
N_{Name}	80			
Observations	174			
Marginal R^2 / Conditional R^2	0.657 / 0.844			

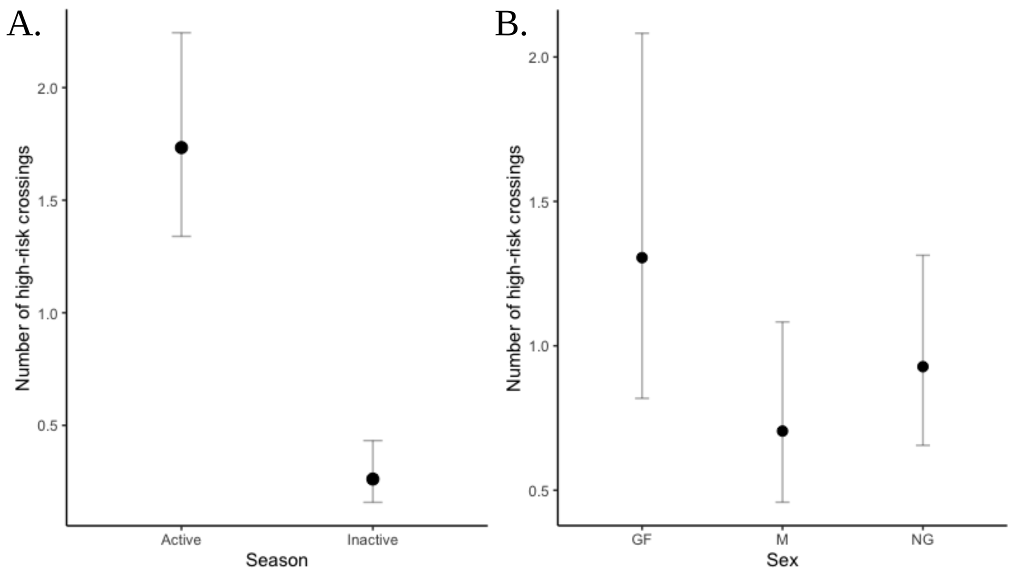

Figure 4. Predicted Number of High-Risk Crossings by Season and Sex
Predicted number of high-risk crossings for adult eastern diamondback rattlesnakes from a coastal South Carolina sea island (2016-2020) by A) season and B) sex. M = male, GF = gravid female, and NG = non-gravid female. Note that sex was not a significant effect in the crossing model.

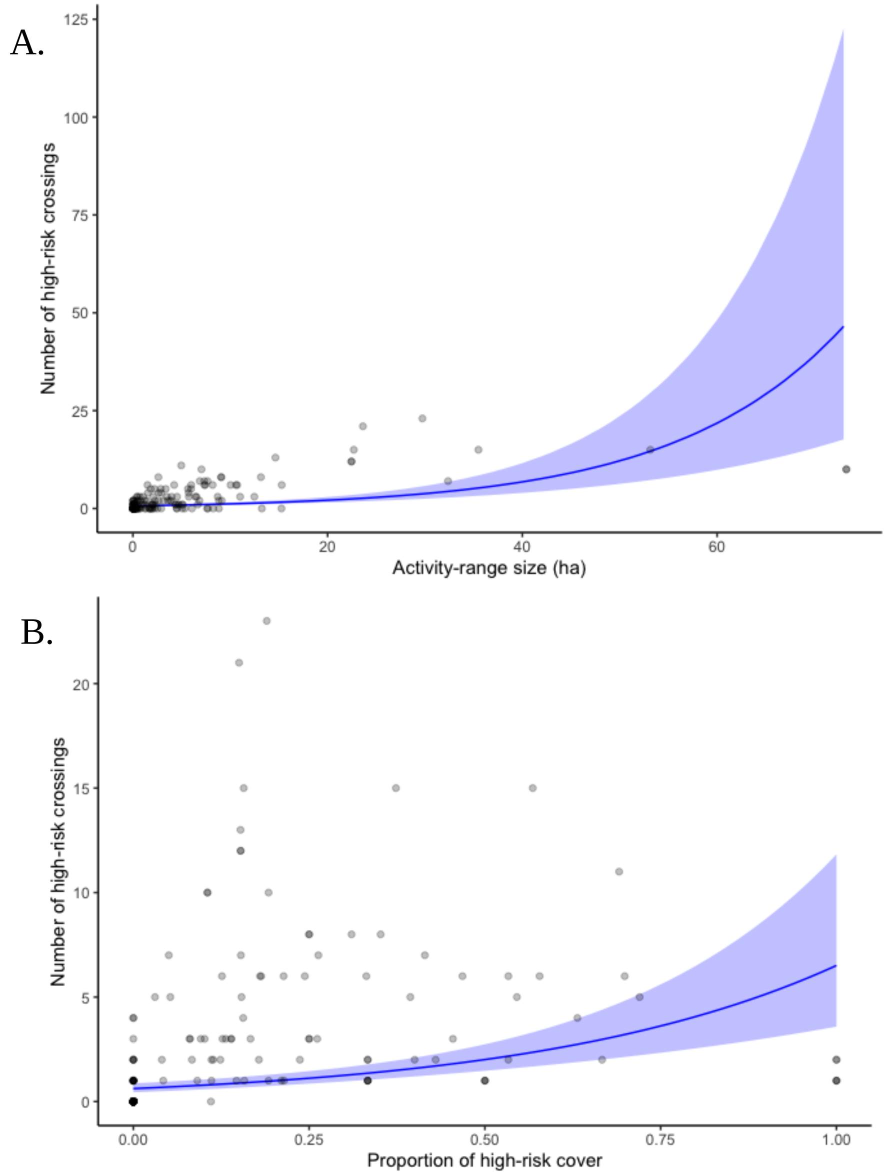

Figure 5. Predicted Number of High-Risk Crossings by Activity-Range Size and Proportion of High-Risk Cover

Predicted number of high-risk crossings for adult eastern diamondback rattlesnakes from a coastal South Carolina sea island (2016-2020) by A) activity-range size (ha) and B) proportion of high-risk landcover within the activity range. The blue line represents predicted values with the 95% confidence interval shaded in blue, and the semi-transparent black dots are observed values.

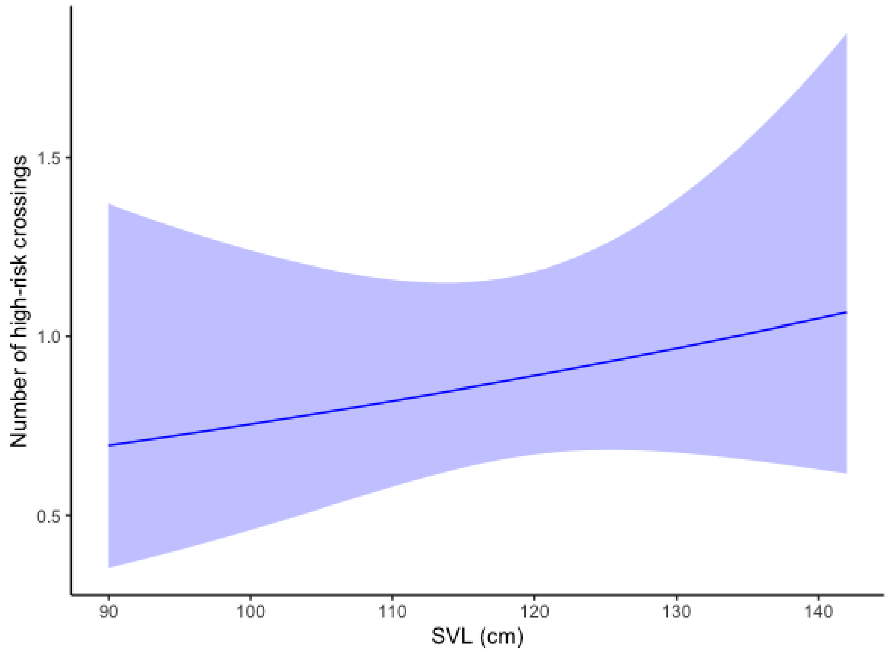

Figure 6. Predicted Number of High-Risk Crossings by SVL
Predicted number of high-risk crossings by SVL (back transformed to cm) for adult eastern diamondback rattlesnakes from a coastal South Carolina sea island (2016-2020). Note that SVL was not a significant effect in the crossing model.

DISCUSSION

As expected, season significantly affected EDB crossings of high-risk habitats (Fig. 4). The active season was characterized by more frequent crossings, likely reflecting greater surface activity compared to the inactive season. Human-caused EDB mortality increases during the active season, but overall mortality does not differ seasonally, likely reflecting naturally elevated winter morality rates due to cooler temperatures limiting digestion capacity for malnourished individuals (Waldron et al. 2013a). There is little support that patterns of human activity influence the occurrence of human-rattlesnake encounters, and that patterns of EDB phenology and movement ecology best predict encounter rates (Waldron et al. 2013b). Other snake species suffer significant increases in mortality and/or crossing rates during periods associated with long-distance movements, such as during the breeding season when males increase mate-searching efforts (Bonnet et al. 1999; Roe et al. 2006; Jochimsen et al. 2014). Few snake radio-telemetry studies have explicitly investigated occurrence and frequency of road crossings, but Row et al. (2007) indicated that ratsnake (*P. obsoletus*) road crossings in Ontario did not vary monthly within the active season. Monthly crossing rate may have been too fine a scale to detect seasonal shifts in activity or behaviors as were observed in this study, or perhaps smaller more active-foraging species of snake at northern latitudes exhibit different shifts in seasonal behaviors. Ambush foraging snakes tend to have smaller range sizes and shorter movement distances than active foraging species (Secor and Nagy 1994; Todd and Nowakowski 2021), and are generally observed less frequently crossing roads than active foragers (Roe et al. 2006; Row et al. 2007; Jochimsen et al. 2014; Rincon-Aranguri et al. 2019).

As predicted, activity-range size and proportion of high-risk cover were positively associated with high-risk crossings (Fig. 5). In this study, high-risk cover included roads and paved areas as well as lawns, golfing greens, and active training areas. Other studies examining the effects of habitat cover on snake home-ranges indicate that agricultural cover significantly influences range size (Kapfer et al. 2010; Breininger et al. 2011), but the MCRDPI did not have any agricultural areas, making direct comparisons with this population difficult. Snakes with larger activity range sizes in this study did not necessarily have a greater proportion of high-risk cover within their range, however other studies have observed variable relationships between home-range size and proportion of high-risk cover. For example, bullsnakes (*Pituophis catenifer sayi*) increased their home-range size with increasing density of agricultural fields within their ranges (Kapfer et al. 2010), whereas eastern indigo snakes (*Drymarchon couperi*) and EDBs exhibited smaller home-ranges in highly fragmented suburban/agricultural landscapes (Hoss et al. 2010;

Breininger et al. 2011). A relationship between range size and proportion of high-risk cover may have been obscured by greater spatial limitations experienced by island populations compared to inland populations. Morphological and foraging differences between insular and mainland populations of snakes have been documented (*reviewed in*: Luiselli 2015), but explicit comparison of the spatial ecology of snakes on islands versus inland populations have not been conducted. One study however, documented later ingress dates, greater diversity of hibernacula, and less winter surface activity in a coastal population of *C. horridus* compared to an inland site in South Carolina (Andrews and Waldron 2017). Another study observed EDB preferences for secondary dune habitat on barrier islands at a 1000-m scale, a habitat structurally similar to the preferences of inland EDB populations for pine savanna habitat (Stohlgren 2013). It is likely that coastal and inland populations of EDBs also differ in phenology and spatial ecology due to climatic and habitat differences. For example, the active season range sizes were much smaller in this study than the range estimates for inland EDB populations (Kain 1995; Timmerman 1995; Waldron et al. 2006).

Six records of individual EDBs having their activity range categorized as 100% high-risk cover were observed (Fig. 5B). All of those observations were females during the inactive season, likely reflecting a limitation in the spatial scale of this analysis, accuracy of GPS equipment, as well as the habitat preferences and behavior of individual EDBs. During the inactive season, EDB range sizes were smaller due to limited surface activity during periods of cooler temperatures, and most range estimates calculated in this study were less than 1 ha during the inactive season. During this period of inactivity, many EDBs seek shelter under exposed root tip-ups from fallen trees and tip-ups that receive direct sunlight may be more attractive for thermal regulation than those that are shaded on the interior of dense canopied forests. This may have brought some EDBs closer to the edge of forested habitats, for example where a low-risk forest meets a high-risk training area.

The observed individuals may have found shelter along the edge of a high-risk and low-risk area, and because of the spatial resolution of the habitat raster and the limitations in the accuracy of the GPS equipment used, some individual ranges may have been categorized entirely as high-risk because their ranges were encompassed by one or two raster cells.

A significant effect of body size (SVL) on the frequency of high-risk crossings was not detected in the analysis, indicating that body length of adult EDBs may not influence the likelihood of risky crossings. Generally, the number of crossings increased slightly as SVL increased (Fig. 6), but the contribution of SVL to the model was not significant (Table 2). Previous observations of within-species body size and road mortality have been reported where larger individuals of some species were more likely to be observed in roads (Andrews and Gibbons 2008; Mccardle and Fontenot 2016), but some species displayed the opposite trend (Andrews and Gibbons 2005). Few studies have explicitly investigated body size as a variable of risky movement, however. Body size is often a sexually dimorphic trait in many snake species, including rattlesnakes where females tend to be smaller than males likely due to differences in energetic costs of reproduction (e.g., Gibbons 1972; Beaupre 2002; Taylor and Denardo 2005). There was no correlation between sex and SVL in this study however, and neither factor significantly influenced the number of high-risk crossings. Altering the types of land cover included in the high-risk category may reveal subtle patterns in habitat preferences of different sexes or individuals of different body length. For example, males may be more likely to cross through some types of high-risk cover than other types while searching for receptive females. Given the data presented here, it is unlikely such fine-scale patterns would be detected, as EDBs generally had a low number of crossings and it is likely that most crossings occurred over roads specifically, since they often represent the narrowest path to reach another patch of low-risk cover. Previous studies have found positive relationships between body size and home-range size, likely reflecting changes in reproduction-related shifts in movement ecology (Todd and Nowakowski 2021). Again, activity-range size and body length were not significantly correlated in this study, but the relationship could have been obscured by reduced habitat area available on the island. Previous studies relating adult EDB survival to body size found that survival decreased as SVL increased (Waldron et al. 2013a). The generally positive trend between EDB body size and propensity to cross high-risk areas would provide an interesting explanation for observed differences in adult survival, though further analysis is needed to clearly define how variables such as exposure to human activity, perception of risky cover, age, reproductive status, and prey availability influence EDB crossings.

Insights into how the EDB navigates fragmented habitat is essential for improving management efforts. For example, insight into factors that influence whether EDBs cross high-risk habitats and the phenology of when snakes enter areas with high human activity, can equip managers with a better understanding of when and where to implement conservation actions to minimize human-wildlife conflict. For snakes with slow life histories, even annual road mortality rates of 6% are projected to caused significant declines in a protected population of *Crotalus oreganus* only exposed to low traffic volume roads (Winton et al. 2020). It is likely that the combined pressures of habitat loss, climate change, and human persecution have caused significant declines in many snake populations, and the addition of human-caused mortality directly removing important reproductive adults from the population can lead to local extirpations (Row et al. 2007; Andrews et al. 2015; Brehme et al. 2018; Winton et al. 2020).

The results of this study indicate that adult EDBs increased the frequency of high-risk crossings when surface activity and movement increased, as well as in the presence of a greater proportion of high-risk cover within their activity range. Comparisons with other studies were difficult given the unique study design using a large long-term dataset not typically available for snake research. The explicit classification of a “risk” landscape for rattlesnakes that includes roads as well as other anthropogenic land use types is also unique and has not previously been assessed for any other snake species in this way. To improve our understanding of the ecology of a declining rattlesnake population within a fragmented human-dominated landscape is critical for informing managers about how these populations are able to persist, as well as how to reduce human-rattlesnake conflict. Understanding when and where snakes decide to cross through areas of high human activity could inform managers about where to place signs or build crossing structures, and how to minimize human activity when and where encounters are most likely. Further analyses should investigate other potentially motivating factors associated with long-distance movements, such as conspecific density (particularly of reproductive adults), prey abundance, and the population level effects of road mortality on EDBs in particular.

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APPENDIX A

MARSHALL UNIVERSITY.

www.marshall.edu

Office of Research Integrity

November 14, 2022

Mya Wiles
15 Fairview Terrace
Windsor, VT 05089

Dear Mya:

This letter is in response to the submitted thesis abstract entitled *"Mortality Risk Associated With Urban Land Use for Adult Eastern Diamondback Rattlesnakes (Crotalus Adamanteus)."* After assessing the abstract, it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocols #614, 640, 703, and 759. 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,

Bruce F. Day

Bruce F. Day, ThD, CIP
Director

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APPENDIX B

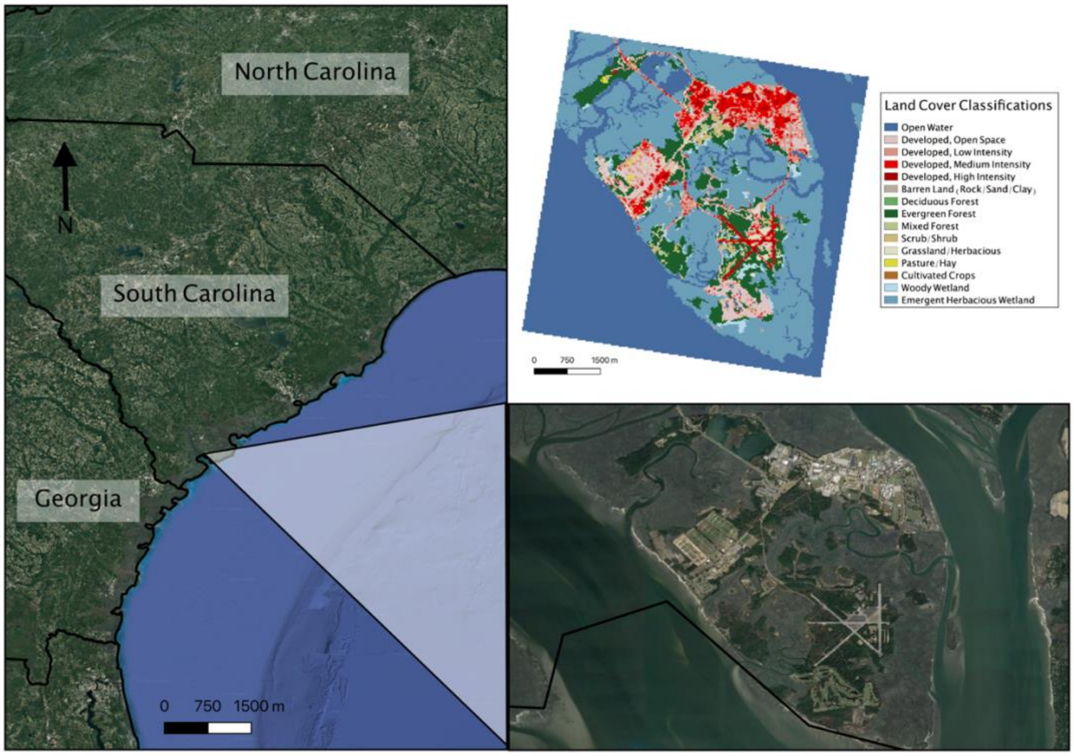
Table of NLCD landcover types present within the MCRDPI and the corresponding ‘high’ or ‘low’ risk categorization of each for adult EDBs.

Cover Type	MRLC Descriptions	Risk	Reasoning
Open Water	Area of open water with vegetation less than 25% total cover.	Low	Natural habitat feature, minimal human activity
Developed – Open Space	Mixture of some constructed materials, mostly vegetation in the form of lawn grasses. Impervious surfaces are less than 20% of total cover.	High	High human activity, some vehicle activity
Developed – Low Intensity	Mixture of constructed materials and vegetation. Impervious surfaces are 20% to 49% of total cover.	High	High human activity, likely includes roadways
Developed – Medium Intensity	Mixture of constructed materials and vegetation. Impervious surfaces are 50% to 79% of the total cover.	High	High human activity, includes roadways
Developed – High Intensity	Areas where people reside or work in high numbers. Impervious surfaces are 80% to 100% of the total cover.	High	High human activity, includes roadways
Barren Land	Areas of bedrock, desert, pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits, and other accumulations of earthen material. Vegetation less than 15% of total cover.	Low	Natural habitat feature, minimal human activity
Deciduous Forest	Area dominated by trees greater than 5m tall and greater than 20% of total vegetation cover. Deciduous species account for greater than 75% of total species.	Low	Natural habitat with cover, minimal human activity
Evergreen Forest	Area dominated by trees greater than 5m tall and greater than 20% of total vegetation cover. Evergreen species account for greater than 75% of total species.	Low	Natural habitat with cover, minimal human activity
Mixed Forest	Area dominated by trees greater than 5m tall and greater than 20% of total vegetation cover. Neither deciduous nor evergreen species account for greater than 75% of total species.	Low	Natural habitat with cover, minimal human activity

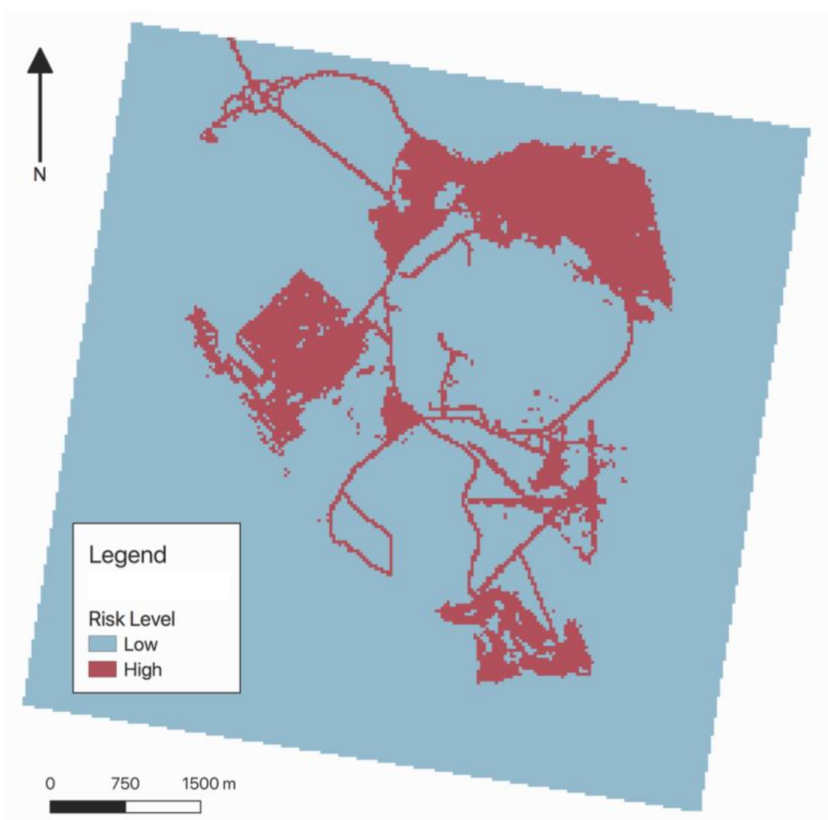
Shrub/Scrub	Dominated by shrubs less than 5m tall greater than 20% total vegetation cover.	Low	Natural habitat with cover, minimal human activity
Grassland/ Herbaceous	Dominated by graminoid or herbaceous vegetation not subject to intensive management.	Low	Natural habitat with some cover
Pasture/Hay	Area of grasses, legumes, or mixed planted species greater than 20% total vegetation for livestock grazing or hay/seed crops on perennial cycle.	High	*No agriculture on the island, cells identified as mowed grass from imagery
Cultivated Crops	Area used for production of annual crops greater than 20% total vegetation cover.	High	*No agriculture on the island, cells identified as mowed grass from imagery
Woody Wetlands	Areas where forest or shrubland vegetation are greater than 20% of vegetative cover and soil substrate is periodically saturated or covered with water.	Low	Natural habitat with some cover, minimal human activity
Emergent Herbaceous Wetland	Area with perennial herbaceous vegetation greater than 80% of vegetative cover and soil substrate is periodically saturated or covered with water.	Low	Natural habitat with cover, minimal human activity

APPENDIX C

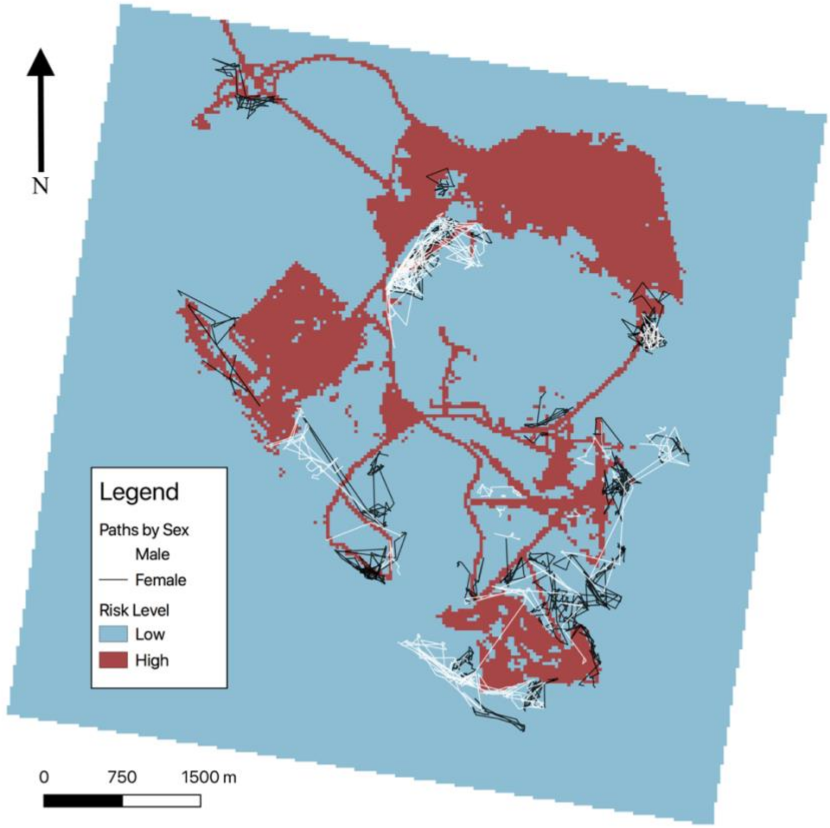
A.



B.



C.



Three raster maps of the A) National Land Cover Dataset (NLCD) 2019, B) reclassified map of 'high'(red) and 'low' (blue) mortality risk, and C) reclassified risk map showing individual paths of adult eastern diamondback rattlesnakes (EDBs) highlighted by sex (Male = white, Female = black) at the study site.