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Nest-Site Selection and Neonate Survival of Eastern Box Turtles (Terrapene carolina carolina) in Michigan's Northern Lower Peninsula

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Nest-Site Selection and Neonate Survival of Eastern Box Turtles (*Terrapene carolina carolina*) in Michigan's Northern Lower Peninsula

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DEDICATION

This thesis is dedicated to my grandmother, Joan G. Cyr, who fostered my early appreciation of the natural world with childhood visits to the beach to search for sea-glass and through our exploration of the small tidal pools on the shores of Mt. Hope Bay. Your dedication to my education through countless reading lessons, long discussions at the kitchen table helped shape my values and my career. Your strength, intelligence, and passionate pursuit of knowledge have acted as a constant source of inspiration for me and will continue to long into the future. I am forever appreciative of your love and support.

With love and thanks,
Joseph

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ABSTRACT

Turtles (Order Testudines) are experiencing global declines largely due to anthropogenic influences such as habitat fragmentation, illegal collection and sales, and the threat of global climate change. Removal of individuals from the adult age-classes means there is now a greater need to understand the survival of neonate and juvenile turtle age-classes. In this study I examined a population of eastern box turtles (*Terrapene carolina carolina*) at the northern limit of their range in Michigan's lower peninsula. The objectives of my thesis were 1. to determine the microhabitat factors that influence nest-site selection by female box turtles and how selected microhabitat and environmental factors affect box turtle nest success and 2. create known-fate annual survival estimates for hatchling box turtles through the first year of life. Box turtles select nest sites with a higher percent of bare soil and lower amounts of understory vegetation compared to random sites and avoid nesting on north facing slopes. Larger clutch sizes as well as a lower percent of bare soil at the nest site increased the probability of nest success. Depredation and exposure to suboptimal environmental conditions were the primary sources of neonate mortality from 2013-2015, and annual survival estimates for neonate box turtles predicted survival to decrease through the first year of life with a steep drop in the probability of survival from nest emergence in the fall, before leveling off at 50% for overwintering (day 50 = .503; SE = 0.067), then gradually decreasing again with spring emergence till reaching 0% survival short of the 1 year mark (day 335 = 0.0). Similar studies should be conducted across the geographic range of Eastern Box Turtles to better understand the major threats to the survival of other box turtle populations.

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KEY TO SYMBOLS

K

The number of parameters in logistic regression models including the intercept.

ω_i

Weight value from Akaike's Information Criterion corrected for small sample sizes.

AIC_c

Rank value of Akaike's Information Criterion corrected for small sample size.

Δ_i

The AIC_c Delta, or the change in AIC_c from the top ranked model and the model of interest.

ABBREVIATIONS

AICc = Akaike’s Information Criterion corrected for small sample sizes

TSD = Temperature-dependent sex determination

MNF = Manistee National Forest

TB = Turtle Bowl forest opening

SV = Savanna forest opening

EW = East-West forest opening

GP = Gravel Pit forest opening

Spp. = species

USFS = USDA Forest Service

GPS = Global Positioning System

CL = Carapace Length

CW = Carapace Width

CH = Carapace Height

PL = Plastron Length

PW = Plastron Width

NASA = National Aeronautics and Space Administration

I.D. = Identification number

SD = Standard Deviation

SE = Standard error of the mean

CI = 95% Confidence intervals

°C = Degrees Celsius

n = sample size

km = kilometers

m = meters

cm = centimeters

mm = millimeters

g = grams

y = years

h = hours

d = days

ha = hectares

CHAPTER I

INTRODUCTION

Turtles (Order Testudines) are experiencing global declines largely due to anthropogenic influences such as habitat fragmentation, illegal collection and sales, and the threat of global climate change (Gibbson et al., 2000; Refsnider and Janzen, 2012). One species of turtle native to the state of Michigan that is experiencing such declines is the eastern box turtle (*Terrapene carolina carolina*) (Harding, 1997; Hall, 1999; Dodd, 2001). The eastern box turtle has a large geographic range across much of the Northeastern and Midwestern United States and inhabits moist, broadleaf woodlands for the majority of their life. Box turtles also migrate to dry grassy plains and forest clearings for nesting while juvenile box turtles are known to prefer open canopy grasslands for the early years of life (Felix et al., 2008; Flitz and Mullin, 2006). The Manistee National Forest (MNF) in Michigan's northern lower peninsula lies at the northern limit of the eastern box turtles species range within the Midwest and makes up the largest area of publicly held box turtle habitat in the state (Laarman, 2017).

Eastern box turtle life history traits, shared by most turtle species, dramatically increase the effects of anthropogenic stressors. Because of their longevity and slow growth rate, it can take up to ten years for juvenile turtles to reach sexual maturity. Once sexually mature, turtles may only produce one or two clutches of eggs a year, typically with low hatchling success (Dodd, 2001). Historically, eastern box turtles compensated for low annual recruitment through multiple reproductive events across an adult's lifetime. This strategy relies on high adult survivorship for the persistence of a population. But with the increased removal of individuals from the adult age-class in many turtle species there is now a greater need to understand the survival of neonate and juvenile age-classes to ensure the continued persistence of current populations. Further, the partitioning of habitat use in eastern box turtles based on life stage and season makes effective habitat conservation particularly difficult.

The primary objective of my thesis was to fill the current gaps in box turtle literature concerning neonate age classes and aid in the conservation of eastern box turtles by providing crucial information on the nesting ecology of these turtles at the northern limit of their range. While the majority of current box turtle literature has focused on adult age classes, I will be the first to use radiotelemetry to construct known fates models for age specific cohorts of neonate box turtles at the northern limit of the species range in the Midwestern United States. These models will inform future research on population viability and in doing so provide a better understanding of the environmental needs of eastern box turtles to ensure their persistence in MNF.

In chapter II my objectives were to determine the microhabitat factors that influence nest-site selection by female Box Turtles and how selected microhabitat and environmental factors affect Box Turtle nest success. In June (2013-2016) following egg deposition, I collected environmental data within a 1-m x 1-m quadrat around Box Turtle nest sites and random sites to determine if nest sites differed from randomly selected sites within four forest openings. I then created logistic regression models using collected microhabitat and environment data to explore the relationship between the microhabitat surrounding nests and microhabitat surrounding random sites. I then compared microhabitat variables from successful nests and unsuccessful nests using logistic regression models. I used Akaike's Information Criterion corrected for small sample sizes

(AIC_c) to rank both models for nest-sites selection and nest success. Chapter II was formatted for submission to the Journal of Herpetology.

The objective in chapter III was to use data collected through radio-telemetry tracking and Kaplan-Meier estimators modified for staggered-entry to create known-fate annual survival estimates for the first year of life of the neonate box turtles that emerged from the nests used in Chapter II. Chapter III was formatted for submission to the American Midland Naturalist following submission of this thesis.

Finally Chapter IV contains an extended review of current literature on eastern box turtles, Extended Methodology of Chapter II and III, and a Bibliography.

PURPOSE

Although Eastern Box Turtles are one of the most recognizable and wide spread species of turtles in the eastern United States, little research has been conducted to understand the effects the microhabitat and environment surrounding a box turtle nest have on the success of nests. Even fewer studies have attempted to estimate the annual survival of neonate Eastern Box Turtles using known-fate models. The purpose of this thesis was to provide valuable information on the environmental and microhabitat characteristics selected for by female Eastern Box Turtles when choosing a nest site (Chapter II). Then to measure the associated probability of nest success based off of the collected microhabitat date to better understand what habitat requirements will promote nest success and the long-term persistence of Eastern Box Turtles in Manistee National Forest. Additionally, through the use of radio-telemetry and Kaplan-Meier modeling (Chapter III) this thesis will provide novel information on the biological and environmental obstacles that prevent the survival of neonate Eastern Box Turtles through the first year of life out of the nest.

SCOPE

This thesis discusses in detail the behavioral ecology surrounding female nest-site selection in Eastern Box Turtles as well as survival estimates for neonate Eastern Box Turtles through the first year of life outside of the nest in Manistee National Forest in Michigan's northern lower peninsula. Additionally this thesis includes morphometric information from neonate and adult female Eastern Box Turtles in the Manistee population.

ASSUMPTIONS

Pilot studies conducted years earlier found that unprotected Eastern Box Turtle nests in Manistee National Forest experienced near 100% depredation. For my thesis I assumed there would be near 100% depredation without the use of nest exclosure boxes around each nest included in my study. Thus, despite Chapter II focus on the measurement of nest success, I installed nest exclosure boxes and excluded predation as potential cause of nest failure. With nest exclosures installed I was able to accurately measure the impact of the microhabitat and environment surrounding the nest on the probability of nest success rather than the probability of a nest being depredated. Additionally during our collection of box turtle nest-site microhabitat variables we placed temperature dataloggers (iButton DS1922L-F5 thermochrons, Maxim Integrated) within each nest programmed to record nest temperatures at hourly intervals for the duration of the incubation period. I assumed that by carefully buried the temperature loggers immediately adjacent to the nest chamber to ensured the temperature logger would collect accurate nest temperatures while not disturbing the nest itself (Jaffé et al., 2008; Morrison et al., 2009).

For Chapter III I assumed depredation of neonates during overwintering would be near zero percent and that the neonates would not move locations once they began overwintering. Based off of these two assumptions I decided to install the nest exclosures around neonate Eastern Box Turtles during overwintering in order to easily relocate each individual in the spring and reinstall radio transmitters on each.

HYPOTHESIS

In Chapter II I hypothesized that nest-sites selected by female Eastern Box Turtles would differ from random sites with nest sites having higher amounts of bare soil present and southern facing aspects within each study forest opening. In Chapter III I hypothesized that at least one neonate from the cohorts monitored during my study period would survive a full 365 days.

SIGNIFICANCE

This thesis presents the first research analyzing the environmental and microhabitat nest-site preferences of female Eastern Box Turtles and the resulting success or failure of box turtle nests at the northern limit of the Eastern Box Turtle's geographic range in the Midwest. Furthermore, this thesis adds to the currently limited number of studies that provide information on the early life stages of North American turtles. Chapter III provides the first detailed survival estimates for a population of neonate Eastern Box Turtles in the Midwest region of the United States. With the increase threat of climate change it is imperative that we continue to collect information on box turtle behavioral ecology as well as annual neonate survival in order to properly address the management needs of Eastern Box Turtles to ensure their persistence throughout the geographic range.

DEFINITIONS

Temperature-dependent sex determination

(TSD) A type of sex determination where the temperatures experienced by the embryos during development determine the sex of the individual.

Genotypic Sex Determination

(GSD) Sex determination in which sex is determined at conception by chromosomal factors.

Neonate

The age class of a hatchling Eastern Box Turtle that is less than 1 year old.

Adult

The age classes of a sexually mature Eastern Box Turtles.

Ecological Edge

Transition zone between two distinct habitat types.

First or Fall activity season

Period of Eastern Box Turtle neonate activity from it's emerge from the nest in late summer or fall to it's first overwintering period.

Forest Opening

A classification term used to describe a suite of upland non-forested areas with little to no canopy cover.

Overwintering

A period of torpor allowing box turtles to survive inclement winter climates (Dodd 2001).

Spring Emergence

The date when an Eastern Box Turtle emerged from overwintering refugia in spring.

Second or Spring activity season

Period of neonate Eastern Box Turtle activity between spring overwintering egress and fall overwintering ingress.

Natal Opening

The forest opening in which the focus individual emerged from it’s nest.

Mother knows best: Nest-site selection and hatching success in Eastern Box Turtles (*Terrapene carolina carolina*) in Michigan

ABSTRACT.— In oviparous species female animals can affect their offspring's survival through genetic as well as non-genetic influences such as nest-site selection. In this study we examined a population of Eastern Box Turtles (*Terrapene carolina carolina*) at the northern limit of their range in Michigan's lower peninsula. Our objectives were to determine the microhabitat factors that influence nest-site selection by female Box Turtles and how selected microhabitat and environmental factors affect Box Turtle nest success. In June (2013-2016) following egg deposition, we collected environmental data within a 1-m x 1-m quadrat around Box Turtle nest sites and random sites to determine if nest sites differed from randomly selected sites within four forest openings. We used logistic regression models using collected microhabitat and environment data to explore the relationship between the microhabitat surrounding nests and microhabitat surrounding random sites. We also used logistic regression to compare microhabitat variables from successful nests and unsuccessful nests. Box Turtles select nest sites with a higher percent of bare soil and lower amounts of understory vegetation compared to random sites and avoid nesting on north facing slopes. The success rate of our observed nests was 50.0% and larger clutch sizes as well as a lower percent of bare soil at the nest site increased the probability of nest success. The complex relationships between the microhabitat surrounding nest-sites and the survival of the embryos to hatch highlights the importance of continued research and conservation to ensure the persistence of Eastern Box Turtles in Michigan's lower peninsula.

Key words: Box Turtle; Microhabitat; Nest-site selection; Nest success; Offspring; *Terrapene c. carolina*;

INTRODUCTION

Female animals can affect their offspring's survival through genetic and non-genetic influence. In oviparous species, non-genetic influences such as the selection of a nest site can have dramatic effects on the survival of the female as well as potential offspring. In addition to selecting a site that will ensure their own survival during oviposition and incubation, females must also select a nest site that will protect the nest from predation and still provide the environmental qualities to maximize the survival of the embryos to hatch (Refsnider and Janzen, 2010; Amat and Masero, 2004). Selection of a nest site can also affect the subsequent dispersal of the offspring. Thus in species with limited dispersal and specialized habitat requirements, nest-site selection and the success of the nests can shape the long-term spatial genetic as well as demographic structure of a population (Hazlitt et al., 2004; Scribner et al., 1993).

In iteroparous species, reproductive success largely depends on multiple reproductive events spread across multiple years, thus maximizing maternal survival during nesting events can be of great importance. In reptiles there is generally less parental investment during the incubation period than in species such as birds, which are vulnerable to predators throughout the entire period of egg incubation (Seltmann et al., 2013; Montgomerie and Weatherheard, 1988). In many species of reptile however, the potential threats to maternal survival are often restricted to constructing the nest and traveling to and from the nesting location. To prevent overheating as well as reduce the threat of depredation many reptiles construct nests and deposit eggs at dusk under the cover of darkness (Angilletta et al., 2009).

Some females may settle for suboptimal nesting sites to reduce the risk of predation to herself or the nest (Miller et al., 2007). For example, in the Australian turtle species *Emydura macquarii,* females will travel farther distances in the absence of predators to find ideal nesting conditions than when predation pressures are higher to either the female herself or her nest (Spencer, 2002; Spencer and Thompson, 2003). Added pressure to select nest sites with optimal environmental conditions over the risk of depredation may be placed on many turtle species in North America that exhibit temperature-dependent sex determination (TSD) (Ewert and Nelson, 1991); where the developmental rate as well as the sex of the offspring is controlled by the environmental conditions of the nest during embryonic development. As a result the demography of entire populations can be affected by the females ability to select optimal nesting habitat (Packard and Packard, 1988; Wilhoft et al., 1983; Valenzuela and Lance, 2004). Thus, for turtles with TSD the advantages of nesting in habitat that is optimal for proper growth and development of the embryos might outweigh the risk of depredation (Refsnider et al., 2015).

Turtles non-randomly select locations to deposit their eggs based on certain habitat characteristics (Shine and Harlow, 1996; Hays et al., 2001; Zappalorti et al., 2015). Further, the microhabitat surrounding nests has been shown to control the thermal environment within the nest thus controlling the sex ratio of the embryos within (Burger, 1976). With the phenotype and survival of her offspring depending on her selection of an appropriate nest site, natural selection should favor female turtles that are able to distinguish between sites with adequate microhabitat properties for optimal nest success and those without.

The Eastern Box Turtle (*Terrapene carolina carolina*) is one of the most common terrestrial turtle species in the eastern United States (Wilson and Ernst, 2008). Eastern Box Turtle nests experience high depredation from a variety of mammalian and insect predators (Dodd, 2001). Additionally, box turtle embryos exhibit both temperature-dependent sex determination as well as temperature-dependent developmental rates making it a model study organism to analyze nest-site selection and the influences it has on nest success. The Eastern Box Turtle has a large geographic range across much of the Northeastern and Midwestern United States (Dodd, 2001). Despite being listed as a protected species in many of the states within their geographic range, including Michigan where it is a species of special concern, Eastern Box Turtles continue to have dramatic population declines (Williams and Parker, 1987; Harding, 1997; Hyde, 1999).

Eastern Box Turtles preferentially select forest openings or open-canopy sites to lay their eggs over heavily forested areas (Williams and Parker, 1987; Burke and Capitano, 2011; Willey and Sievert, 2011). However, it is still unclear as to what microhabitat variables they select for within forest openings to determine where to deposit their eggs. Many species of turtle in the southern United States favor nesting sites with lower vegetation heights, less canopy cover, and greater amounts of exposed soil compared to randomly selected sites within forest openings to maintain optimal thermal and hydrological conditions for egg development (Hughes and Brooks, 2016; Flitz and Mullen, 2006; Janzen and Morjan 2001; Janzen, 1994). However reptiles frequently exhibit variation in the selection of microhabitat features across their geographic range (Doody et al., 2006). For example the Common Snapping Turtle (*Chelydra serpentina*), exhibits varying nest site preferences along a latitudinal gradient across its range, with females at higher latitudes selecting open canopy sites to increase nest temperatures, and females at lower latitudes selecting shaded sites to prevent nests from overheating (Ewert et al., 2005). This suggests that the microhabitat selected for nesting by females likely varies across a species range.

With a variety of habitat types and variation in active season length and temperature across the Eastern Box Turtles expansive range, the available number of days per year for oviposition and incubation vary (Packard et al., 1981; Hughes and Brooks, 2006). Due to the shorter summers at higher latitudes the time to reach development could be the limiting factor in the survival of nests at the Box Turtle's northern range limit (Compton, 1999). As such there is the potential for plasticity in the microhabitat features selected by box turtles among populations as well as across its geographic range to ensure nest and hatchling survival. Despite the large body of information currently available regarding Eastern Box Turtles there is still a great need for statistically rigorous quantitative research on this species at its northern range limit.

In this study we examined a population of Eastern Box Turtles at the northern limit of their range in the lower peninsula of Michigan. Our objectives were to determine 1.) the microhabitat factors that influence nest-site selection by female Eastern Box Turtles and 2.) how selected microhabitat and environmental factors affect Eastern Box Turtle nest success.

MATERIALS AND METHODS

Study Sites.— Our study was conducted in the Manistee National Forest (MNF) which lies at the northern limit of the Eastern Box Turtle's range in northwest lower Michigan (Figure 1). MNF is described as having a wet, temperate climate with four distinct seasons. Yearly maximum temperatures average 13.8 ˚C with yearly minimum temperatures averaging 1.7 ˚C. The yearly average rainfall is 99.6 cm and average snowfall is 322.4 cm (Midwest Regional Climate Center, 2017). MNF is managed for multiple uses including: recreation, wildlife habitat, timber production, watershed quality improvement, and the management of hazardous fuels (USDA, 2006). MNF is composed of primarily secondary growth forest with a mixture of red maple (*Acer rubrum*), jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*), and various oak species (*Quercus spp*.).

Within MNF, we selected four open-canopy nesting sites referred to as Turtle Bowl (TB), Savanna (SV), East-West (EW), and Gravel Pit (GP). The four study sites were located within herbaceous openings comprised of sandy soils largely covered with lichens (*Cladonia spp*.), grasses (*Andropogon spp.*), sedges (*Carex spp.*), bracken fern (*Pteridium spp.*), low bush blueberry (*Vaccinium angustifolium*), and sparse shrubs such as witch-hazel (*Hamamelis spp.*) and cherry (*Prunus spp.*). Sites were areas managed using prescribed fire or mechanical brushing and invasive species treatments by the USDA Forest Service (USFS) (Cadillac-Manistee Ranger District) and ranged in size from roughly 0.6 hectares to 5.5 hectares (EW = 0.90ha, GP = 0.68ha, TB = 1.88ha, SV = 5.55ha). These openings serve as nesting habitat for Eastern Box Turtles within an otherwise heavily forested area and were selected based on their historic use as nesting sites by female Eastern Box Turtles.

Nest-site selection.— Each June from 2013 to 2016 we conducted visual encounter surveys beginning at approximately 1900 h at each of our study sites in MNF to locate nesting females. Nesting females were monitored until egg deposition, upon which time we temporarily covered the nest using a predator-proof exclosure until the following morning. Within 24 hours of egg deposition we collected microhabitat data from within a 1-m × 1-m quadrat placed around the nest. Microhabitat data included percent bare soil, percent understory vegetation, slope angle, slope aspect, canopy cover density, distance from nest to nearest tree within the forest opening, and distance from nest to nearest forest edge. We visually estimated slope to the nearest 5 degrees and measured the aspect of the slope using a standard field compass. We visually estimated percent bare soil and percent understory vegetation within the 1-m × 1-m quadrat. The quadrat was centered over the nest and we considered any vegetation under 1m in height to be understory vegetation. However since percent bare soil and percent understory vegetation were highly correlated (r = -0.92), in our statistical analysis percent bare soil was used in favor of understory vegetation.

We measured canopy cover density using a spherical convex densiometer held at breast height. We took four densiometer readings from the center of the nest while facing north, south, east, and west then averaged the four readings to obtain average canopy cover density. We used a Trimble Geo 7 series GPS to mark the location of each nest to an accuracy within 25 cm. Using a Trimble Geo 7 series GPS, we walked the edge of each opening to create polygons of each of the study sites in ArcGIS (version 10.3). Then using ArcGIS (version 10.3) we measured the distance in meters from each nest to the forest edge closest to the nest. Once we had recorded the microhabitat variables from the Box Turtle nests, we used ArcGIS (version 10.3) to select one random point associated with each Box Turtle nest. To create our random points a constraining layer of each opening (*n* = 4) was constructed, then within each constraining layer random single feature points were created. We then replicated the methods used to collect microhabitat data from actual nests to gather microhabitat data at each random point generated in ArcGIS.

Nest Success.— After all microhabitat data were recorded from the Box Turtle nests we installed predator-proof exclosure boxes made of wood and 1/4in. mesh steel hardware cloth by digging roughly 20cm into the ground and burying the exclosures to assure larger predators could not access the nest for the duration of incubation. Additionally, after the predator-proof exclosures were installed, beginning in June of 2014 we placed temperature dataloggers (iButton DS1922L-F5 thermochrons, Maxim Integrated) within each nest programmed to record nest temperatures at hourly intervals for the duration of the incubation period. We carefully buried the temperature loggers immediately adjacent to the nest chamber at the depth of the center of the clutch within the nest. This ensured the temperature logger would collect accurate nest temperatures while not disturbing the nest itself (Jaffé et al., 2008; Morrison et al., 2009). Fifty-five days after the eggs were laid we began monitoring the nests for emerged neonates. If an emergence had occurred we collected morphometric data on the neonates including carapace length, width, and height as well as plastron length and width using calipers. The mass for all neonates was collected using a digital scale. We released all hatchling Box Turtles on the same day as the observed emergence at the nest site immediately after collecting morphometric data. If an emergence did not occur at a nest during the fall the predator-proof exclosure was left installed till the following spring and was again monitored daily for possible spring emergences. Once all of the live hatchlings had emerged from a nest we excavated each nest to look for any eggs that may have failed to develop or any neonates that had failed to make it to the surface. We categorized a nest as successful if at least one hatchling emerged from the nest on its own and was found at the surface (Kipp, 2003).

Nest-site Selection Statistical Analysis.— We used logistic regression to model the factors affecting nest-site selection and designed 15 models using microhabitat data collected at nest sites selected by Box Turtles and our randomly selected sites (Table 1). Models were constructed using microhabitat variables that would primarily affect nest temperature and all variables had been found to characterize nest-sites in previous turtle nesting studies. Hughes and Brooks (2006) found that painted turtles (*Chrysemys picta*) preferentially selected nest-sites free of vegetation. Nest sites with low vegetation and higher percent bare soil are likely to receive increased solar radiation and could remain warmer throughout the incubation period. Because Box Turtles typically nest in forest openings and previous studies have found canopy cover to be a reliable descriptor of turtle nest sites in other species the percent canopy cover was also included in our models (Janzen and Morjan, 2001; Hughes and Brooks, 2006). The slope and aspect of the ground surrounding a nest site would greatly affect the intensity and duration of exposure a nest could have to the warming effects of solar radiation and as such both were selected as likely important variables to include in our models. Further previous studies on multiple turtle species have found significant support for females preferentially selecting nest sites based on slope aspect (Schwarzkopf, 1984; Garmestani et al., 2000). Predation pressure is known to influence female turtle behavior related to nest-site selection, where younger females will select nest-sites with suboptimal microhabitat characteristic for egg development that are closer to ecological edges to reduce the females risk of predation (Harms, 2005; Spencer and Thompson, 2003). Although we did not measure predation pressure at the nest sites, we included distance from forest edge and distance to nearest tree in our suite of variables used in model construction as they also likely impact the thermal characteristics within the nest by affecting the intensity of solar radiation reaching the nest-site.

We used logistic regression to explore the relationship between the microhabitat surrounding nests and microhabitat surrounding random sites. The binary dependent variable was nest site (1) or random site (0). Additionally, since our study sites are spread across MNF, study site was also included as a variable in an attempt to detect how each site might affect nest-site selection. Akaike's information criterion corrected for small sample sizes (AIC_c) was used to rank all models. Important values included the number of parameters in each model, including an intercept (K), the delta AIC_c or the difference between the highest ranked model and the model of interest, the AICc weight (ω_i), the cumulative weight, the log likelihood where smaller values indicate better model fits, and the evidence ratio between the highest ranked model and the model of interest. All analyses for nest-site selection were conducted in program R Studio (version 0.99).

Nest Success Statistical Analysis.— Since nests in our study were protected from depredation the next likely factors to influence nest success were variables that influenced the thermal properties of the nest site. We used the same models from our nest-site selection analysis to predict nest success with the addition of a "thermal squeeze" model (Table 2). The thermal squeeze model was used by Hughes and Brooks (2006) to predict survival to hatch in Midland Painted Turtles (*Chrysemys picta*) as a function of the date of oviposition. The Thermal Squeeze model was originally recommended by Compton (1999) in response to the constraint shorter growing seasons placed on the development of embryos of northern populations of Painted Turtles, resulting in nests laid later in the season not having enough time to develop before winter. In addition to the "thermal squeeze" model, we used the duration of incubation in days as an additional model. Clutch size will likely influence the thermal makeup inside the nest with some eggs from larger clutches potentially being deeper and thus cooler (Schwarzkopf and Brooks, 1987). Additionally the clutch size will also affect the probability of a nest being successful as a larger clutch size has a higher probability of an egg to hatch regardless of environmental characteristics. Because we are unable to separate this probability from the environmental characteristics selected to predict nest success we included clutch size as a covariate in all models related to nest success. Due to our study spanning multiple breeding seasons within the same population, some females nested more than once across years. We included female identity as a variable in an additional series of our models for nest success, as there could be an underlying effect on nest success by particular females that the microhabitat data alone could not address. Our nest success analysis included 32 models with a binary dependent variable where successful nests were assigned a (1) and unsuccessful nests were assigned a (0). We used logistic regression for our analysis between microhabitat data and Box Turtle nest success. We ranked our nest success models using AIC_c and important values included the number of parameters in each model including an intercept (K), the delta $AIC_{c,}$ the AIC_c weight (ω_i), the cumulative weight, the log likelihood, and the evidence ratio of the AIC_c weight (ω_i) between the highest ranked model and the model of interest. All analyses for nest success were conducted in program R Studio (version 0.99).

Nest Temperature and Nest Success.— Although temperature loggers were implanted in all nests monitored from 2013-2016, we were only able to collect temperature data spanning the entire duration of incubation from 43 of our total 58 nests. Due to the smaller sample size, we did not include temperature in our primary models, but rather our models for nest-temperature were run as a separate exploratory analysis. We used logistic regression for our analysis between nest temperature data and Box Turtle nest success with a binary dependent variable where successful nests were assigned a (1) and unsuccessful nests were assigned a (0). We constructed 4 models in an attempt to explore the relationship between direct nest temperatures in the field during incubation and nest success and ranked our models using AIC_c (Table 3). Important values included the number of parameters in each model including an intercept (K), the delta $AIC_{c,}$ the AIC_c weight (ω_i), the cumulative weight, the log likelihood, and the evidence ratio. Models included the average nest temperature over the incubation period, the minimum temperature of the nest during the incubation period, the maximum temperature reached by the nest during the incubation period, and the number of hours a nest was above 22.5 °C. The hours above 22.5 °C model was created in response to a Ewert and Nelson (1991) study which found that the minimum constant egg temperatures that permitted embryonic development in Eastern Box Turtles was 22.5°C. Thus females should select nest-sites that have a suite of microhabitat variables that facilitate nest temperatures above 22.5°C. Further exploratory analysis included the use of Wilcoxon Signed Rank tests to directly compare temperature parameters between successful and unsuccessful nests. We compared all four variables used in our logistic models including average nest temperature over the incubation period, the number of hours a nest was above 22.5 °C, and minimum and maximum temperatures experienced by the nest during incubation. All means of our data are reported as the mean ± 1 standard deviation and the analyses for nest success and nest temperature were conducted in program R Studio (version 0.99).

RESULTS

Nest Surveys and Data Collection.— From 2013 to 2016 we protected and recorded microhabitat data on 58 Eastern Box Turtle nests (EW n = 11, GP n = 13, SV n = 10, TB n = 24) from 40 individual females, as well as 58 random sites (EW n = 11, GP n = 13, SV n = 10, TB n = 24) across our four study sites in MNF (Figure 2). We recorded carapace length, width, and height as well as plastron length, width, and mass for 29 of the total 40 female Eastern Box Turtles that nested between 2013 and 2016. We were not able to record morphometric data for all females as disrupting the individual could have resulted in the female abandoning her nesting attempt. The average carapace length and width for nesting females was 14.6 ± 1.1cm and 11.4 ± 1.3cm respectively. Average carapace height was 69.9 ± 1.2cm and average plastron length and width was 14.1 ± 1.3cm and 9.0 ± .54cm respectively. The average mass of nesting females was 613.2 ± 90.2g.

Thirteen of the 40 female Eastern Box Turtles observed nested across multiple years of the study. The average distance between nests from the same female was 204.5m ± 231.6m from 2013-2016, but ranged from only 1.09m to 715.3m between nesting locations. Further, 11 of the 13 observed females nested within the same opening in consecutive years and only 2 of the 13 females did not nest within the same opening at least once from 2013-2016. Clutch size across sites ranged from 1–11 eggs with an average of 5.6 ± 2.2 eggs and we found no correlation between female carapace length and clutch size (r = -0.13, P = 0.39), female carapace width and clutch size (r = 0.04, P = 0.80), between female mass and clutch size (r = -0.01, P = 0.94), or between female carapace length and average neonate mass per nest (r = -0.03, P = 0.91). All nests with predator-proof exclosures installed (n = 58) were successfully protected from depredation over the course of the incubation season. From 2013-2016 the average incubation time for a nest was 97.83 ± 12.05 days with the shortest incubation period only lasting 71 days

(Table 8). Of the 58 nests observed from 2013 to 2016, 29 nests had at least one hatchling emerge for a nest success rate of 50.0%.

Nest-site selection.— Of our original 15 models, four models were supported in predicting between Box Turtle nest sites and randomly selected sites (Table 3). The top competing models were all within 2 AIC_c units of one another with the next closest model having a Δ_i of 2.19. Top models included the "Thermal+" model ($K = 8$, $AIC_c = 134.24$, $\omega_i = 0.35$, evidence ratio = 1.00), the "Distance to Edge+" model ($K = 8$, $AIC_c = 135.85$, $\Delta_i = 1.61$, $\omega_i = 0.15$, evidence ratio = 2.24), the "Thermal" model ($K = 7$, $AIC_c = 135.93$, $\Delta_i = 1.69$, $\omega_i = 0.15$, evidence ratio = 2.33), and the "Aspect+" model ($K = 6$, $AIC_c = 136.14$, $\Delta_i = 1.91$, $\omega_i = 0.13$, evidence ratio = 2.59). The next closest model to our four top ranked models was the "Global" model ($K = 10$, $AIC_c = 136.14$, $\Delta_i = 2.19$, $\omega_i = 0.12$, evidence ratio = 2.99) (Table 4). The "Thermal+" model was composed of the variables degree of slope, slope aspect, canopy, and percent bare soil. While the next closest three competing models "Distance to Edge+", "Thermal", and "Aspect+" all contained the microhabitat variables percent bare soil and slope aspect. Nest sites had a higher percent bare soil than our randomly selected sites (Figure 3) and females avoided northern facing slopes (Figure 4).

Nest Success.— From the original 33 models we constructed, 3 competing models were well supported in predicting successful and failed nests. Our top ranked model was the univariate model "Clutch Size" ($K = 2$, $AIC_c = 81.07$, $\omega_i = 0.20$, evidence ratio = 1). The next two highest ranked models were the "Bare Soil and Clutch Size" model ($K = 3$, $AIC_c = 82.18$, $\Delta_i = 1.11$, $\omega_i = 0.12$, evidence ratio = 1.74) as well as the "Distance to Edge and Clutch Size" model ($K = 3$, $AIC_c = 82.66$, $\Delta_i = 1.59$, $\omega_i = 0.09$, evidence ratio = 2.21). The next closest model to our three top ranked models was the "Slope and Clutch Size" model ($K = 3$, $AIC_c = 83.13$, $\Delta_i = 2.06$, $\omega_i =$

0.07, evidence ratio = 2.80) (Table 5). Each of the top ranked models contained the variable clutch size; with the probability of nest success increasing as clutch size increases (Figure 5).

Nest Temperature and Nest Success.— The average incubation temperatures of successful nests from 2013-2015 ranged from 20.4 °C to 25.2 °C with a mean of 22.7 °C (Table 6). Average incubation temperatures of successful and unsuccessful nests did not differ significantly ($P = 0.43$). Further, minimum nest temperatures did not differ between successful and unsuccessful nests ($P = 0.93$); nor did maximum nest temperatures ($P = 0.43$). Successful nests incubation temperatures were above 22.5 °C for on average 1260 hours and there was no significant difference ($P = 0.21$) between the number of hours successful nests and unsuccessful nests incubation temperatures were above 22.5 °C (Figure 6).

Of our 4 models constructed to explore nest temperatures effects on nest success only one model had sufficient support in predicting the probability of a nest being successful. The model including only the parameter of maximum temperature reached by a nest during incubation had almost six times the weight as the next closest model ($K = 2$, $AIC_c = 60.46$, $\omega_i = 0.62$, evidence ratio = 1). Our model predicts that as the maximum temperature reached by a nest increases the probability of the nests success decreases. All other models had AIC_c scores greater than 2 AIC_c above the maximum temperature model and lack sufficient support to be considered informative (Table 7).

DISCUSSION

Nest-site selection.— By measuring a suite of microhabitat characteristics from Eastern Box Turtle nests our objective was to examine what microhabitat characteristics influence nest-site selection compared to random locations within the same opening. Lamb et al. (2013) suggested that for a female turtle to be able to preferentially select for a particular microhabitat characteristic that characteristic must occur along a detectable gradient providing information on the direct or indirect influence the characteristic will have on the survival or fitness of the female's offspring. Adhering to this tenet, the microhabitat characteristics we selected to measure were continuous in nature and could provide a gradient of quantity and quality in a natural system. The amount of understory vegetation is often used by many species of turtles to select superior nesting sites (Kolbe and Janzen, 2002; Hughes and Brooks, 2006). In the MNF Eastern Box Turtles preferentially select for nest site locations with a higher percent of bare soil and lower amounts of understory vegetation compared to random sites. Eastern Box Turtles expend great amounts of energy digging nests in the cooler hours of the evening and a greater percent of bare soil could offer an easier place to dig; requiring less energy expenditure than soil with a high root or organic matter content.

Roots from vegetation could pose an obstacle for newly hatched turtles to navigate as they dig toward the surface. While we were unable to measure the root mass found in the soil surrounding nests, failed nests often appeared to have thick root mats enveloping the clutch of eggs which had many hatchlings partially emerged from their eggs, but the majority of the body still within the egg. As previously mentioned, Eastern Box Turtles exhibit temperature-dependent sex determination as well as temperature-dependent developmental rates and by selecting for nest sites with great amounts of bare soil Box Turtles could be selecting for specific thermal qualities that exposed soil provide; such as increased heating capacity. Further, lower vegetation also limits the moisture retention of the soil, which also increases the heating capacity of the soil (Briggs et al., 1997). For Common Green Sea Turtles as well as Loggerhead Sea Turtles the temperature-holding capacity of soil results in varying levels of nest success depending on the soil thermal characteristics (Garmestani et al., 2000; Hays et al., 2001). Thus by selecting for areas of greater percent bare soil Box Turtles are likely selecting areas with optimal thermal properties for embryonic development.

In addition to bare soil, the microhabitat variable aspect was included in all of our top-ranked models. Female Box Turtles in MNF preferentially selected against nest-sites that had north facing slopes. By constructing nests on slopes that are not facing north it is likely that females are selecting sites that would provide a longer period of solar exposure during the day. This extended exposure would increase the heat provided to the nest by solar radiation and could increase the rate of development of the offspring (Brady and Weil, 2000). At higher latitudes where MNF is located, the hours of sunlight a day are often longer than at lower latitudes during nesting season (June in MNF) and could make up for the fewer number of growing degree days experienced at higher latitudes. Because the MNF population of Box Turtles is one of the furthest northern located Box Turtle populations in the United States, slope aspect could have an even more profound effect on the thermal properties of nests (Ewert and Nelson, 2005); making slope aspect a strong basis for nest-site selection in Eastern Box Turtles.

Canopy cover was also a parameter in our top-ranked model as well as our 3rd-ranked model, which predicts female Eastern Box Turtles are less likely to nest in areas with a high percent of canopy cover (Figure 7). It is well documented that Eastern Box Turtles almost exclusively nest in forest openings and thus areas with low canopy cover (Dodd, 2001). By limiting our selection of random sites to within the forest openings used by Eastern Box Turtles we may have induced a sampling effect by limiting the variability of canopy cover due to the overall low canopy cover within the openings. As a result the limited variation within the openings could have resulted in a low power of detection of differences between nest sites and random sites with our sample size. Within certain species of turtles though there is great variation in the selection of canopy cover. For example American Snapping Turtles select for higher canopy cover at lower latitudes and individuals at northern latitudes will select for lower canopy cover (Wilson, 1998; Ewert et al., 2005; Hughes and Brooks, 2006). Similar studies to ours conducted in Illinois found that canopy cover best predicted Painted Turtle nest-site selection even though the percent canopy cover selected for by females varied greatly within the same population (Janzen and Morjan, 2001). Like Eastern Box Turtles, Painted Turtles also exhibit temperature-dependent sex determination and canopy cover has predictable effects on the thermal environment of turtle nests. Thus it is possible that within openings Eastern Box Turtles are using canopy cover to manipulate the sex ratio of their offspring by selecting sites with a range of thermal properties. This sex ratio selection would be the result of the evolution of macro-spatial as well as micro-spatial natal philopatry in conjunction with temperature-dependent sex determination (Bulmer and Bull, 1982; Bull et al., 1982; Janzen, 1994; Janzen and Morjan, 2001, Refsnider and Janzen, 2010).

While the distance from a nest to the forest edge was included as a parameter in our 2nd ranked model, the 'Distance to Edge+" model also included degree of slope, slope aspect, canopy, and bare soil as the remaining parameters. Since all but two of those parameters (degree of slope and distance to forest edge) are included in our top model and all of our top-ranked models were > 2 AIC_c units apart from one another, it is likely that the parameters not included in our top-ranked model are not biologically meaningful. Using AIC_c ranking it is possible to take any well-supported model and add a single nonsensical parameter and the result would be a new model that is < 2 AICc units from the well-supported model. As such the addition of parameters in lower ranked models that are absent from the top ranked model should not be used to make strong biological inferences (Arnold, 2010).

Despite our results, distance to edge should not be abandoned in future studies as a potentially important microhabitat variable in regard to nest-site selection. According to our model female Box Turtles do preferentially select nesting locations closer to the forest edge (Figure 8). The reason for this however could be more related to the female's survival than the nest's success. Box turtles are a long-lived, iteroparous species and may shift nest-site priorities as they age. While nesting further from the forest edge may decrease the chance of nest predation (Temple, 1987; Dijak and Thompson, 2000; Herkert et al., 2003), it increases the energy required from the female to travel the increased distance and places her at a greater risk of predation and potentially desiccation as she moves through the opening with little cover.

Female Box Turtles could also shift their focus from their own survival to the survival of the nest as they age and the number of potential reproductive events dwindles increasing the relative value of each remaining event. For example, female Painted Turtles have been observed to nest farther from the safety of the water's edge for nest sites with environmental characteristics more conducive to embryonic development as they increase in age (Harms et al., 2005). Whether this increase in distance travelled for nesting is the result of increased risk taking or possibly the result of an increase in the female's nesting experience, depending on the average age of the population one is observing, the relationship between distance to forest edge and nest-site selection could change and should only be considered in conjunction with female age. Females in our study also displayed evidence of nest site fidelity; selecting nesting sites within as little as 1.09m from the previous years nest-site further complicating our understanding of the relationship between distance to forest edge, nest-site selection, and female age.

Nest Success.— The success rate of our observed nests was 50.0% which is similar to values of Box Turtle survival to hatching in current literature that excluded depredation (Willey and Sievert, 2012). Studies that did not exclude depredation have recorded success rates from 24-94% with large variation both between and among populations of Eastern Box Turtle (Ewing, 1933; Congello, 1978; Dodge et al., 1978; Stuart and Miller, 1987; Burke and Capitano, 2011). AIC_c scores indicate the highest-ranking model in predicting nest success was the single variable model of clutch size. Our top ranked model reveals that as the clutch size of a nest increases the probability of nest success also increase. There are many possible reasons for clutch size and nest success to display a positive relationship and previous studies of other reptiles have observed similar results. Turtles from larger clutches may be better able to dig through the compacted soil covering the nest to escape the nest chamber (Nagle et al., 2004). At our field sites in particular we anecdotally noticed considerable root growth from grasses over the course of the incubation period and it is possible that a larger clutch size might be better able to dig through not only the soil but also the root structures covering the nest to reach the surface.

Clutch size can also influence the hydrological conditions surrounding each egg within the nest. Brown and Shine (2009) found that clutch size affected the ability of the eggs to uptake water in Keelback Snakes (*Tropidomophis mairrii*) and allowed for larger clutches to retain more water through incubation increasing nest success. While Radder and Shine (2007) found that in scincid lizard nests less water was available to eggs in larger clutches and resulted in smaller offspring size. We were unable to measure nest humidity or water retention of the Box Turtle nests in the field but it is likely that there is a more complicated relationship between clutch size and nest success in the MNF population that should be explored further in future studies.

While many species of reptiles exhibit a positive correlation between female size and clutch size (Iverson, 1992), this correlation seems to vary depending on the population in Eastern Box Turtles (Congdon and Gibbons, 1985; Tucker et al., 1999; Kipp, 2003; Burke and Capitano, 2011). Our population over the four years of observation displayed no correlation between female carapace length or width and the size of the clutch produced. However, no measurements of clutch mass were collected during our study which may be correlated with female size. Further, because of food or environmental limitations clutch sizes and egg mass can vary from year to year on an individualized basis depending on the energy available to the female for egg development (Rowe, 1994; Madsen and Shine, 1999; 2000). In order to accurately measure these changes in reproductive success on an individualized level we would need to observe the MNF population of Box Turtles for a longer period of time than this study allowed.

Percent bare soil was included in our second ranked model along with clutch size in predicting nest success. Our model found that as the percent bare soil increased at a nest site the probability of nest success decreased (Figure 9). The explanation for the negative relationship between the percent of bare soil at a nest site and the probability of nest success is likely related to nest temperatures and the moisture retention of the nest. As mentioned in our nest-site selection models, which displayed a positive relationship between the percent of bare soil at a site and the probability of a female selecting that site to nest, the percent of bare soil at a site will greatly influence the thermal environment of the nest. While a high percentage of bare soil might be appealing to a female Box Turtle for the ease of digging it provides as well as it's increased retention of solar radiation, in large openings with little canopy and low vegetation cover nest temperatures could become too high for embryos to survive.

Other soil characteristics that we were unable to directly measure, such as the potential moisture retention of the soil, could also be important to the successful development of Box Turtle embryos. Packard et al. (1987) found that one of the substrate characteristics that had the greatest effect on hatching success in Common Snapping Turtles was the moisture level of the substrate with moist soils yielding the highest hatchling success. Further, the survival of Painted Turtle nests is positively correlated with higher soil moisture rather than within-nest temperatures (Cagle et al., 1993, Morjan, 2003). However it is still unclear how Box Turtle eggs respond to varying levels of soil moisture as increased soil moisture decreases nest temperatures during incubation (Morjan, 2003). Thus, soil moisture, like other microhabitat variables, may be selected for differently across a species range or within populations. Although Box Turtles are generally more resistant to desiccation compared to more aquatic pond turtle species, adult Box Turtles frequently rely on refuges with microhabitats that provide greater ambient humidity as well as lower surface temperatures than random sites from May through August, often the driest and hottest months of the year (Rossell et al., 2006). These months are also the time in which Box Turtle nests are incubating and could be susceptible to desiccation. The high temperatures and decreased rain fall during these months would make soil moisture an important characteristic to the survival of Box Turtle nests and future studies should focus on collecting information on the moisture retention of soils found in nesting sites. Examining the soil composition in and around Box Turtle nests would provide more information and insight into what microhabitat characteristics influence the survival of a Box Turtle nest or if female Box Turtles are somehow able to distinguish superior soil compositions for embryonic development.

Our third ranked model for predicting nest success included the distance from a nest to the forest edge and clutch size; with the probability of nest success increasing as the distance from the forest edge increases. Previous studies have explained this relationship as the result of nest predation where nests closer to ecological edges were easier and more readily found by predators than nests laid further from ecological edges (Temple, 1987; Dijak and Thompson, 2000; Herkert et al., 2003). However our study excluded the potential for nest predation by protecting our nests with exclosures. A likely explanation for this pattern could be that the distance to the nearest tree relates to the amount of shade a nest experiences throughout the day and thus alters the potential thermal properties of a nest site initially selected for by the female regardless of the quality of microhabitat immediately surrounding the nest.

Conclusions.— Our study adds to the current foundation of literature on the life history of Eastern Box Turtles but from the northern extent of its range, which has received little attention. Our study provides greater insight into which microhabitat parameters are most important when attempting to manage forests to facilitate the persistence of the Eastern Box Turtle at it's northern range limit. Through the use of manual clearing managers could increase the amount of bare soil as well as maintain openings in the forest canopy while limiting the amount of fire-related injuries and deaths of Box Turtles caused by traditional clearing methods such as prescribed burning. Additionally our models show that larger openings with greater distances to the forest edge as well as an increase in the amount of bare soil could affect the survival of Box Turtle nests. Thus by maintaining the size of the current forest openings and creating new larger clearings in MNF, managers could increase the number of available openings to nesting females and ideally increase the current survival of Eastern Box Turtle nests to ensure the persistence of this species into the future.

While our study revealed a clear preference for nesting Eastern Box Turtles to select areas with greater percent bare soil and to avoid sites with northern facing slopes, the relationship between the selected microhabitat variables and those we did not collect (e.g., soil, characteristics, clutch size impacts on microclimate and humidity in the nest chambers) require future investigation. Our study also revealed complex relationships among the same parameter when comparing its effect on nest-site selection versus nest success. While a greater percent of bare soil was a strong predictive parameter in nest-site selection in the observed Box Turtle population, a greater percent bare soil had a negative affect on the survival of the nests. While the reason for this contrasting result is unclear, it is possible that it is the result of yearly variations in summer temperatures and season length. Using a subset of our data we explored the impact of nest temperatures at our field sites on nest success and found that the maximum temperature within the nest cavity displayed a negative relationship with nest success. Thus it is possible that unusually hot days with little rain could have had a negative impact on the survival of the nests during our study period. To gain a greater understanding of the impact of seasonal variation on the persistence of the MNF population longer-term studies should be conducted. The complex relationship between the microhabitat at nest-sites and the survival of the embryos to hatch highlights the precarious position Eastern Box Turtles occupy as the threat of climate change increases. Studies like ours should be conducted more frequently and over greater periods of time to better understand how to mitigate the impacts of habitat destruction and fragmentation as well as climate change on not just Eastern Box Turtles but all species that exhibit TSD and require a specific suite of environmental variables to successfully reproduce and persist.

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TABLE 1.— List of models constructed to differentiate nest sites selected by female Eastern Box Turtles and randomly selected sites in the Manistee National Forest from 2013-2016. Model titles as well as habitat parameters are listed.

Model Title	**Habitat Parameters**
Bare Soil	Bare soil
Aspect	Aspect
Canopy	Canopy
Slope	Slope
Distance to Tree	Distance to tree
Distance to Edge	Distance to edge
Aspect+	Aspect + bare soil
Canopy+	Canopy + bare soil
Thermal	Aspect + canopy + bare soil
Thermal+	Aspect + canopy + bare soil + slope
Distance to Tree+	Distance to tree + aspect + bare soil + slope
Distance to Edge+	Distance to edge + aspect + bare soil + slope
Slope+Aspect	Slope + aspect
Slope:Aspect interaction	Slope : aspect
Global Model	Aspect + canopy + bare soil + distance to edge + distance to edge

TABLE 2.— List of models constructed to differentiate successful Eastern Box Turtle nests and unsuccessful nests in the Manistee National Forest from 2013-2016. Model titles as well as habitat parameters are listed.

Model Title	**Habitat Parameters**
Clutch Size	Clutch size
Bare Soil and Clutch Size	Bare soil + clutch size
Bare Soil and Female	Bare soil + clutch size + female I.D.
Aspect and Clutch Size	Aspect + clutch size
Aspect and Female	Aspect + clutch size + female I.D.
Canopy and Clutch Size	Canopy + clutch size
Canopy and Female	Canopy + clutch size + female I.D.
Slope and Clutch Size	Slope + clutch size
Slope and Female	Slope + clutch size + female I.D.
Distance to Tree and Clutch Size	Distance to tree + clutch size
Distance to Tree and Female	Distance to tree + clutch size + female I.D.
Distance to Edge and Clutch Size	Distance to edge + clutch size
Distance to Edge and Female	Distance to edge + clutch size + female I.D.
Aspect+ and Clutch Size	Aspect + bare soil + clutch size
Aspect+ and Female	Aspect + bare soil + clutch size + female I.D.
Canopy+ and Clutch Size	Canopy + bare soil + clutch size
Canopy+ and Female	Canopy + bare soil + clutch size + female I.D.
Thermal and Clutch Size	Aspect + canopy + bare soil + clutch size
Thermal and Female	Aspect + canopy + bare soil + clutch size + female I.D.
Thermal+ and Clutch Size	Aspect + canopy + bare soil + slope + clutch size
Thermal+ and Female	Aspect + canopy + bare soil + slope + clutch size + female I.D.
Distance to Tree+ and Clutch Size	Distance to tree + aspect + bare soil + slope + clutch size
Distance to Tree+ and Female	Distance to tree + aspect + bare soil + slope + clutch size + female I.D.
Distance to Edge+ and Clutch Size	Distance to edge + aspect + bare soil + slope + clutch size
Distance to Edge+ and Female	Distance to edge + aspect + bare soil + slope + clutch size + female I.D.
Slope+Aspect and Clutch Size	Slope + aspect + clutch size
Slope+Aspect and Female	Slope + aspect + clutch size + female I.D.
Slope:Aspect interaction and Clutch Size	Slope : aspect + clutch size
Slope:Aspect interaction	Slope : aspect + clutch size + female I.D.
Thermal Squeeze	Oviposition date + clutch size
Thermal Squeeze and Female	Oviposition date + clutch size + female I.D.

TABLE 3.— List of models constructed to differentiate successful Eastern Box Turtle nests and unsuccessful nests by in nest temperature in the Manistee National Forest from 2013-2016. Model titles as well as temperature parameters are listed.

Model Title	**Temperature Parameters**
Average Temp	Average nest temperature
Min Temp	Minimum nest temperature
Max Temp	Maximum nest temperature
Hours Above	Total hours nest was above 22.5˚C

TABLE 4.— Akaike's information criterion corrected for small sample size and the log likelihood for the top four competing models to predict nest-site choice of Eastern Box Turtles in the Manistee National Forest over randomly selected sites from 2013-2015. Models with AICc scores greater than two AICc units above the top-ranked model were excluded. K is the number of parameters plus an intercept, Δ_i is the AIC_c delta or change in AIC_c from the top ranked model and the model of interest, and (ω_i) is the AIC_c weight.

Model Title	**Model Variables**	**K**	**AIC_c**	**Δ_i**	**ω_i**	**Log Likelihood**	**Evidence Ratio**
Thermal+	Aspect, Slope, Canopy, Bare soil	8	134.24	--	0.35	-58.45	1
Distance to Edge+	Distance to edge, Aspect, Slope, Bare soil	8	135.85	1.61	0.15	-59.26	2.24
Thermal	Aspect, Canopy, Bare soil	7	135.93	1.69	0.15	-60.45	2.33
Aspect+	Aspect, Bare soil	6	136.14	1.91	0.13	-61.69	2.59

TABLE 5.— Akaike's information criterion corrected for small sample size and the log likelihood for the three competing models to predict to predict nest success for Eastern Box Turtles in the Huron-Manistee National Forest from 2013-2015. Models with AIC_c scores greater than two AIC_c units above the top-ranked model were excluded. K is the number of parameters plus an intercept, Δ_i is the AIC_c delta or change in AIC_c from the top ranked model and the model of interest, and (ω_i) is the AIC_c weight.

Model Name	**K**	**AIC_c**	**Δ_i**	**ω_i**	**Log Likelihood**	**Evidence Ratio**
Clutch Size	2	81.07	--	0.20	-38.43	1.00
Bare Soil and Clutch Size	3	82.18	1.11	0.12	-37.87	1.74
Slope and Clutch Size	3	82.66	1.59	0.09	-38.11	2.21

TABLE 6.— Temperature attributes of successful and unsuccessful Eastern Box Turtle nests recorded on temperature loggers placed within nests for the duration of incubation.

	Successful		**Unsuccessful**	
	Mean ± SD	Range	Mean ± SD	Range
Avg. Nest Temp. (°C)	22.7 ± 1.4	20.4 – 25.2	23.0 ± 2.2	18.3 – 26.9
Avg. Minimum Nest Temp. (°C)	10.6 ± 2.9	4.1 – 14.6	10.0 ± 4.7	-1.4 – 15.6
Avg. Maximum Nest Temp. (°C)	36.2 ± 2.9	31.1 – 43.6	37.7 ± 3.9	32.1 – 47.1
Avg. Hours Above 22.5 °C	1243 ± 252	881 - 2047	1276 ± 271	673 - 1691

TABLE 7.— Akaike's information criterion corrected for small sample size and the log likelihood for all models used to predict nest success for Eastern Box Turtles in the Manistee National Forest by temperature parameters from 2013-2015. Models with AIC_c scores greater than two AIC_c units above the top-ranked model were excluded from discussion. K is the number of parameters plus an intercept, Δ_i is the AIC_c delta or change in AIC_c from the top ranked model and the model of interest, and (ω_i) is the AIC_c weight.

Model Name	**K**	**AIC_c**	**Δ_i**	**ω_i**	**Log Likelihood**	**Evidence Ratio**
Max Temp	2	60.46	--	0.62	-28.08	1
Average Temp	2	63.57	3.11	0.13	-29.63	4.73
Min Temp	2	63.64	3.18	0.13	-29.67	4.91
Hours Above	2	63.71	3.25	0.12	-29.70	5.09

TABLE 8.— The range as well as mean and standard deviation (SD) of each habitat parameter collected from Eastern Box Turtle nests in Manistee National Forest from 2013-2016.

*Collected from a June 2015 nest in which neonates overwintered and first emerged in late May of 2016. Because exact date of hatch could not be determined the value was removed from the calculations for mean incubation period.

Habitat Parameter	**Range**	**Mean ± SD**
Percent Bare Soil	5 – 95%	54.82 ± 29.22%
Percent Canopy Cover	0 – 100%	12.99 ± 16.54%
Degree of Slope	0 – 35˚	8.79 ± 8.99˚
Distance to Forest Edge	0.57 – 69.27 m	13.18 ± 13.06m
Distance to Nearest Tree	2.50 – 18.80 m	8.25 ± 3.71m
Incubation Period	71 – 358* d	97.83 ± 12.05 d
Minimum Nest Temperature	-1.44 – 15.56˚C	10.31 ± 3.93˚C
Average Nest Temperature	18.29 – 26.91˚C	22.89 ± 1.87˚C
Maximum Nest Temperature	31.13 – 47.10˚C	36.93 ± 3.51˚C
Hours Above 22.5˚C	673 – 2047 h	1259.84 ± 259.56 h
Clutch Size	1 – 11 eggs	5.6 ± 2.2 eggs

Figure Legends

I Map displaying the State of Michigan with the boundary of Manistee National Forest (MNF) and a zoomed view of the location of the 4 study sites relative to one another within MNF.

II Map displaying the 4 study site boundaries for the Turtle Bowl (A), East-West (B), the Gravel Pit (C), and the Savanna (D). As well as the location of all Eastern Box Turtle nests recorded from 2013 – 2016.

III Plot displaying Eastern Box Turtle nest-site selection probability versus the microhabitat parameter percent bare soil using our logistic regression models for nest-site selection.

IV Plot displaying Eastern Box Turtle nest-site selection probability versus the microhabitat parameter slope aspect using our logistic regression models for nest success. The x-axis is labeled with the four cardinal directions of the compass (N= north, S= south, E = east, W= west) and the category X that represents nest-sites that had no micro-slope and thus no slope aspect.

V Plot displaying the probability of Eastern Box Turtle nest success versus the nest parameter clutch size using our logistic regression models for nest success.

VI Four plots of comparisons of nest temperature parameters between successful and unsuccessful nests. A = comparison of maximum nest temperatures reached by successful and unsuccessful nests; B = comparison of minimum nest temperatures reached by successful and unsuccessful nests; C = comparison of average nest temperatures over the incubation period of successful and unsuccessful nests; D = comparison of the number of hours nest incubation temperatures were above 22.5 °C between successful and unsuccessful nests.

FIG.1.—

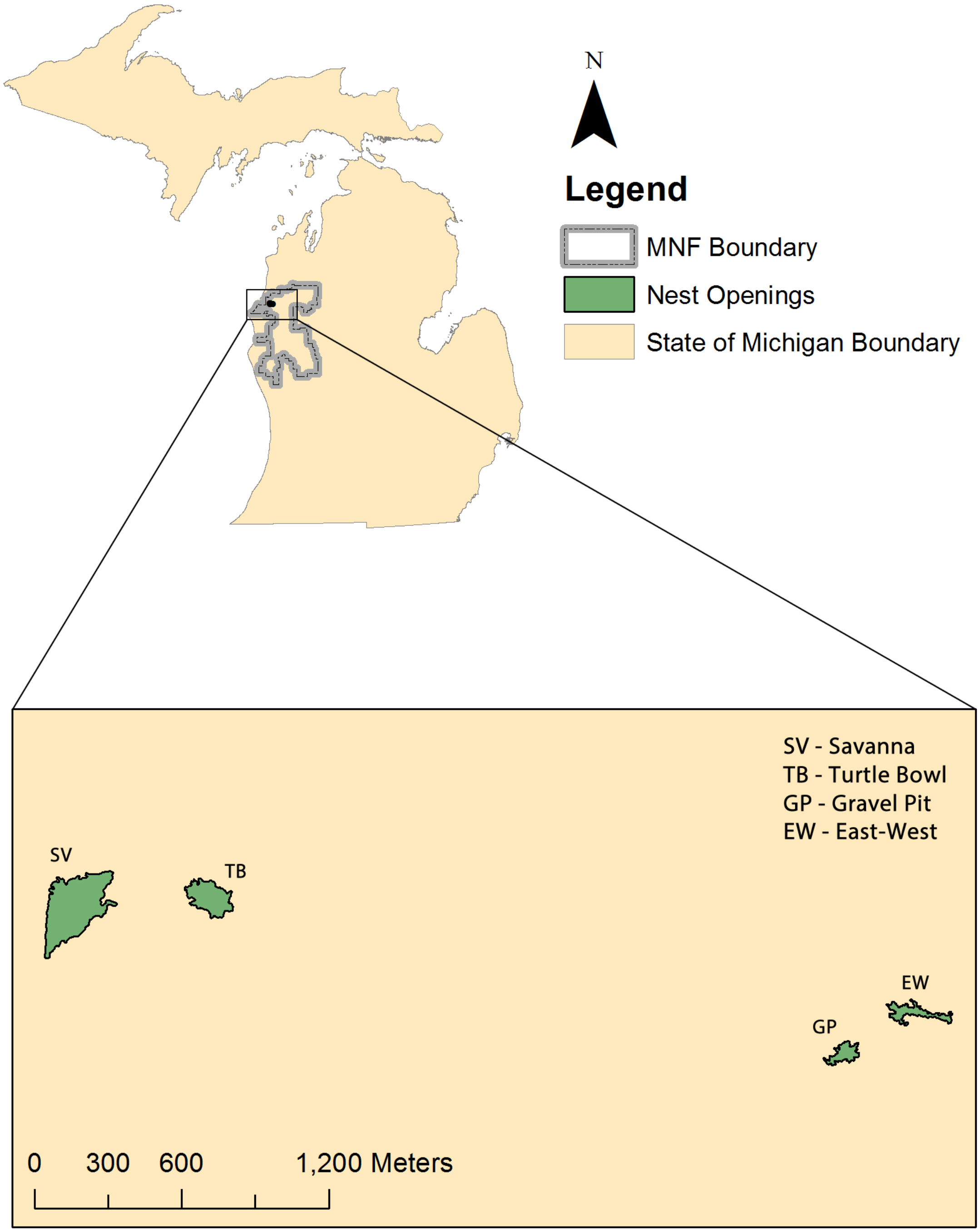

N
Legend
MNF Boundary
Nest Openings
State of Michigan Boundary
SV - Savanna
TB - Turtle Bowl
GP - Gravel Pit
EW - East-West
SV
TB
EW
GP
0
300
600
1,200 Meters

FIG.2.—

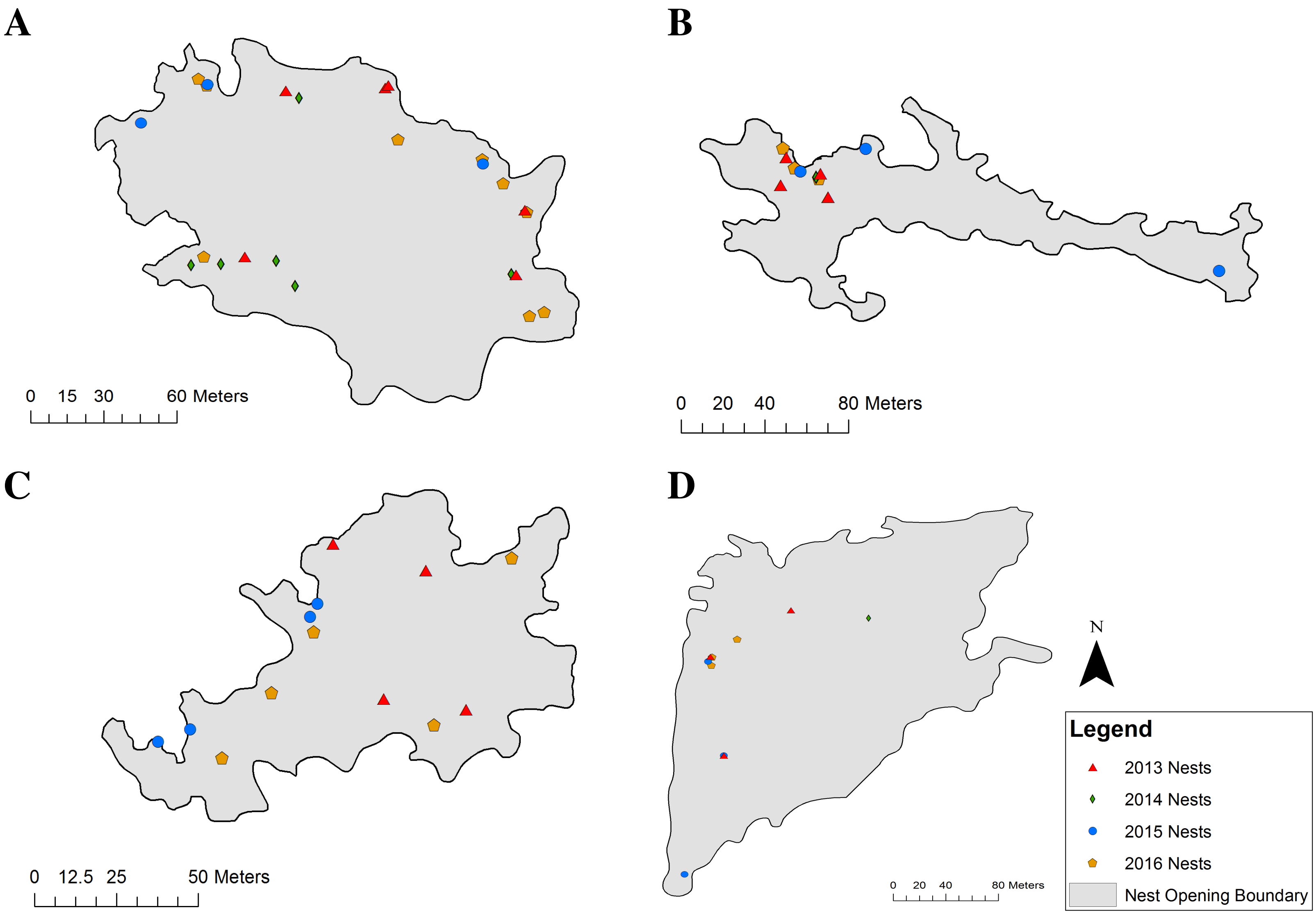
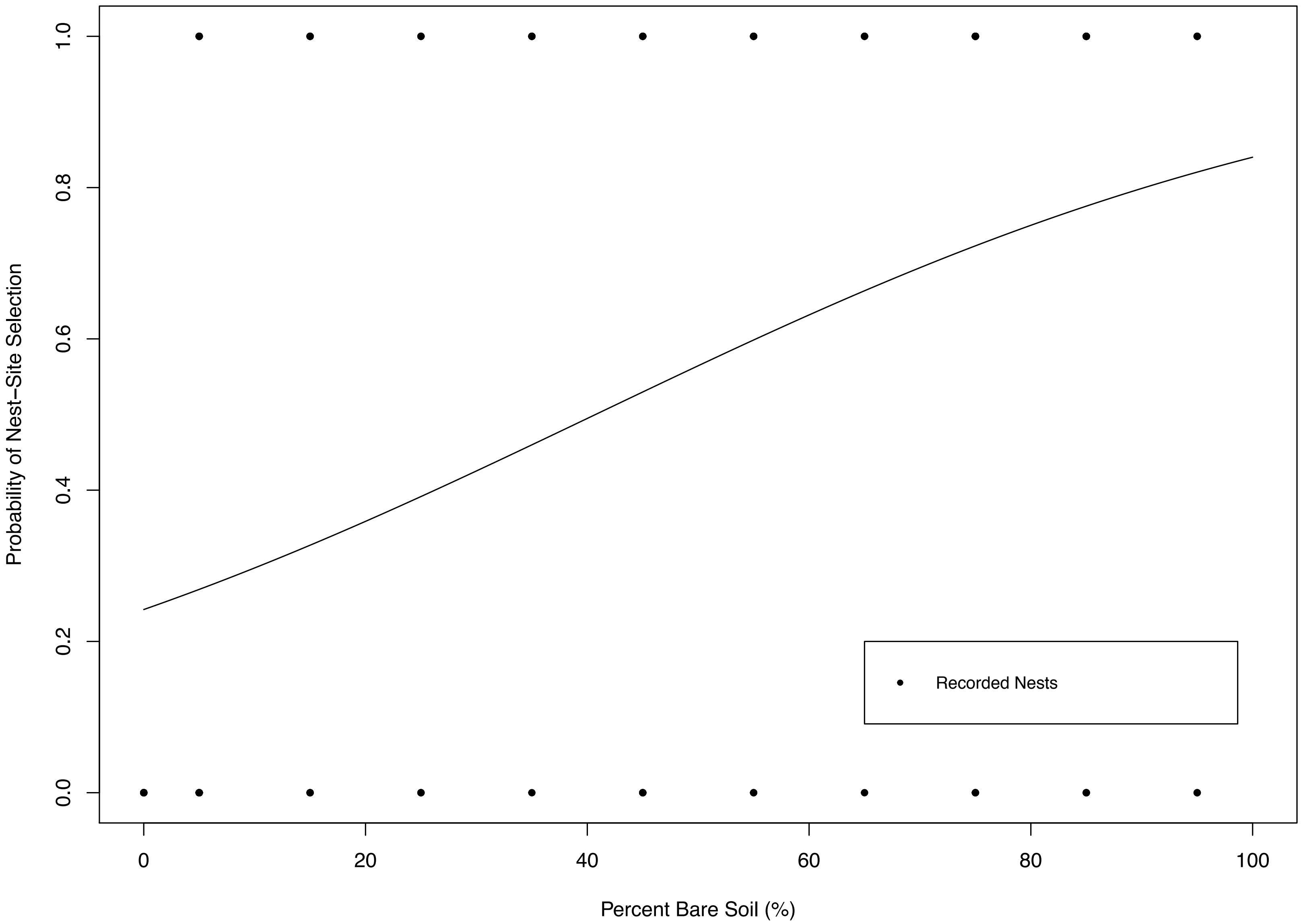

A
0 15 30 60 Meters
B
0 20 40 80 Meters
C
0 12.5 25 50 Meters
D
0 20 40 80 Meters
N
Legend
▲ 2013 Nests
◆ 2014 Nests
● 2015 Nests
⬟ 2016 Nests
▨ Nest Opening Boundary

FIG.3.—



Probability of Nest−Site Selection
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Percent Bare Soil (%)
Recorded Nests

FIG.4.—

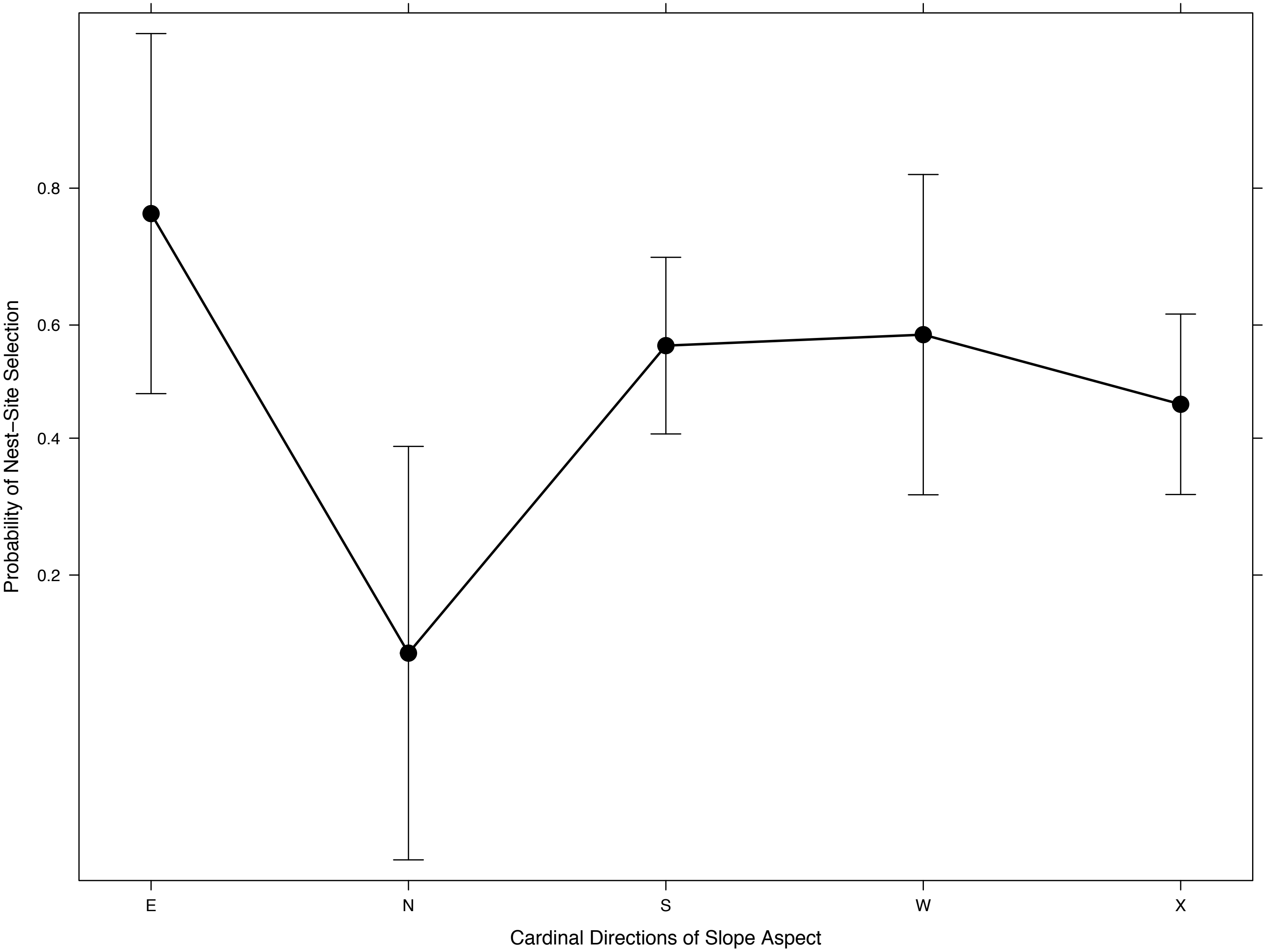

Probability of Nest−Site Selection
0.8
0.6
0.4
0.2
E
N
S
W
X
Cardinal Directions of Slope Aspect

FIG.5.—

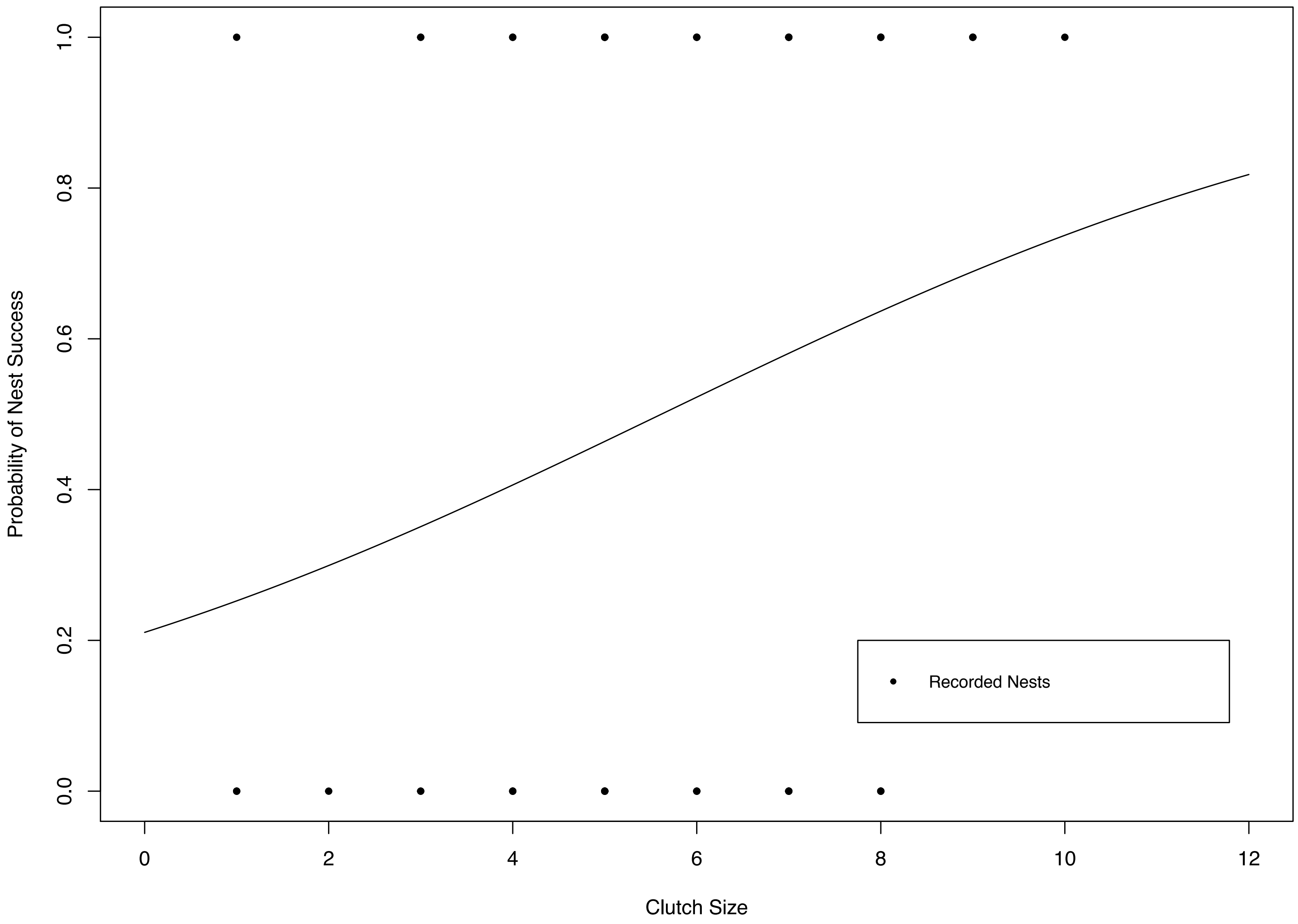

Probability of Nest Success
0.0
0.2
0.4
0.6
0.8
1.0
Clutch Size
0
2
4
6
8
10
12
● Recorded Nests

FIG.6.—

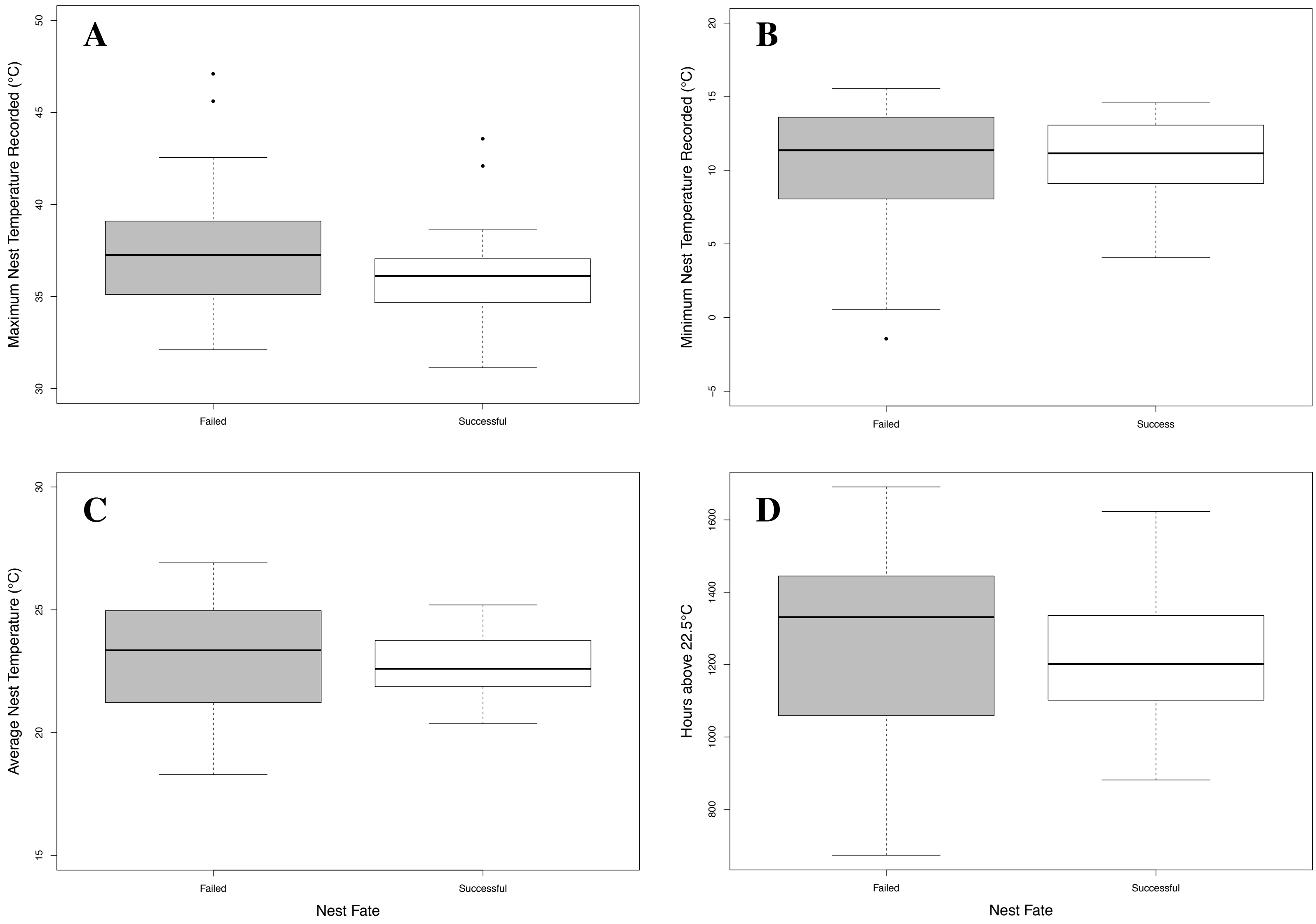

A
Maximum Nest Temperature Recorded (°C)
30
35
40
45
50
Failed
Successful
B
Minimum Nest Temperature Recorded (°C)
-5
0
5
10
15
20
Failed
Success
C
Average Nest Temperature (°C)
15
20
25
30
Failed
Successful
Nest Fate
D
Hours above 22.5°C
800
1000
1200
1400
1600
Failed
Successful
Nest Fate

FIG.7.—

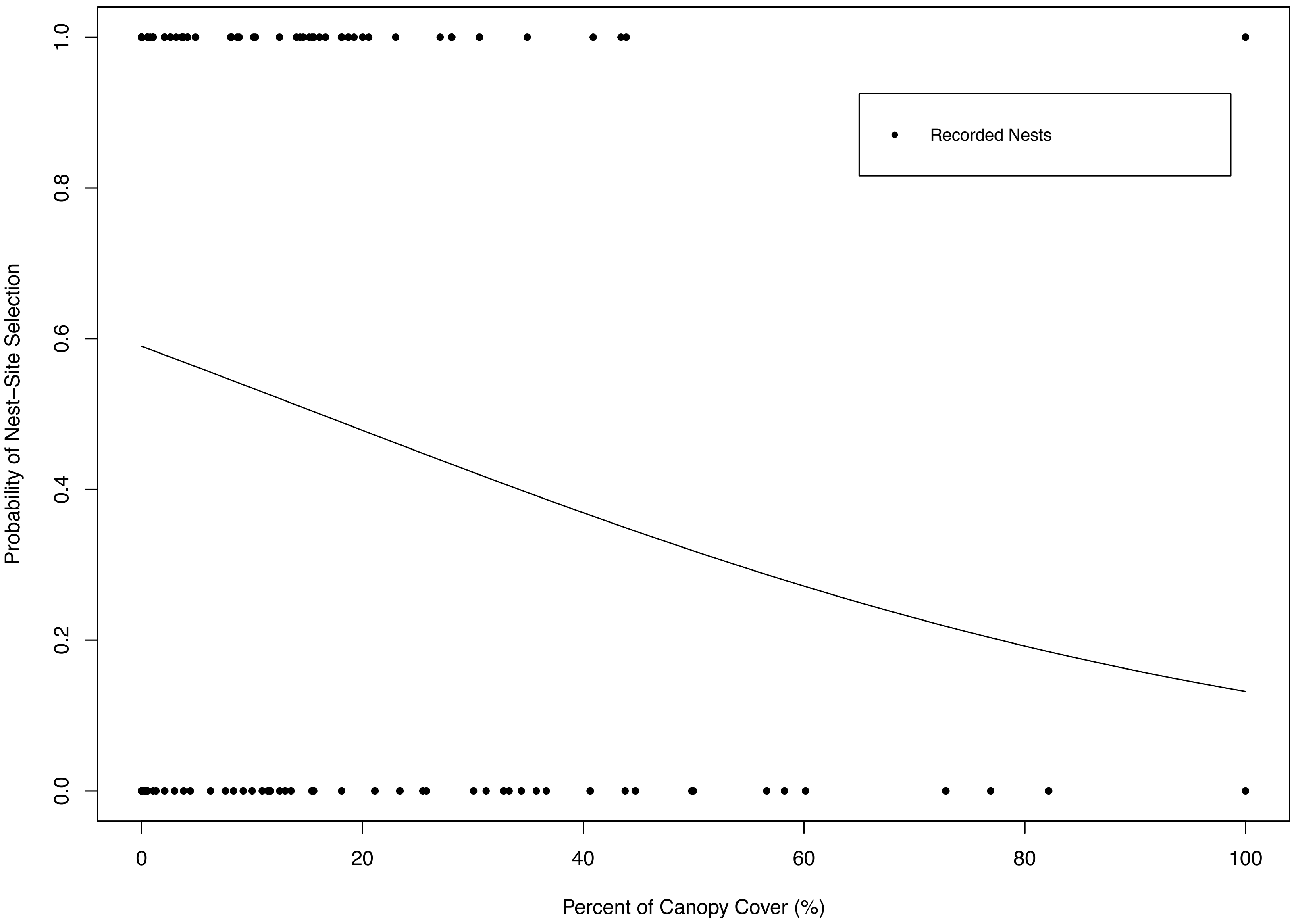

Probability of Nest−Site Selection
1.0
0.8
0.6
0.4
0.2
0.0
Percent of Canopy Cover (%)
0
20
40
60
80
100
● Recorded Nests

FIG.8.—

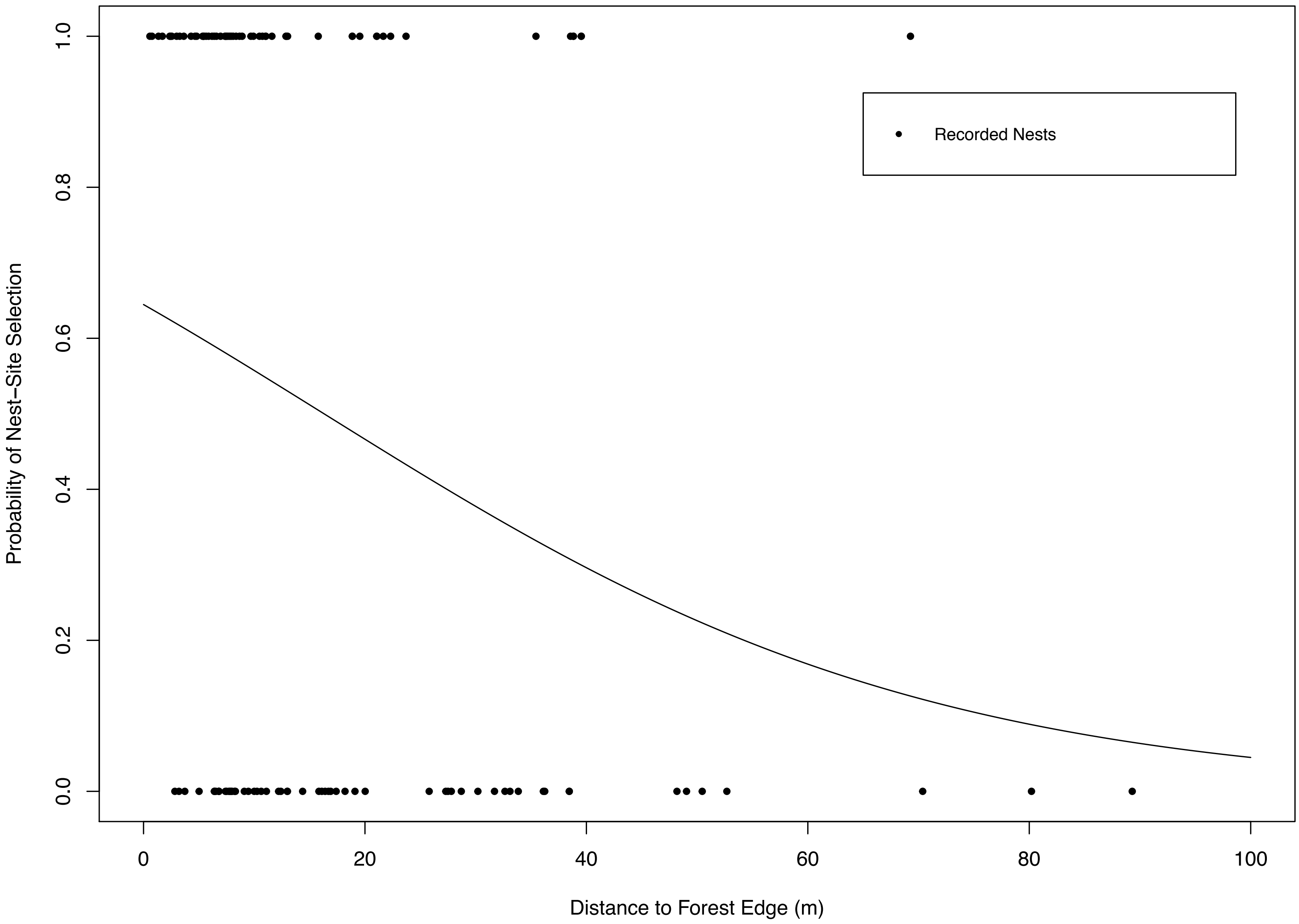
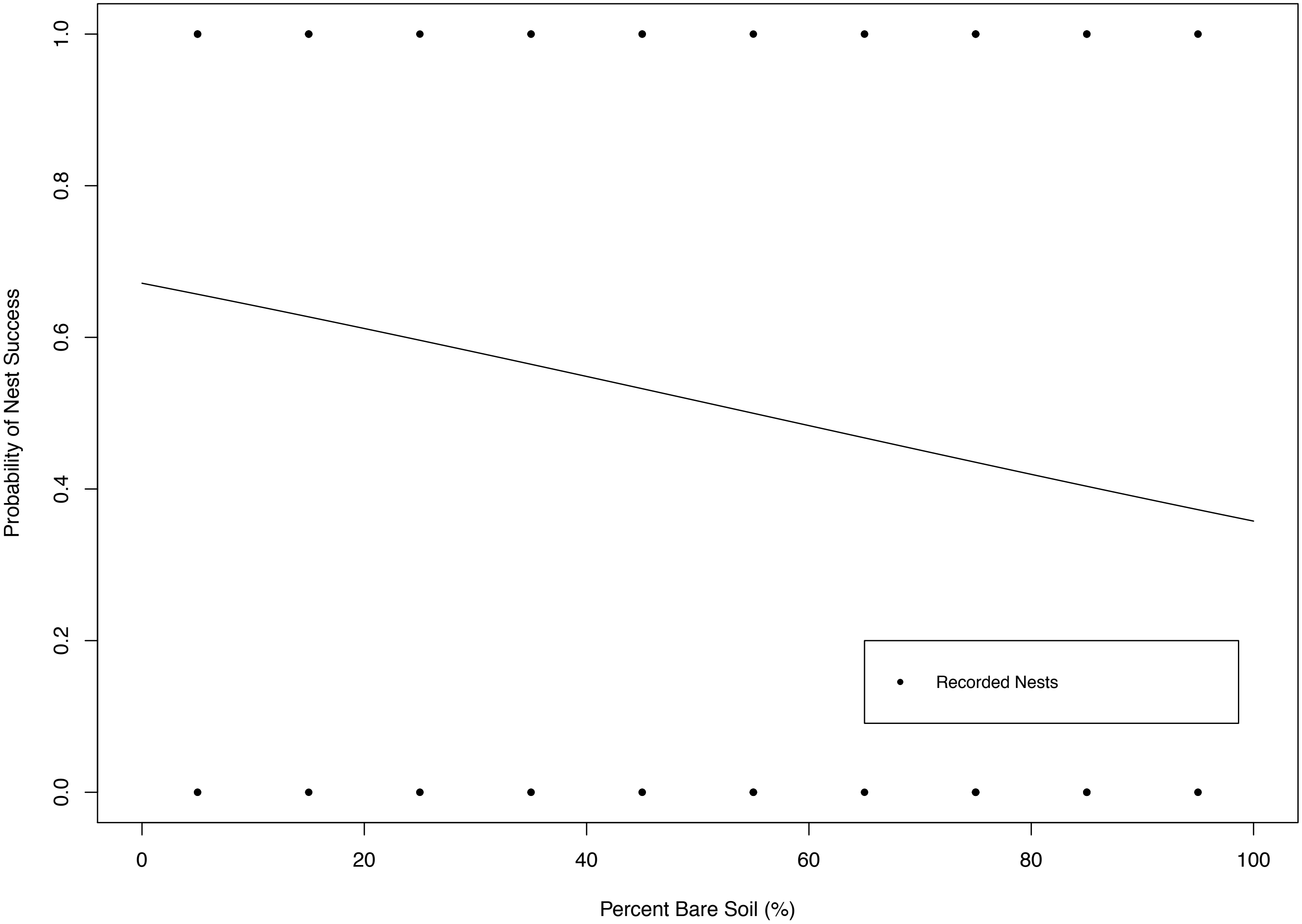

Recorded Nests
Probability of Nest–Site Selection
0.0
0.2
0.4
0.6
0.8
1.0
Distance to Forest Edge (m)
0
20
40
60
80
100

FIG.9.—



Probability of Nest Success
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Percent Bare Soil (%)
● Recorded Nests

First year survival of neonate Eastern Box Turtles (*Terrapene carolina carolina*) at their northern range limit in Michigan

ABSTRACT

Turtles (Order Testudines) are experiencing global declines largely due to anthropogenic influences such as habitat fragmentation, illegal collection and sales, and the threat of global climate change. Historically, turtles compensated for low annual recruitment through multiple reproductive events across an adult's lifetime. This strategy relies on high adult survivorship for the persistence of a population but with the increased removal of individuals from the adult age-class in many turtle species there is now a greater need to understand the survival of neonate and juvenile age-classes to ensure the continued persistence of current populations. The objective of our study was to estimate annual survival of neonate Eastern Box Turtles (*Terrapene carolina carolina*) through the first year of life at the northern limit of the species range. We collected radio telemetry data and used Kaplan-Meier estimators modified for staggered-entry to create known-fate annual survival estimates. Annual survival estimates for the 2013-2015 neonate cohorts estimated neonate survival to decrease through the first year of life with a steep drop in the probability of survival from nest emergence in the fall, before leveling off at 50% for overwintering (day 50 = .503; SE = 0.067), then began to gradually decrease again with spring emergence till reaching 0% survival short of the 1 year mark (day 335 = 0.0). Our study identified depredation and exposure to suboptimal environmental conditions as the main sources of neonate mortality. The challenges to a species survival at the limits of its range are often different from those faced by individuals more centrally located within the species range. Thus while our study found that exposure to sub-freezing temperatures during the first activity season out of the nest negatively affected a neonates probability of survival, similar studies should be conducted across the geographic range of the Eastern Box Turtle to determine the threat severe weather occurrences pose to the persistence of other populations. Additionally managers should increase the size and number of forest openings within the national forest to provide more suitable nesting habitat for female Eastern Box Turtles and distribute neonates across the landscape to prevent large-scale depredation events in future cohorts.

Key words: Box Turtle; Neonate; Survival; Kaplan-Meier; depredation; *Terrapene c. carolina*

INTRODUCTION

The Order Testudines (turtles) represents some of the most morphologically unique and longest-lived species in the world. Despite many species' extreme longevity, turtles are experiencing global declines largely due to anthropogenic influences such as habitat fragmentation, illegal collection and sales, and the increased threat of global climate change (Gibbson et al., 2000; Dodd 2001, Refsnider and Janzen, 2012). Life history traits shared by most turtles dramatically increase the effects of these stressors. Because of their long life span it can take up to ten years for juvenile turtles to reach sexual maturity. Once sexually mature, turtles may only produce one or two clutches of eggs a year, typically with low hatching success (Dodd, 2001). Historically turtles compensated for low annual recruitment through multiple reproductive events across an adult's lifetime. This strategy relies on high adult survivorship for the persistence of a population (Congdon et al., 1993).

These commonly held life history traits intensify the impact anthropogenic stressors have on turtle populations by reducing adult survivorship in many species of turtle making viability of populations in disturbed areas especially difficult (Nazdrowicz, 2008). With the increased removal of individuals from the adult age-class of turtle populations there is now a greater need to understand the survival of neonate and juvenile age-classes to ensure continued recruitment and the persistence of the populations.

Much of the previous research concerning yearly survival of North American turtles has focused on the adult age-class (Metcalf and Metcalf, 1979; Iverson, 1991; Hall et al., 1999; Fredericksen, 2014; Agha et al., 2017). Far fewer studies have focused on the current state of neonate and juvenile turtle survival. Neonate turtles are often highly cryptic and small in size making traditional capture and tracking techniques ineffective. Further, neonate and juvenile turtle age-classes historically experience the highest rate of mortality. In a 3-day study of neonate emergence conducted in northwestern Illinois, 41% of neonate Snapping Turtles (*Chelydra serpentina*) in the study were presumed dead by the end of a 3-day observation period (Janzen, 1993a). In a multi-year study conducted by Hammer (1969), only 3% of neonate Snapping Turtles survived the first year and of those only 17% of the yearlings survived to 2 years of age. However after the first two years out of the nest survival rates in snapping turtles typically increase to greater than 93% (Galbraith an Brooks, 1987). Low hatchling survival is not limited to aquatic turtle species. Survivorship of neonate gopher tortoises (*Gopherus polyphemus*) in southern Mississippi is 65% 30 days after nest emergence and only one of forty-eight hatchlings survived for two years (Epperson and Heise, 2003). The combination of delayed sexual maturity, low annual recruitment, increased adult mortality, and low neonate survival post emergence has created a great and urgent need to fill existing information gaps regarding the survival of the early life stages of North American turtle populations to prevent further loss.

The Eastern Box Turtle (*Terrapene carolina carolina*) is one of the most common terrestrial turtle species in the United States with a large geographic range across much of the Northeastern and Midwestern United States (Dodd, 2001; Wilson and Ernst, 2008). Despite the species expansive geographic range, Eastern Box Turtles are protected in many states within the species' range due to continued declines (Williams and Parker, 1987; Harding, 1997; Hyde, 1999). Little is known about the survival of hatchling Eastern Box Turtles, particularly at the northern limits of the species' range. The information currently available regarding the neonate life-stage of Eastern Box Turtles is incomplete and often outdated or has limited application. Madden (1975) attempted to monitor 2 neonate box turtles using radio telemetry in New York. The radios used weighed 20% of the neonate's total body mass, well over the recommended 8% of total body mass used today (Beaupre et al., 2004). Further, information from the study spans only from the time of nest emergence in the fall to the start of overwintering the same year, for a total time of 17-20 days. However advances in radio-telemetry technology such as decreased radio size have allowed investigators greater opportunities to collect measures of neonate turtle survival over a biologically relevant period of time (Forsythe et al., 2004).

The objective of our study was to estimate annual survival for neonate Eastern Box Turtles through the first year of life at the northern limit of the species range in the Midwestern United States. We used radio telemetry data and Kaplan-Meier estimators modified for staggered-entry to create known-fate survival estimates. Our study represents the first of it's kind to report on neonate eastern box turtle survival through the first year of life and can be applied to inform future box turtle population viability analyses throughout the eastern box turtle's range.

STUDY AREA

Our study area was located within Manistee National Forest (MNF) in Michigan's lower peninsula (Figure 1). MNF lies at the northern limit of the box turtle's known range and is comprised of primarily federally owned (United States Forest Service - USFS) land fragmented by private plots. MNF is managed for multiple uses including: recreation, wildlife habitat, timber production, watershed quality improvement, and the management of hazardous fuels (USDA, 2006). MNF is densely forested with primarily secondary growth forest comprised of red maple (*Acer rubrum*), jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*), and various oak species (*Quercus spp*.) with small (0.5 ≤ 50 ha) forest openings located throughout the study area.

Forest openings within MNF are managed by the USFS through prescribed fire, mechanical brushing, mowing, and non-native invasive species treatments. Openings are comprised of sandy soils largely covered with lichens (*Cladonia spp*.), grasses (*Andropogon spp.*), sedges (*Carex spp.*), bracken fern (*Pteridium spp.*), low bush blueberry (*Vaccinium angustifolium*), and sparse shrubs such as witch-hazel (*Hamamelis spp.*) and cherry (*Prunus spp.*). MNF has four distinct seasons with a generally wet, temperate, climate. Yearly average rainfall is 99.6 cm and average snowfall is 322.4 cm with a yearly maximum average temperature of 13.8 ˚C and a yearly minimum temperature averaging 1.7 ˚C (Midwest Regional Climate Center, 2017).

We selected four openings within MNF historically used by box turtles as nesting sites, referred to as Turtle Bowl (TB), Savanna (SV), East-West (EW), and Gravel Pit (GP). Although the openings had previously been managed by the USFS no management treatments were implemented during the duration of our study (2013-2015). The Turtle Bowl opening was a 1.9 ha, oval shaped, geological depression dominated by grasses (*Andropogon spp*.) mixed with large stretches of bare ground colonized by lichens. The TB featured low canopy cover with few trees (*Quercus alba, Pinus banksiana,* and *Pinus strobus*) and a small number of shrubs within the opening (*Prunus virginiana* and *Vaccinium angustifolium*). The Savanna opening was the largest of the study openings (5.6 ha) with relatively flat topography. The transition between forest opening and closed canopy forest was the least abrupt in the Savanna and held the largest number of trees (*Quercus alba, Pinus banksiana,* and *Pinus strobus*) as well as the thickest shrub coverage (*Prunus virginiana, Vaccinium angustifolium, Andropogon spp., and Carex pennsylvania)* of any of the openings. The East West opening was a 0.9 ha linear shaped opening with a 30° south-facing slope running the entirety of the opening. Trees were sporadic around the margins of the opening (*Quercus alba, Quercus velutina, and Pinus banksiana) and the center of the opening was a mosaic of* open ground colonized by lichens and patches of *Andropogon spp*. The Gravel Pit opening, similar to the TB opening, was a 0.7 ha oval shaped opening with a bowl-like topography. Vegetation in the GP is considerably lower in abundance compared to the TB and the vegetation that is present is considered invasive in the state of Michigan (*Centaurea maculosa, Hypericum perforatum, Verbascum thapus*). This opening was the most heavily modified of the study openings and is considered highly disturbed due to its frequent use by the public for recreational off-road vehicles.

METHODS

NEST LOCATION AND RADIO-TELEMETRY

From 2013 to 2015 during the first and second week of June, the selected forest openings were monitored beginning at approximately 1900 h for nesting female box turtles. Once a nesting female was spotted, we monitored the turtle throughout the night until the eggs were deposited and covered. Within 24 hours of egg deposition predator proof exclosure boxes were dug roughly 20cm into the ground surrounding the nests. Exclosure boxes were constructed using a wood frame with 1/4in. mesh steel hardware cloth fixed to the sides and top and remained in the ground until a nest emergence occurred. After fifty-five days of incubation we began monitoring the nests daily for emerged neonates. Once a neonate was found at the surface, morphometric data including carapace length (CL), carapace width (CW), carapace height (CH), and plastron length (PL) and width (PW) were collected. In order to accurately monitor neonate survival during the first year of life, each was fitted with a .35g or .5g R1614 radio-transmitter (Advanced Telemetry Systems®) to the right rear side of the carapace using clear two-part epoxy (Gorilla Epoxy adhesive®). Radio-transmitter and epoxy weight did not surpass 8% of the neonate's total body mass and did not impede neonate movements in anyway (Beaupre et al., 2004). Immediately following data collection all neonates were released at the nest site the day the emergence was detected.

During the fall activity season (from nest emergence to overwintering) we located each neonate two to three times per week using radio-telemetry. Once located, we used a Trimble® Geo 7x Global Positioning System unit to mark the location of each neonate with an accuracy of ± 25cm. If a signal could not be detected for a particular neonate we would visually inspect the last recorded location for signs of life or depredation. If no evidence of activity or depredation were found at the last known location we would scan the surrounding area for 1-2 hours in an attempt to pick up a signal.

Once the neonates ceased movement for roughly two weeks signaling the beginning of overwintering, the predator-proof exclosures were reinstalled surrounding each neonate and were monitored daily beginning each spring for reemergence. All neonates that survived through overwintering were fitted with a new .35g or .5g R1614 radio-transmitter using clear two-part epoxy and morphometric data (neonate weight, CL, CW, CH, PL, and PW) were again collected to record any changes in growth that may have occurred since emergence. Neonates were then located three to four times a week until radio contact was lost or a mortality event occurred.

STATISTICAL ANALYSIS

We used the data collected through radio-telemetry tracking of the neonate box turtles to estimate annual neonate survival probability using the nonparametric Kaplan-Meier survival estimator (Kaplan and Meier, 1958). Because the emergence time of each nest as well as the emergence time of each individual within the nests often varied, we utilized a modified version of the Kaplan-Meier procedure that allowed for new individuals to be added after the study period had begun with the emergence of the first neonate (Pollock et al., 1989). This staggered-entry method also allowed for the right censoring of individuals whose fates were unknown due to radio failure or loss of signal possibly due to large-scale movements. Right censoring occurs when an animal is no longer under observation and is removed from the study before the study period ends and should not induce bias on the Kaplan-Meier point estimates of survival (DeCeasar et al., 2016). All neonates were considered at risk until a death occurred and was confirmed by the retrieval of a carcass or radio communication was lost in which case the individual was censored. Survival curves were constructed to examine annual neonate survival across all years of the study (2012-2015) as well as for each year. Additional survival curve models included estimated annual survival by month of emergence, opening (TB, SV, EW, GP), and by neonate weight (g) at emergence. Because the Kaplan-Meier procedure does not support the use of continuous variables we constructed three weight classes: small (5.5 - 7.0 g), medium (7.1 - 8.6 g), and large (8.7 - 10.2 g) to separate neonates for our survival analysis. Our weight classes were constructed using the 1st and 3rd quartiles as well as the median of our range of weights measured from the neonates from 2013-2015. All analyses of neonate survival were conducted using the Survival package (Therneau et al., 2015) for program R version 2.15.1 (R Development Core Team, 2012).

RESULTS

NEONATE MORPHOMETRIC DATA

A total of 62 neonate Eastern Box Turtles were fitted with radio transmitters over 3 years, 27 neonates in 2013, 18 neonates in 2014, and 17 in 2015. From 2013 to 2015 we radio tracked 11 neonates in the East-West opening, 11 neonates in the Savannah opening, 4 in the Gravel Pit opening, and 36 in the Turtle Bowl opening. The average carapace length for marked neonates at hatch was 31.9 mm (SD = 1.98) and ranged from 28.1 mm to 37.9 mm. Average neonate carapace width was 28.8 mm (SD = 1.91) and ranged from 24.3 mm to 33.0 mm. Average neonate carapace height was 16.1 mm (SD = 1.60) and ranged from 11.0 mm to a maximum height of 18.3 mm. Average neonate plastron length was 28.8 mm (SD = 2.34) with a minimum of length of 18.3 mm and a maximum length of 34.0 mm. Average neonate plastron width was 23.1 mm (SD = 1.80) and ranged from 19.3 mm in length to 28.5 mm. Average neonate weight at emergence was 7.97 g (SD = 1.00) with the smallest individual weighing 5.73 g and the largest weighing 10.1 g (Table 1).

NEST EMERGENCE AND NEONATE SURVIVAL

Neonate emergence began in late August and ended by late October; with the exception of one neonate in 2015 in the Turtle Bowl opening that overwintered within the nest and did not emerge till 1 June of 2016. Only one neonate emerged in the month of August across our study period and was the earliest neonate emergence recorded on 22 August in 2013. We recorded the largest number of emergences in September with 48 neonates total emerging from 2013-2015. There were 12 emergences in October from 2013-2015 with the latest recorded fall emergence on 25 October of 2015.

We were unable to track a neonate for a full year (365 d). However we were able to successfully track a single neonate from nest emergence on 18 September 2013 to 18 August 2014 for a total of 335 days. The neonate was eventually depredated as the carcass was never recovered but the transmitter was located covered in visible bite marks. From 2013 to 2015 a total of 14 neonates (22.6%) were depredated (10 in 2013, 2 in 2014, and 2 in 2015), 19 neonates (30.6%) died due to exposure (3 in 2013, 15 in 2014, and 1 in 2015), and 2 neonates (3.2%) were found on the side of a dirt road adjacent to the Turtle Bowl study opening crushed by motor vehicles (2 in 2013, 0 in 2014, and 0 in 2015). From 2013 to 2015 a total of 27 neonates (43.6%) were censored from our study due to loss of a transmitter signal or the absence of evidence to determine whether the neonates had been depredated (12 in 2013, 1 in 2014, and 14 in 2015).

KAPLAN-MEIER SURVIVAL ESTIMATES

Our Kaplan-Meier annual survival estimate for the 2013-2015 neonate cohorts (Fig. 2) estimated neonate survival to decrease through the first year of life with a steep drop in survival from nest emergence in the fall, before leveling off at 50% for overwintering (day 50 = .503; SE = 0.067), then survival began to gradually decrease again with spring emergence till it reached 0% survival short of the 1 year mark (day 335 = 0.0; Table 2). Although all survival estimates predict a decrease in neonate survival across the first year of life, survival estimates varied among years (Fig. 3). The 2015 cohort had the highest predicted survivorship through fall, overwintering, and into spring; with predicted neonate survival only dropping from 0.938 (SE = 0.061) during the fall to 0.750 (SE = 0.128) by day 228 (Table 3). However after day 228 we lost the transmitter signal from the last neonate and as a result were censored from the study (Fig. 4). The 2013 cohort provided the longest survival estimate with an estimated survival of 0.207 (SE = 0.163) at day 307 before the last remaining neonate was depredated at day 335 (Fig. 5). Our survival estimate for 2014 varied considerably from both 2013 and 2015, as no neonate (*n* = 15) across our four study openings (TB, SV, EW, GP) survived through overwintering (Fig. 6). Thus our survival estimate only accounts for the estimated survival during the fall activity period ending on day 49 with a survival estimate of 0.444 (SE = 0.117); which was similar to the 2013 fall survival estimate (0.630; SE = 0.093), but lower than 2015 (0.938; SE = 0.061).

Kaplan-Meier survival estimates for neonates across 2013-2015 also varied by study opening (Fig. 7). Neonates that emerged from nests within the Gravel Pit (*n* = 4) and Savanna (*n* = 11) openings had similar survival estimates separated by 0.06 (GP = 0.667, SE = 0.272; SV = 0.727, SE = 0.134). However at both GP and SV openings survival estimates end with neonates being censored from further estimates (Table 4). The Turtle Bowl opening (*n* = 36) produced the longest survival estimate with estimated survival of 0.531 (SE = 0.086) in the fall on day 38 and ending in the spring on day 355 at 0%. Neonates that emerged in the East-West opening (*n* = 11) had the lowest estimated fall survival at 0.202 (SE = 0.096). Out of the 11 neonates tracked in the East-West opening from 2013-2015, 6 died during overwintering and an additional 3 were depredated during the fall activity season. Of the two neonates to survive to spring in the East West opening 1 neonate died due to exposure and was found entirely desiccated in a sandy clearing within the opening and the other was censored from the study.

Although nest emergences took place in August, September, October, and June our Kaplan-Meier neonate survival estimates show a clear survival advantage to neonates that emerge during September when compared to all other months (Fig. 8). Neonates that emerged from nests in the month of September had a higher rate of survival across the first 355 days outside of the nest (Table 5). Further, neonates that emerged in September were predicted to survive an additional 107 days over neonates that emerged during the month of October.

Although emergences in August and June were recorded, each estimate was only supported by a single individual. The neonate that emerged in August was found desiccated a day after nest emergence near the nest opening; while the neonate that overwintered within it's nest and emerged in June was censored from the study a day after emergence due to transmitter failure. Thus while estimates for both August and June emergences are represented in Figure 7 both should be interpreted as biased estimates.

Of the 62 neonates fitted with radio transmitters for our study from 2013 to 2015, 14 (22.6%) neonates were separated into our small weight class (5.5 – 7.0g), 31 (50.0%) in our medium weight class (7.1 – 8.5g), and 17 (27.4%) in our large weight class (8.7 10.2g). Our Kaplan-Meier survival estimates of neonate survival by weight class display a positive relationship between neonate weight and survival (Fig. 9); with the large neonate weight class having the highest probability of survival from emergence through overwintering and into the spring activity season before dropping sharply to 0% by day 355 (fall = 0.941± 0.057; overwinter = 0.882 ± 0.078; spring = 0.819 ± 0.095). The small neonate weight class had the lowest survival throughout the year with survivorship dropping sharply from fall emergence before leveling off near zero for overwintering and into the spring activity season (fall = 0.923 ± 0.074; overwinter = 0.084 ± 0.08; spring = 0.084 ± 0.08). The survival estimate for the medium neonate weight class fell between the large and small weight class estimates with survivorship dropping gradually through fall and leveling off at 0.467 ± 0.095 for overwintering, then gradually decreasing again during spring till reaching 0%.

DISCUSSION

Previously, the use of large radio transmitters with short battery life spans had made it difficult to collect and analyze natural neonate survivorship trends over a period of time that would allow for biologically relevant results. Through the use of smaller more efficient radio technologies, results from our study have greatly increased the typical observation period provided through the current literature for neonate turtles. Despite being unable to track a neonate for the entire first full year of life, our results show that neonate Eastern Box Turtles typically experience the largest drop in survival during the fall activity season from nest emergence to overwintering where a 50% drop in survivorship was observed from 2013-2015. There are potentially multiple forces causing the steady drop in neonate survivorship during the fall activity season but our study identified predation and exposure to suboptimal environmental conditions as the main sources of neonate mortality.

Of the 34 neonates that did not survive the fall activity season during our study, over 50% of the deaths (*n* = 18) were for reasons associated with environmental conditions. This was the case during the 2014 fall activity season when all of the observed neonates (*n* = 15) that survived to overwintering in November were later found dead from an early season freeze cross the 4 study openings (Fig. 6). While many species of hatchling turtles in North America are known to employ super-cooling most neonates only posses a modest capacity for super-cooling in the first few weeks after hatching (Packard and Packard, 2001). Neonate turtles often ingest quantities of soil and eggshell during the hatching process (Packard et al., 2001) and the moisture in the soil can freeze at relatively high sub-freezing temperatures. As a result ice forms in the gut of the neonate turtle and then propagates across the lining of the stomach resulting in the formation of ice in the extracellular fluids ending in the death of the neonate (Costanzo et al., 1998, 2000a, 2000b; Packard and Packard, 2001). A combination of an unseasonably cool summer and early fall and the resulting mid-October nest emergence of many of the neonates also likely did not provide the hatchlings with enough time to dig into the soil below the frost line (Costanzo et al., 1995). Further the early fall freeze did not allow the neonates time to purge the contents of their gut typically signaled by the gradual decrease in temperatures through fall and into winter to prevent the internal spread of ice (Packard et al., 2001). While the complete loss of a cohort was initially startling, these natural variations in winter weather could be limiting this species' range expansion at the northern edge of the Eastern Box Turtles range (Root, 1988; Stevens, 1989).

Predation was the second largest cause of neonate mortality during the fall activity seasons from 2013-2015. Roughly 15% of neonate Eastern Box Turtles (n = 9 out of 62) were verified as depredated during our study. Neonates remained within their natal forest openings or took shelter along the edge of the forest opening leaving them concentrated together and potentially more vulnerable to detection from predators. Suspected predators of the neonates included small mammals and mesopredators due to clear bite marks found on recovered radio-transmitters often with scute scales still attached to the recovered radio-transmitters as if chewed off and left meters away from the last recorded neonate location. Additionally, two transmitters were tracked up into trees that were located greater than double the longest recorded distance traveled by any neonate during our study. Historically avian predation of neonate turtles has been observed and that is likely the explanation for both the large distance between the last observed neonate location and the tree in which the transmitter was found as well as how high in the tree the transmitter was located (Wilson, 1991; Janzen et al., 2000).

While neonate survival declined throughout the fall activity season survival probabilities during overwintering were stable. However it should be noted that the chance of overwinter depredation of the neonate box turtles was eliminated through the use of nest exclosures. We reinstalled nest exclosures around each neonate box turtle during overwintering in order to easily relocate each individual in the spring and reinstall radio transmitters on each. For all cohorts with the exception of the 2014 cohort, when a neonate survived to overwintering, the survival probability remained constant to spring emergence (Table 3). Unlike 2014, which exposed neonates to an early freeze with little to no snow cover until late into the winter season, 2013 and 2015 saw a mild fall with snowfalls beginning earlier in winter. Snow cover provides an important insulating layer and likely facilitates steady neonate survival through the winter by preventing sudden drops in temperature or frost from developing on the outer and inner tissue of neonates that would otherwise lead to death (Breitenbach et al., 1984).

Survival estimates also decreased through the spring activity season. However the interpretation of the survival estimates during the spring activity season are complicated by the large increase in censoring neonates from our study because their fates could not be determined. Of the 29 Eastern Box Turtle neonates that survived into the spring activity season from 2013-2015, the fates of 22 could not be determined due to radio failures, potentially undetected predation, or the individuals moved out of the study area. Thus, our survival estimates in the spring activity season could be overly conservative since we were only able to use the survival data from the 7 individuals of which fates were known. It is possible that many of the lost neonates survived the spring activity season, but with the increased ambient temperatures of spring the battery life of the radio-transmitters decreased and the radio signals often unexpectedly disappeared. Neonate movements and dispersal from the forest openings also dramatically increase during the spring activity season and could have negatively influenced our ability to track and locate the neonates through the forest (Laarman, 2017). Of the neonates whose fates were known for the spring, 5 were depredated by mammalian predators, 1 individual was found desiccated within the East-West forest opening, and 1 individual was crushed by a motor vehicle after exiting it’s natal opening and attempted to cross a gravel road into the forest.

Separating the neonate survival estimates by the month of nest emergence indicate that the month of emergence also affected the probability of survival through the first year of life for neonate Eastern Box Turtles. Despite our survival estimates indicating that neonates that emerged from nests in the month of September had a higher rate of survival across the first 355 days outside of the nest than those that emerged in October by an additional 107 days (Fig. 8), it should be noted that there is a strong probability of sampling bias in this particular comparison. Because the majority of the neonates observed in our study from 2013-2015 emerged in the month of September (n = 48) compared to the month of October (n = 12) the higher survival probabilities could be a result of four-times the number of individuals emerging in September and increasing the probability that more individuals survived longer. In addition, the late emergence and subsequent die off of our 2014 cohort, many of which emerged during the month of October, likely also influenced our results. It is also possible that neonates that emerge earlier in the fall do have higher survivorship as they have time to purge the soil from their gut and more days to find refuge before the arrival of winter (Packard et al., 2001).

While neonate survival estimates for each of our study openings also varied, we believe this variation was largely the result of the uneven sample sizes of neonates monitored at each opening (TB = 36, SV = 11, EW = 11, GP = 4). While the differences in sample sizes between openings were dramatic, the survival estimates for neonate survival across the openings were similar with the exception of the East-West opening (See Table 4). Although the level of disturbance and microhabitat characteristics within each opening appear to be quite different, our survival estimates appear to support the current literature showing that Eastern Box Turtles can persist across a wide variety of habitat types when anthropogenic pressures are limited (Nazdrowicz, 2008).

Although we were unable to successfully produce survival estimates for the first full year of life for neonate Eastern Box Turtles in Manistee National Forest, we were able to identify the major threats facing neonate box turtles at the northern limit of their range within the Midwestern United States. The challenges to a species' survival at the limits of its range are often different from those faced by the same species more centrally located within the species range (Parmesan et al., 2005). Thus while our study found that exposure to suboptimal temperatures during the first activity season out of the nest negatively affected a neonates probability of survival, studies similar to ours should be conducted across the geographic range of the Eastern Box Turtle to determine the possible threat severe weather occurrences pose to the persistence of other box turtle populations. However unlike the variation in weather severity across the Eastern Box Turtle's range, the threat from depredation to neonate survival does align with the current literature and is likely a primary cause of mortality in most populations affected by human disturbance. We hypothesize that with the limited number of large forest openings available to nesting female box turtles in MNF, the study openings we monitored could be concentrating the nesting activities of the current population of box turtles and as a result increase the probability of neonate depredation above the natural level found in locations with less frequent recreational use. Thus the impact of a few predators surrounding those nest sites could dramatically affect the survival of the neonate age-class for the entire MNF box turtle population. To mitigate the effects of depredation, managers should increase the size and number of forest openings within the national forest to provide more suitable nesting habitat for female Eastern Box Turtles thus distributing the nests and neonates across the landscape to prevent large-scale depredation events in future cohorts.

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Table 1. Morphometric measurements for 2013-2015 neonate Eastern Box Turtles in Manistee National Forest at emergence. CL = carapace length in mm; CW = carapace width in mm; CH = carapace height in mm; PL = plastron length in mm; PW = plastron width in mm.

Dimension	Mean	SD	Range
CL	31.9	1.98	28.1 – 37.9
CW	28.8	1.91	24.3 – 33.0
CH	16.1	1.60	11.0 – 18.3
PL	28.8	2.34	18.3 – 34.0
PW	23.1	1.80	19.3 – 28.5
Weight (g)	7.97	1.00	5.73 – 10.1

Table 2. Kaplan-Meier survival estimates, standard error, and 95% confidence intervals for Eastern Box Turtle neonate annual survival in MNF for all individuals radio-tracked from the 2013-2015 cohorts. Day 1 represents earliest neonate emergence for study period (22 August).

Time (Day	No. at Risk	No. Deaths	No. Censored	Survival	SE	95% CI
1	62	1	1	0.984	0.016	0.953 – 1.0
3	60	1	0	0.967	0.023	0.924 – 1.0
4	59	1	0	0.951	0.028	0.899 – 1.0
5	58	1	0	0.935	0.032	0.875 – 0.999
6	56	1	1	0.918	0.035	0.852 – 0.990
10	55	1	0	0.901	0.038	0.829 – 0.980
13	51	1	0	0.884	0.041	0.806 – 0.969
20	50	5	0	0.795	0.053	0.698 – 0 .906
21	45	1	0	0.778	0.055	0.678 – 0.892
27	44	1	1	0.760	0.056	0.658 – 0.878
29	42	1	0	0.742	0.058	0.637 – 0.864
34	41	1	0	0.724	0.059	0.617 – 0.849
36	40	1	0	0.706	0.061	0.597 – 0.834
38	39	4	1	0.633	0.064	0.520 – 0.772
50	34	7	0	0.503	0.067	0.387 – 0.653
200	27	1	0	0.484	0.067	0.369 – 0.636
228	26	1	0	0.466	0.067	0.351 – 0.618
233	25	1	6	0.447	0.067	0.333 – 0.600
263	18	1	13	0.422	0.068	0.308 – 0.578
293	4	1	1	0.317	0.104	0.166 – 0.605
307	2	1	0	0.158	0.124	0.034 – 0.731
335	1	1	0	0.000	--	---

Table 3. Comparison of Kaplan-Meier endpoint survival estimates, standard error, and 95% confidence intervals for the 2013, 2014, and 2015 Eastern Box Turtle neonate cohorts. Survival estimates are separated into the fall activity period, overwintering period, and spring activity period for each year.

	2013 (*n* = 27)			**2014 (*n* = 18)**			**2015 (*n* = 17)**		
	Survival	SE	95% CI	Survival	SE	95% CI	Survival	SE	95% CI
Fall	0.630	0.093	0.472 – 0.841	0.444	0.117	0.265 – 0.745	0.938	0.061	0.826 – 1.0
Overwinter	0.593	0.095	0.433 – 0.810	0.000	--	---	0.844	0.104	0.662 – 1.0
Spring	0.000	--	---	0.000	--	---	0.750	0.128	0.537 – 1.0

Table 4. Comparison of Kaplan-Meier endpoint survival estimates, standard error, and 95% confidence intervals for neonate Eastern Box Turtles by the forest opening in which the neonate emerged. Survival estimates are separated into the fall activity period, overwintering period, and spring activity period for each forest opening. Addition signs (+) indicate periods where all individuals were censored thus no estimate was provided.

	Turtle Bowl (*n* = 36)			**Savanna (*n* = 11)**			**East-West (*n* = 11)**			**Gravel Pit (*n* = 4)**		
	Survival	SE	95% CI	Survival	SE	95% CI	Survival	SE	95% CI	Survival	SE	95% CI
Fall	0.531	0.086	0.387 – 0.728	0.727	0.134	0.506 – 1.0	0.202	0.127	0.059 – 0.696	0.667	0.272	0.300 – 1
Overwinter	0.500	0.086	0.356 – 0.700	0.727	0.134	0.506 – 1.0	0.101	0.096	0.016 – 0.647	0.667	0.272	0.300 – 1
Spring	0.000	--	---	++	++	+++	0.000	--	---	++	++	+++

Table 5. Comparison of Kaplan-Meier survival estimates, standard error, and 95% confidence intervals for neonate Eastern Box Turtles in MNF for the 2013-2015 cohorts separated by month of emergence. Addition signs (+) indicate periods where all individuals were censored thus no estimate was provided.

	September (*n* = 48)			**October (*n* = 12)**			**August (*n* = 1)**			**June (*n* = 1)**		
Day	Survival	SE	95% CI	Survival	SE	95% CI	Survival	SE	95% CI	Survival	SE	95% CI
1	0.979	0.021	0.940 –1.0	1.000	--	1.0 – 1.0	1.000		1.0 – 1.0	++	++	+++
3	0.979	0.021	0.940 –1.0	1.000	--	1.0 – 1.0	0.000	--	---			
50	0.576	0.065	0.619 – 0.877	0.208	0.130	0.061 – 0.710						
200	0.576	0.065	0.619 – 0.877	0.104	0.098	0.016 – 0.663						
228	0.576	0.065	0.619 – 0.877	0.000	--	---						
263	0.522	0.077	0.391 – 0.696									
307	0.196	0.152	0.043 – 0.899									
355	0.000	--	---									

FIGURE LEGENDS

Figure 1. Map displaying the State of Michigan with the boundary of Manistee National Forest and inset zoom of the location of the 4 study openings relative to one another within the boundaries of Manistee National Forest.

Figure 2. Kaplan-Meier estimation of Eastern Box Turtle neonate annual survival in MNF for the 2013-2015 cohorts. Dashed lines indicate 95% confidence intervals with addition signs (+) representing dates when neonates were censored from study. Day 1 represents earliest neonate emergence for study period (22 August).

Figure 3. Kaplan-Meier estimations of Eastern Box Turtle neonate annual survival in MNF separated by cohort year. 95% confidence intervals were removed for ease of legibility. Addition signs represent dates when neonates were censored from study. Day 1 represents the first date of neonate emergence per study year.

Figure 4. Kaplan-Meier estimation of Eastern Box Turtle neonate annual survival in MNF for the 2015 cohort. Dashed lines indicate 95% confidence intervals with addition signs (+) representing dates when neonates were censored from study. Day 1 represents earliest neonate emergence for the 2015 study period (18 September).

Figure 5. Kaplan-Meier estimation of Eastern Box Turtle neonate annual survival in MNF for the 2013 cohort. Dashed lines indicate 95% confidence intervals with addition signs (+)

representing dates when neonates were censored from study. Day 1 represents earliest neonate emergence for the 2013 study period (8 September).

Figure 6. Kaplan-Meier estimation of Eastern Box Turtle neonate annual survival in MNF for the 2014 cohort. Dashed lines indicate 95% confidence intervals with addition signs (+) representing dates when neonates were censored from study. Day 1 on graph represents earliest neonate emergence for the 2014 study period (14 September). The x-axis was reduced to 60 days for legibility since no neonates survived overwintering.

Figure 7. Kaplan-Meier estimations of Eastern Box Turtle neonate annual survival in MNF for the 2013-2015 cohorts separated by forest opening in which neonate emergence took place. 95% confidence intervals were removed for ease of legibility. Addition signs represent dates when neonates were censored from study. Day 1 represents the first date of neonate emergence in each opening.

Figure 8. Kaplan-Meier estimations of Eastern Box Turtle neonate annual survival in MNF for the 2013-2015 cohorts separated by month of emergence. 95% confidence intervals were removed for ease of legibility. Addition signs represent dates when neonates were censored from study. Day 1 represents the date of first neonate emergence in each month. Estimates for both August and June emergences are displayed but should be interpreted as potentially biased estimates as both are based on a single emergence observation.

Figure 9. Kaplan-Meier estimations of Eastern Box Turtle neonate annual survival in MNF for the 2013-2015 cohorts separated by weight class (small 5.5 - 7.0 g, medium 7.1 - 8.6 g, and large 8.7 - 10.2 g). 95% confidence intervals were removed for ease of legibility. Addition signs represent dates when neonates were censored from study. Day 1 represents the first date of neonate emergence for each weight class.

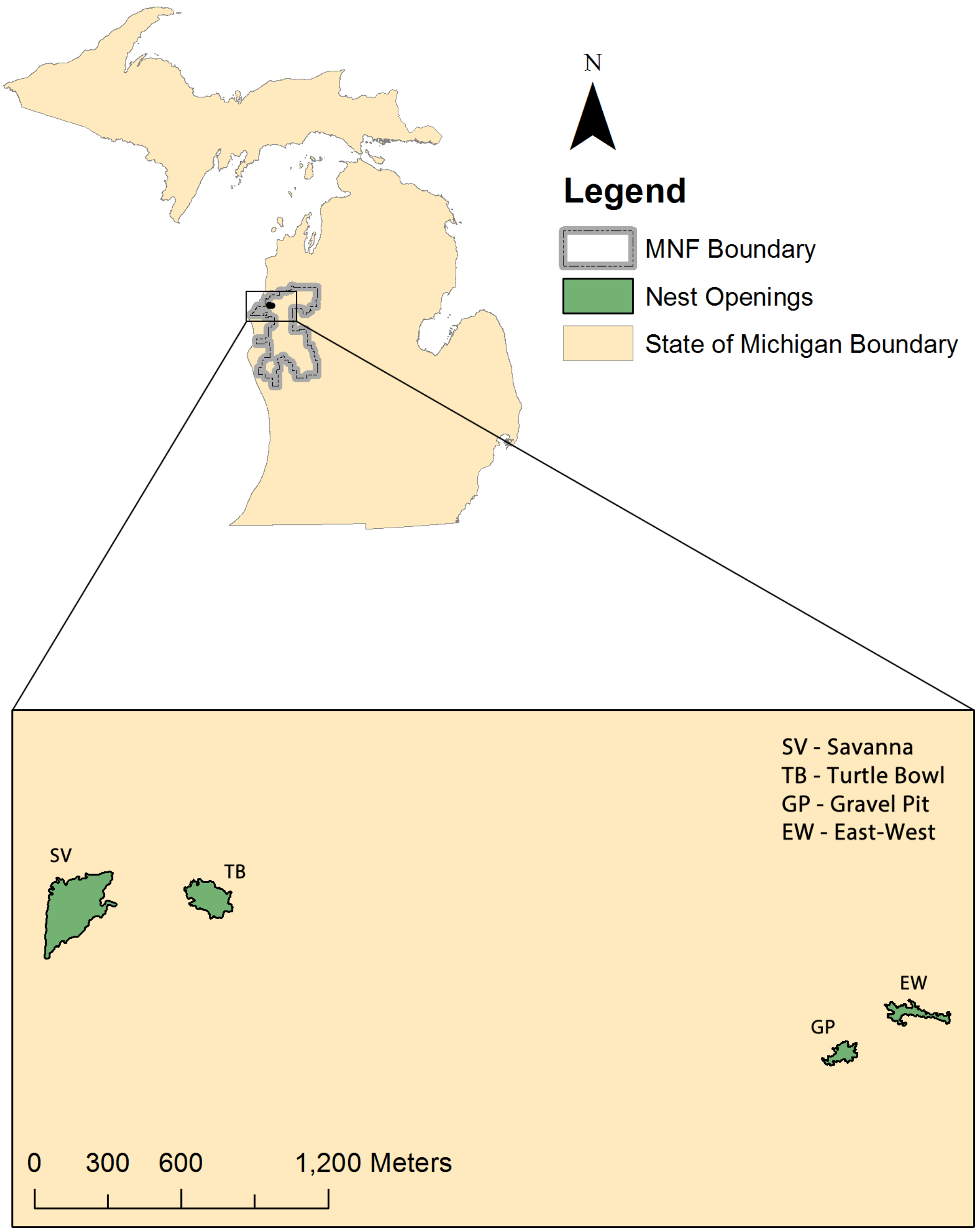

N
Legend
MNF Boundary
Nest Openings
State of Michigan Boundary
SV - Savanna
TB - Turtle Bowl
GP - Gravel Pit
EW - East-West
SV
TB
EW
GP
0 300 600 1,200 Meters

Figure 1.

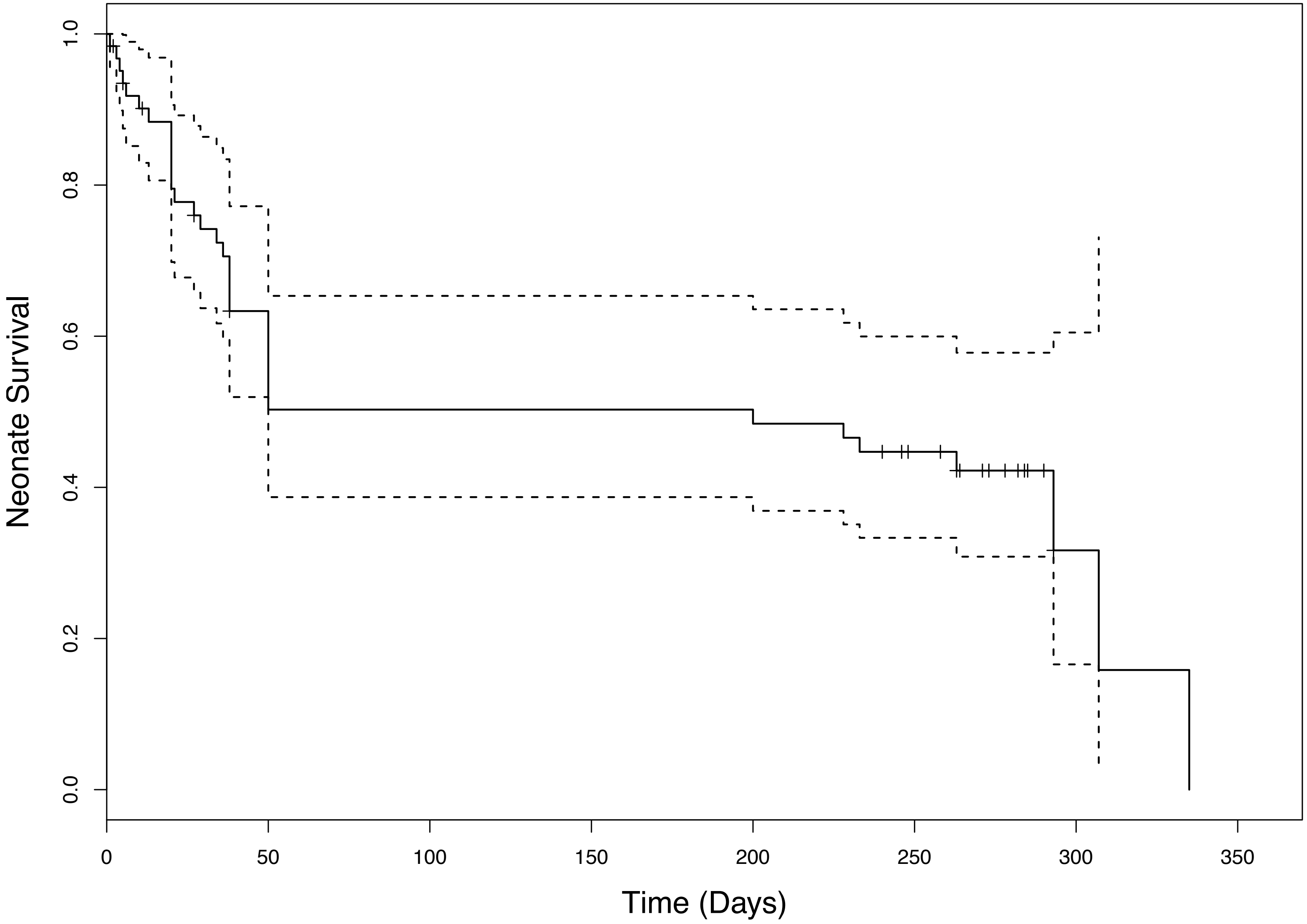
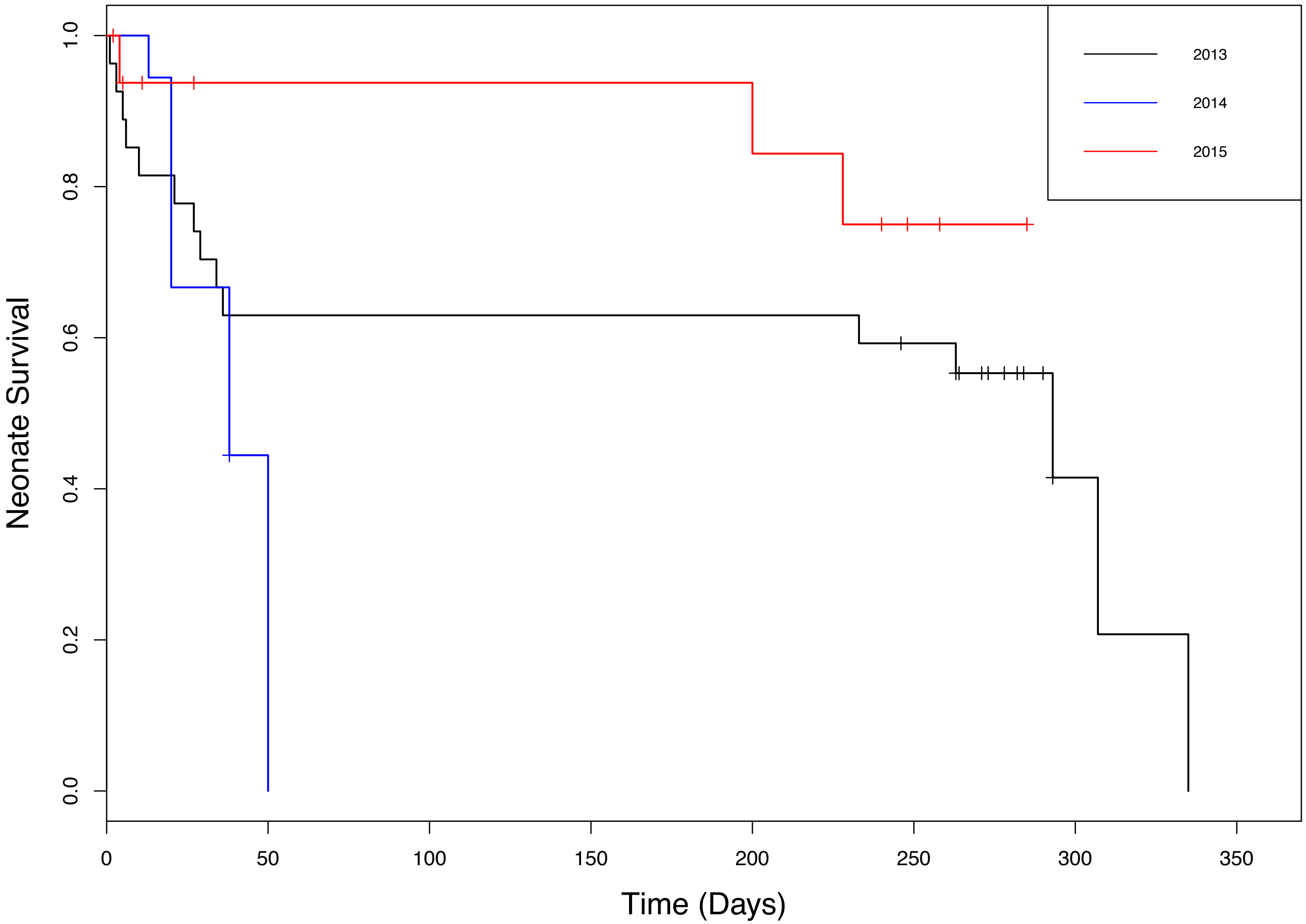
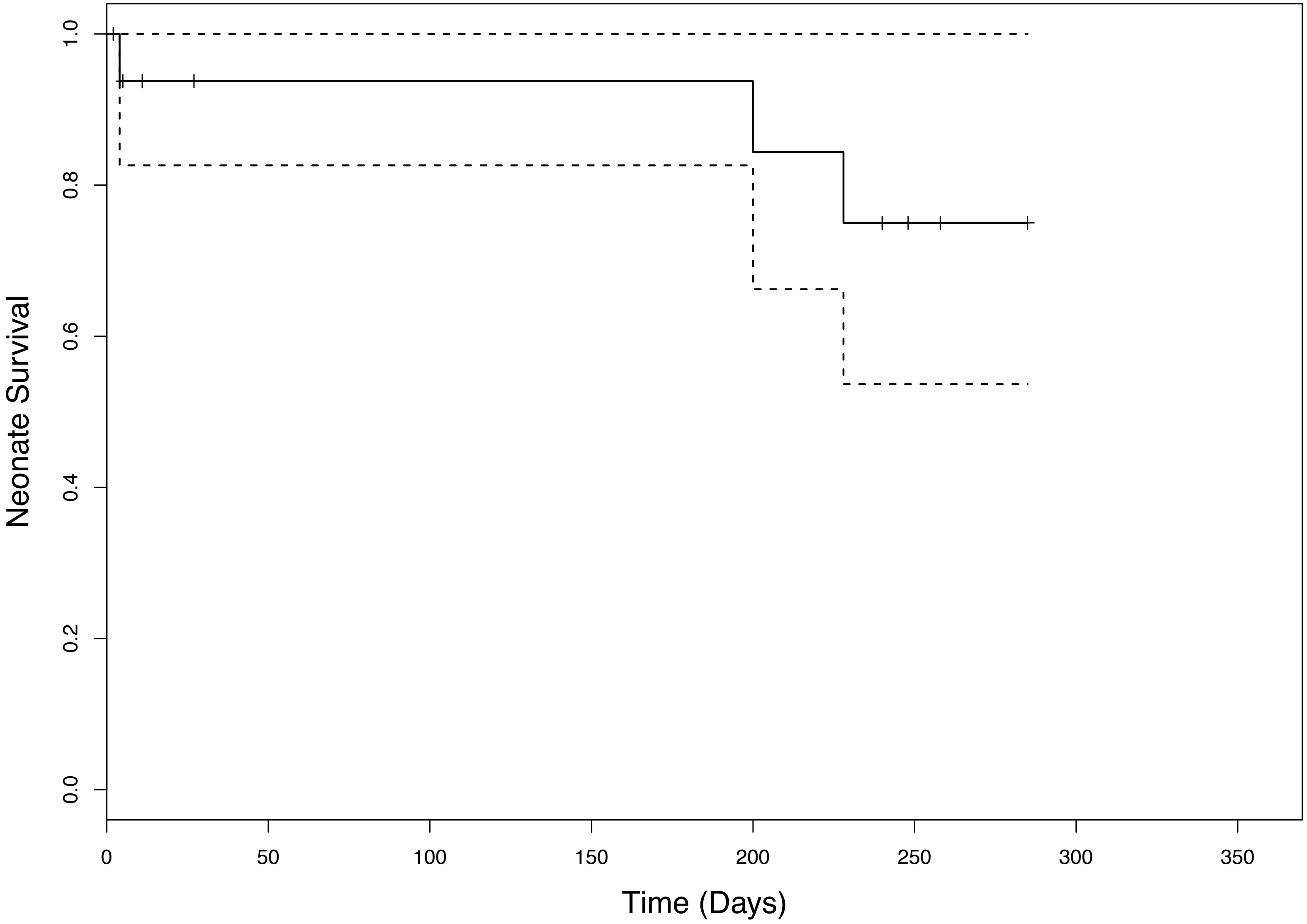

1.0
0.8
0.6
0.4
0.2
0.0
Neonate Survival
0
50
100
150
200
250
300
350
Time (Days)

Figure 2.



Neonate Survival
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
Time (Days)
2013
2014
2015

Figure 3.



Neonate Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
Time (Days)

Figure 4.

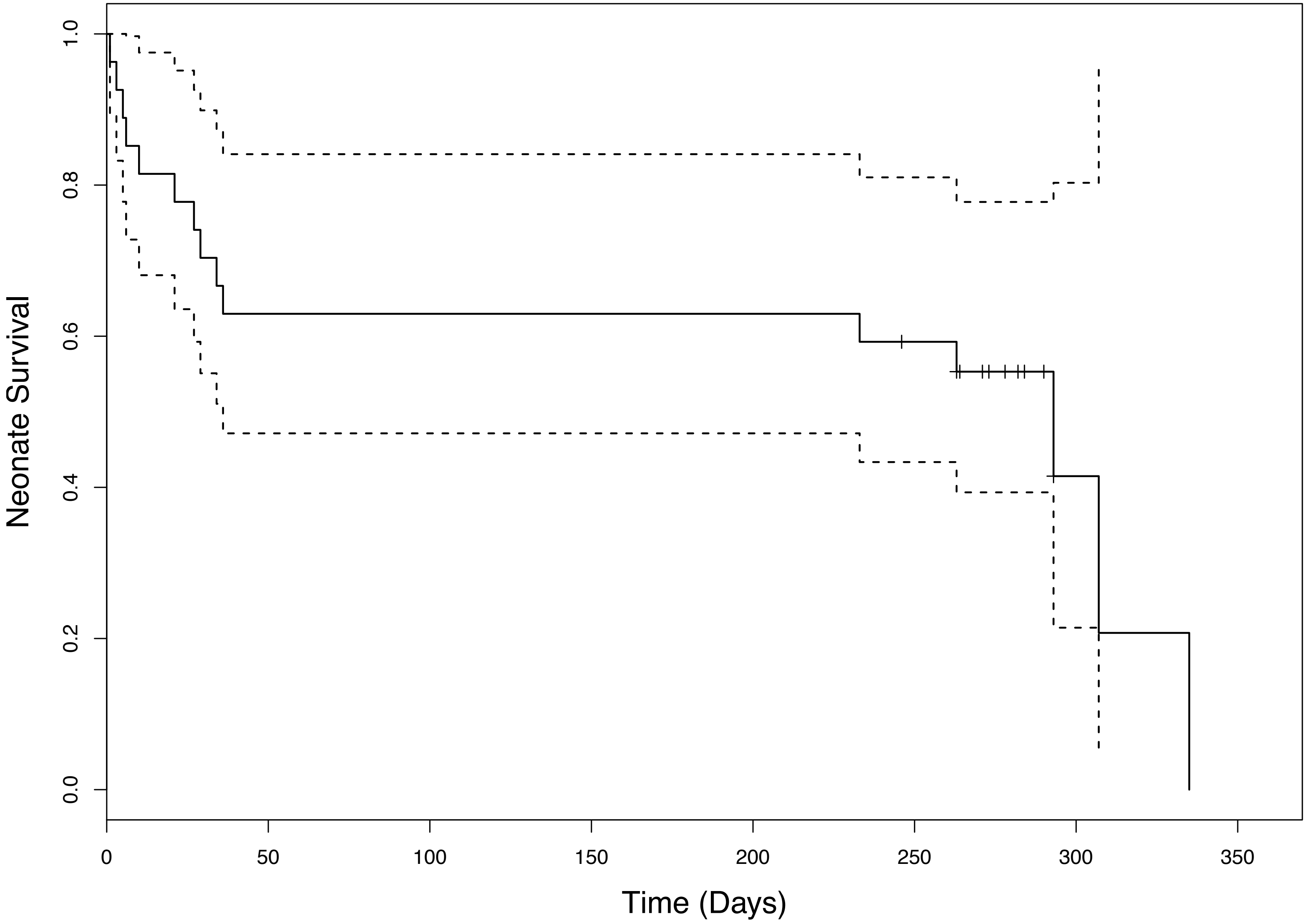

Neonate Survival
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
Time (Days)

Figure 5.

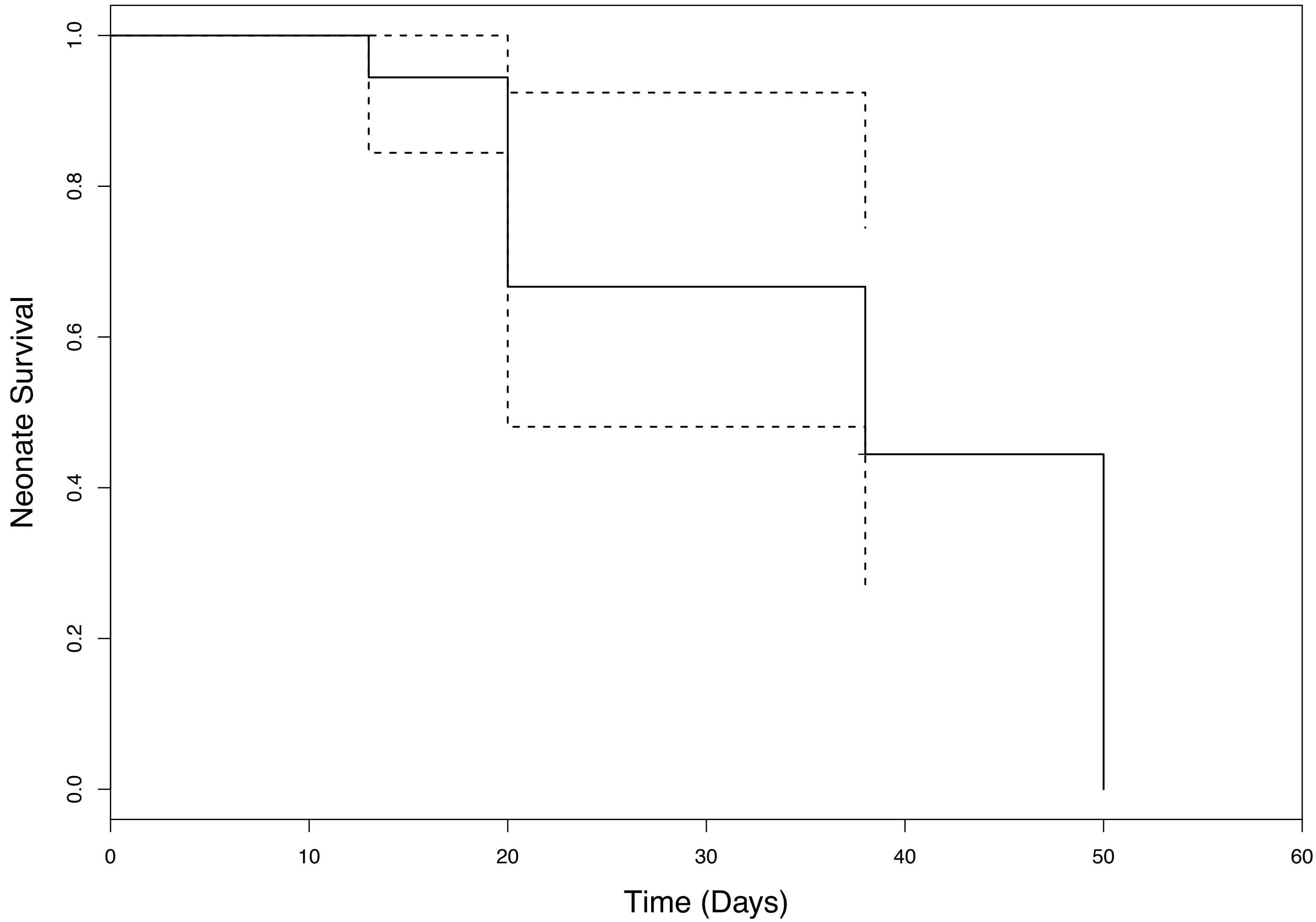


Figure 6.

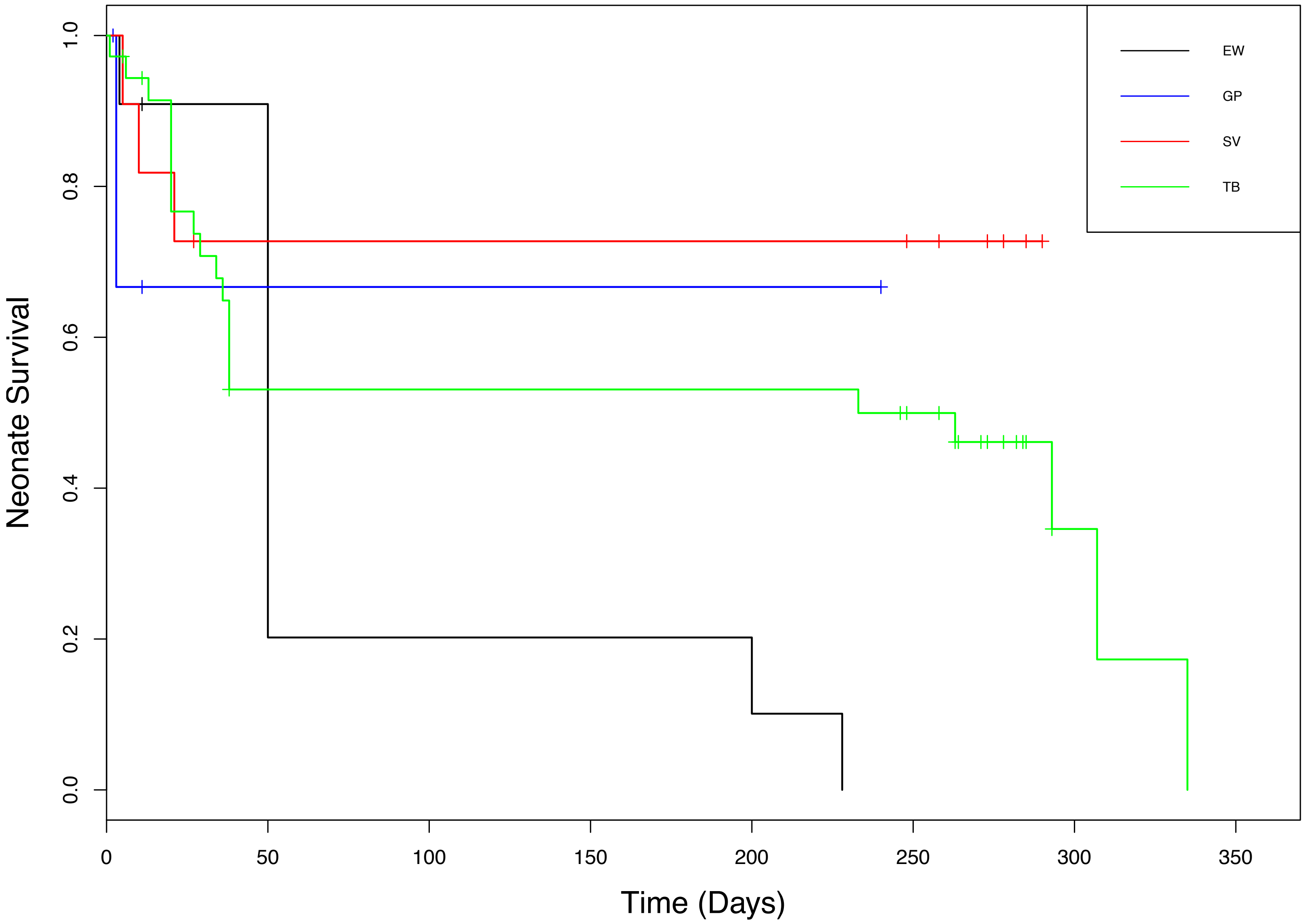

EW
GP
SV
TB
Neonate Survival
0.0
0.2
0.4
0.6
0.8
1.0
Time (Days)
0
50
100
150
200
250
300
350

Figure 7.

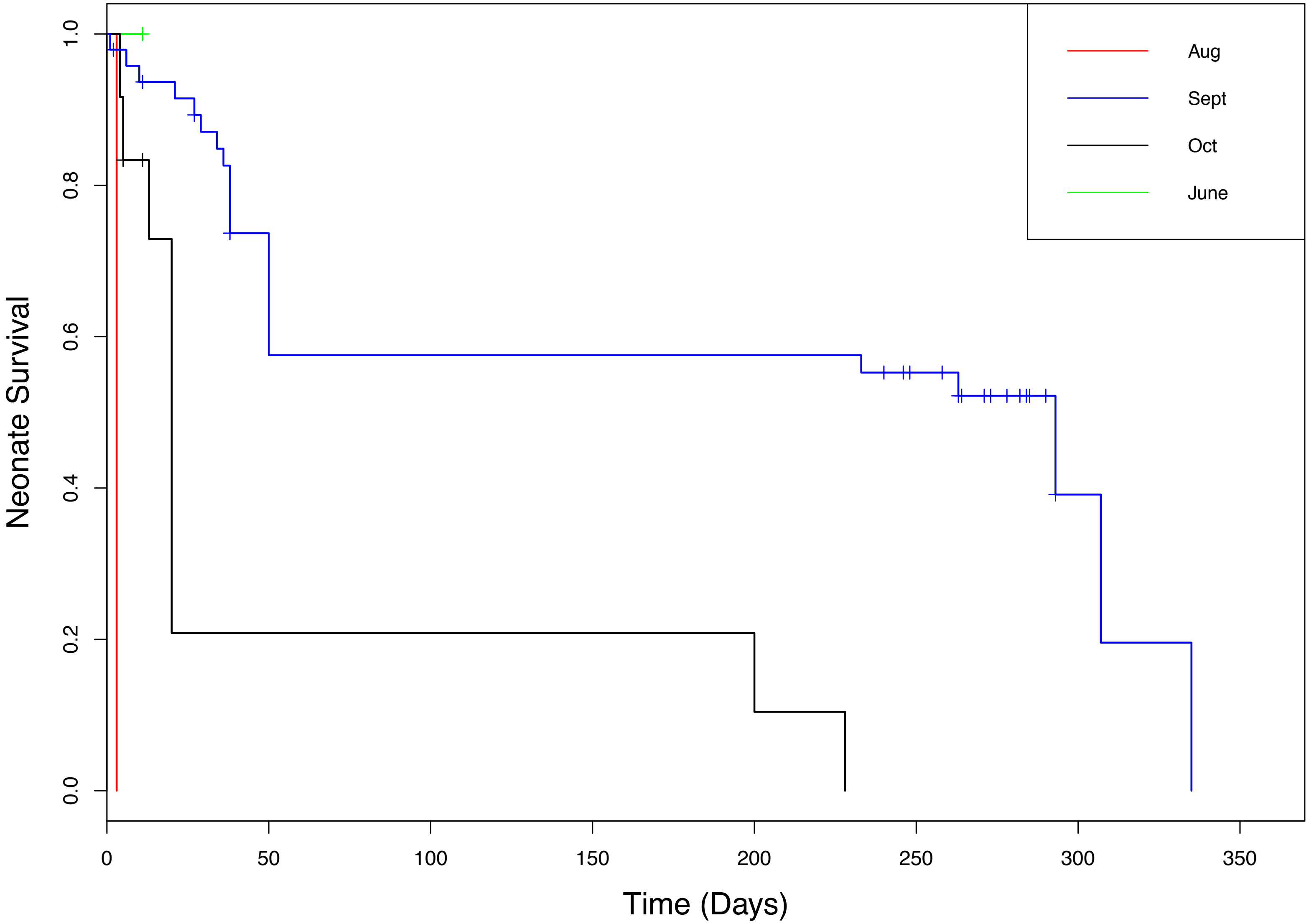

Aug
Sept
Oct
June
Neonate Survival
0.0
0.2
0.4
0.6
0.8
1.0
Time (Days)
0
50
100
150
200
250
300
350

Figure 8.

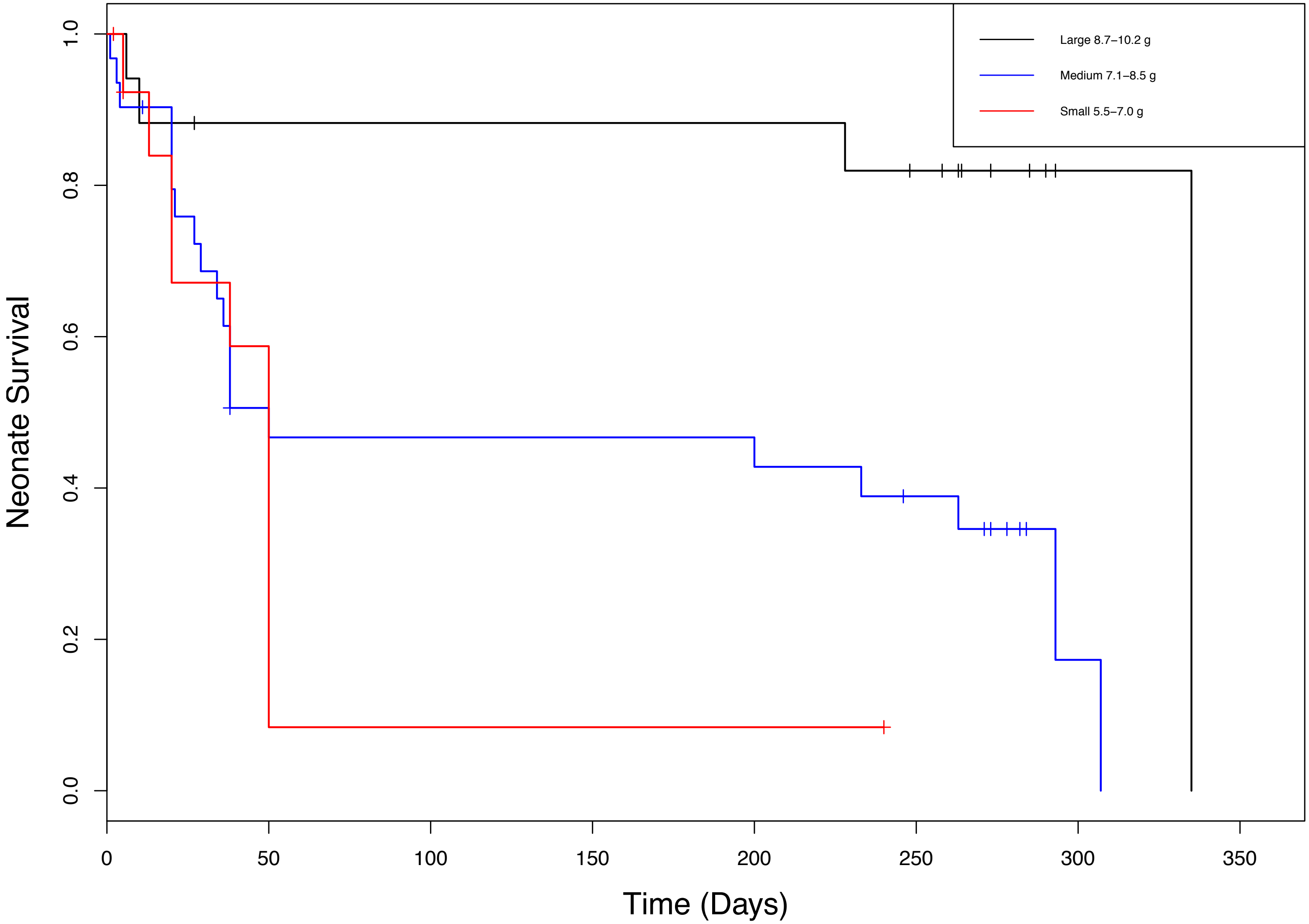

Neonate Survival
Time (Days)
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
Large 8.7–10.2 g
Medium 7.1–8.5 g
Small 5.5–7.0 g

Figure 9.

CHAPTER IV

EXTENDED REVIEW OF LITERATURE

Natural History

The eastern box turtle (*Terrapene carolina carolina*) is one of the most common and recognizable terrestrial turtle species in the United States. Eastern box turtles are members of the family Emydidae and in the Suborder Cryptodira, meaning they are capable of pulling their heads straight back into the shell. Additionally, adult box turtles possess a hinged plastron that allows them to retract their head as well as all other appendages into the shell protecting them from all but the largest predators. Unlike the older age-classes, neonate box turtles cannot retract their appendages immediately following nest emergence leaving them vulnerable to predation (Dodd, 2001). The eastern box turtle has a large geographic range across much of the North Eastern and Midwestern United States and is the only primarily land-based turtle in the Northern United States. Despite being protected in many of the northern states within their habitat, eastern box turtles continue to have dramatic population declines in many locations throughout their range (Williams and Parker, 1987). Eastern Box Turtles, like many other species of turtles, are extremely long-lived, some reaching 80 years old in captivity (Williams and Parker, 1987). However because of their long life span development to sexual maturity can take up to 10 years (Dodd, 2001). Once sexually mature, box turtles may only produce one or two clutches of eggs a year, typically with low hatchling success (Dodd, 2001). Historically box turtles compensated for low annual recruitment through multiple reproductive events across an adult's lifetime. This strategy relies on high adult survivorship for the persistence of a population (Congdon et al., 1993). With the increased removal of individuals from the adult age-class of turtle populations there is now a greater need to understand the survival of neonate and juvenile age-classes to ensure continued recruitment and the persistence of the populations. Much of the previous research concerning yearly survival of North American turtles has focused on the adult age-class (Metcalf and Metcalf, 1979; Iverson, 1991; Hall et al., 1999; Fredericksen, 2014; Agha et al., 2017). Far fewer studies have focused on the current state of neonate and juvenile turtle survival.

The information currently available regarding the neonate life-stage of Eastern Box Turtles is incomplete and often outdated or has limited application. Madden (1975) attempted to monitor 2 neonate box turtles using radio telemetry in New York. The radios used weighed 20% of the neonate's total body mass, well over the recommended 8% of total body mass used today (Beaupre et al., 2004). Further, information from the study spans only from the time of nest emergence in the fall to the start of overwintering the same year, for a total time of 17-20 days. However advances in radio-telemetry technology such as decreased radio size have allowed investigators greater opportunities to collect measures of neonate turtle survival over a biologically relevant period of time (Forsythe et al., 2004).

Temperature-Dependent Sex Determination

Like many reptiles, box turtle sex ratios are temperature-dependent. Temperature-dependent sex determination (TSD) means the sex of offspring is determined by incubation temperatures experienced by the embryo during the middle one-third of development rather than by genetic means (Janzen, 1995). Temperature-dependent sex determination was first identified by Charnier (1966) and the first proposal of an adaptive explanation was by Bull (1980).

TSD is known to occur in all crocodilians, multiple lizard species, tuatara, and many turtle species; however there are few detailed explanations for how this phenomenon arose in turtles (Bull 1980, 1983; Charnov and Bull, 1977; Ewert and Nelson, 1991; Janzen, 1995; Janzen and Paukstis 1991a,b). There are two primary forms of TSD common among turtles. The first being type Ia, defined by a single transition zone of temperatures where above said zone yields 100% female hatchlings and below yields 100% male hatchlings. The second form is Type II where there are two transition zones and males are primarily produced at intermediate temperatures and females are produced at both extreme high temperature as well as the extreme low temperatures. It is worth noting that no constant temperature is known to yield 100% males in Type II species (Ewert and Nelson, 1991). While eastern box turtles are typically considered to follow Type Ia TSD, female hatchlings are occasionally produced at lower temperatures suggesting an opportunity for variation in temperature response (Ewert and Nelson, 1991).

In order for TSD to persist it must have affected the fitness of the ancestors of the species that today possess it. The fitness of an individual could be related to body size, which correlates to female fecundity as well as a male's ability to acquire mates (Ewert and Nelson, 1991). Head et al. (1987) was the first to suggest that patterns of adult sexual dimorphism could be related to type of sex determination of the species of reptile. While there is a pattern associated between the modes of TSD and adult sexual dimorphism, there is currently no evidence to support why similar sexual dimorphism occurs in both GSD species as well as TSD species (Ewert and Nelson, 1991). However it should be mentioned neonate American alligators (*Alligator mississippiensis*) do exhibit sex-specific growth rates that are correlated with their incubation temperature, which could explain the evolutionary advantage of TSD in that species (Joanen et al., 1987).

TSD might also aid in sibling mating avoidance due to many turtle species' generally small home range sizes and nest-site fidelity by increasing the number of single sex clutches (Bull et al., 1982). For example experiments on leopard geckos (*Eublepharis macularius*) found that females from nests that mostly produced males either were not able or did not mate or lay eggs, suggesting they were functionally sterile (Gutzke and Crews, 1988). However these results have not been reproduced (Viets et al., 1993). The probability of parent-offspring mating remains high in turtles and TSD would not prevent this occurrence and thus would not benefit many turtle species. Due to the advantages of TSD in sibling avoidance only occurring when within-clutch sex ratios become strongly skewed it is likely the persistence of TSD is maintained by other means (Ewert and Nelson, 1991).

The wide distribution of TSD across multiple groups of turtles suggests that Genotypic Sex Determination (GSD), in which sex is determined at conception by chromosomal factors (Janzen, 1995), could be the derived mode (Ewert and Nelson, 1991). Ultimately TSD could have evolved independently multiple times in reptiles and different selective pressures could have driven each case so each hypothesis must be examined in each species in order to draw the clearest conclusions (Janzen, 1995). TSD leaves the survival of box turtle populations particularly vulnerable to even minor fluctuations in temperatures since the sex ratio of reproductive individuals is a strong determinant of population demography. Thus nest location, depth, and access to ideal nesting habitat are highly influential to a nest's success (Ewart and Nelson, 1991; Ewert et al., 1994). A better understanding of which variables influence nest selection by female box turtles at their northern range limit and the effects these variables have on nest success could play a key role in increasing population numbers in disturbed as well as undisturbed locations.

The Evolution of TSD and Neonate Survival

TSD has also been show to affect the survival of neonates long after they have hatched and left the nest. For example, Janzen (1995) found that cooler incubation temperatures (28°C) resulted in faster snapping turtle hatchlings. Neonate snapping turtles were released into experimental pond enclosures. Of the 121 individuals released into the enclosures, 16 (13.2%) were recaptured the following year. While turtles incubated at 28°C were faster in the lab, they had significantly lower survivorship in the natural enclosures compared to neonates that derived from single sex incubation treatments (26°C or 30°C). In other words, the more likely a neonate was to react to stimulus through locomotion the less likely it was to survive in a simulated semi-natural environment (Janzen, 1995). The current hypothesis linking TSD and these physiological responses postulates that incubation temperatures affect hypothalamic control of gonadotropin releasing hormone and thus gonadal differentiation through a linking of hormonal effects (Deeming and Ferguson, 1989). The strongest link to TSD is that this cascade of hormonal effects results in gonadal maturation through the electro-stimulation of the hypothalamus, which modifies secretion of gonadal steroids (Licht, 1984; Woods, 1987; Kawakami et al., 1981). Further, the hypothalamus also functions in influencing thermoregulation, aggression, and motivation (Berne and Levy, 1983). Combined, this suggests that sex, behavior, and fitness of neonates are all strongly linked in reptiles with TSD (Janzen, 1995).

Studies of other orders of reptiles however, have found varying results. Jayne and Bennett (1990) conducted similar locomotive performance experiments on garter snakes (*T. sirtalis*) and found the faster neonates had greater probabilities of survivorship. Thus the probability of survivorship in regards to neonate locomotion relies greatly on underlying antipredator strategy of each species. Further, incubation temperatures also influence neonate behavior in multiple other species of reptile (Lang, 1987; Gutzke and Crews, 1988; Burger, 1989, 1990, 1991; Van Damme et al., 1992; Janzen 1993b). As stated, most neonate turtles do not survive the first year of life, first winter, or ever emerge from the nests (Congdon et al., 1987, Janzen, 1993a, 1995). Because of this even traits that only minutely pose an advantage to neonate survival should result in selective forces strongly favoring that trait. So it is possible the interaction between incubation temperature, sex, and antipredator behavior affected the evolution of TSD in many reptile species (Janzen, 1995).

Nest-Site Selection

In oviparous species with temperature of the nest determining not just the sex of the offspring but also the developmental rate of the embryos the location of the nest becomes a matter of great importance to the persistence of many turtle populations. Thus, female eastern box turtles can affect their offspring's survival through genetic and non-genetic influence. Non-genetic influences such as the selection of a nest site can have dramatic effects on the survival of the female as well as potential offspring. Eastern box turtles non-randomly select locations to deposit their eggs based on certain habitat characteristics (Shine and Harlow, 1996; Hays et al., 2001; Zappalorti et al., 2015). Further, the microhabitat surrounding many turtle species' nests has been shown to control the thermal environment within the nest thus controlling the sex ratio of the embryos within (Burger, 1976). With the phenotype and survival of her offspring depending on her selection of an appropriate nest site, natural selection should favor female turtles that are able to distinguish between sites with adequate microhabitat properties for optimal nest success and those without.

Selection of a nest site can also affect the subsequent dispersal of the offspring. Thus in species with limited dispersal and specialized habitat requirements, nest-site selection and the success of the nests can shape the long-term spatial genetic as well as demographic structure of a population (Hazlitt et al., 2004; Scribner et al., 1993).

Some females may settle for suboptimal nesting sites to reduce the risk of predation to herself or the nest (Miller et al., 2007). For example, in the Australian turtle species *Emydura* *macquarii,* females will travel farther distances in the absence of predators to find ideal nesting conditions than when predation pressures are higher to either the female herself or her nest (Spencer, 2002; Spencer and Thompson, 2003). Added pressure to select nest sites with optimal environmental conditions over the risk of depredation may be placed on many turtle species in North America that exhibit TSD (Ewert and Nelson, 1991; Refsnider and Janzen, 2010; Amat and Masero, 2004).

For eastern box turtles the advantages of nesting in habitat that is optimal for proper growth and development of the embryos might outweigh the risk of depredation as there is less parental investment during the incubation period than in species such as birds, which are vulnerable to predators throughout the entire period of egg incubation (Montgomerie and Weatherheard, 1988; Seltmann et al., 2013; Refsnider et al., 2015). Additionally the potential threats to maternal survival in box turtles are often restricted to constructing the nest and traveling to and from the nesting location (Angilletta et al., 2009).

Nest-site Selection in Eastern Box Turtles

Eastern Box Turtles preferentially select forest openings or open-canopy sites to lay their eggs over heavily forested areas (Williams and Parker, 1987; Burke and Capitano, 2011; Willey and Sievert, 2011). However, it is still unclear as to what microhabitat variables they select for within forest openings to determine where to deposit their eggs. Many species of turtle in the southern United States favor nesting sites with lower vegetation heights, less canopy cover, and greater amounts of exposed soil compared to randomly selected sites within forest openings to maintain optimal thermal and hydrological conditions for egg development (Hughes and Brooks, 2016; Flitz and Mullen, 2006; Janzen and Morjan 2001; Janzen, 1994). However reptiles frequently exhibit variation in the selection of microhabitat features across their geographic range (Doody et al., 2006). For example the Common Snapping Turtle (*Chelydra serpentina*), has been found to exhibit varying nest site preferences along a latitudinal gradient across its range, with females at higher latitudes selecting open canopy sites to increase nest temperatures, and females at lower latitudes selecting shaded sites to prevent nests from overheating (Ewert et al., 2005). This suggests that the microhabitat selected for nesting by females likely varies across a species range.

With a variety of habitat types and variation in active season length and temperature across the eastern box turtles expansive range, the available number of days per year for oviposition and incubation vary (Packard et al., 1981; Hughes and Brooks, 2006). Due to the shorter summers at higher latitudes the time to reach development could be the limiting factor in the survival of nests at the Box Turtle's northern range limit (Compton, 1999). As such there is the potential for plasticity in the microhabitat features selected by box turtles among populations as well as across its geographic range to ensure nest and hatchling survival. Despite the large body of information currently available regarding eastern box turtles there is still a great need for statistically rigorous quantitative research on this species at its northern range limit.

EXTENDED METHODOLOGY

Study Sites

My study was conducted in the Manistee National Forest (MNF), which lies at the northern limit of the Eastern Box Turtle's range in northwest Lower Michigan. MNF is described as having a wet, temperate climate with four distinct seasons. Yearly maximum temperatures average 13.8 ˚C with yearly minimum temperatures averaging 1.7 ˚C. The yearly average rainfall is 99.6 cm and average snowfall is 322.4 cm (Midwest Regional Climate Center, 2017). MNF is managed for multiple uses including: recreation, wildlife habitat, timber production, watershed quality improvement, and the management of hazardous fuels (USDA, 2006). MNF is composed of primarily secondary growth forest with a mixture of red maple (*Acer rubrum*), jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*), and various oak species (*Quercus spp*.) with small (0.5 ≤ 50 ha) forest openings located throughout the study area.

Forest openings within MNF are managed by the USFS through prescribed fire, mechanical brushing, mowing, and non-native invasive species treatments. Openings are comprised of sandy soils largely covered with lichens (*Cladonia spp.*), grasses (*Andropogon spp.*), sedges (*Carex spp.*), bracken fern (*Pteridium spp.*), low bush blueberry (*Vaccinium angustifolium*), and sparse shrubs such as witch-hazel (*Hamamelis spp.*) and cherry (*Prunus spp.*).

Selected Openings

Within MNF, I selected four open-canopy nesting sites referred to as Turtle Bowl (TB), Savanna (SV), East-West (EW), and Gravel Pit (GP). Although the openings had previously been managed using prescribed fire or mechanical brushing and invasive species treatments by the USDA Forest Service (USFS) (Cadillac-Manistee Ranger District), no management treatments were implemented during the duration of my study (2013-2015). My focus openings ranged in size from roughly 0.6 hectares to 5.5 hectares and included:

The Turtle Bowl opening was a 1.9 ha, oval shaped, geological depression dominated by grasses (*Andropogon spp*.) mixed with large stretches of bare ground colonized by lichens. The TB featured low canopy cover with few trees (*Quercus alba, Pinus banksiana,* and *Pinus strobus*) and a small number of shrubs within the opening (*Prunus virginiana* and *Vaccinium angustifolium*).

The Savanna opening was the largest of the study openings (5.6 ha) with relatively flat topography. The transition between forest opening and closed canopy forest was the least abrupt in the Savanna and held the largest number of trees (*Quercus alba, Pinus banksiana,* and *Pinus strobus*) as well as the thickest shrub coverage (*Prunus virginiana, Vaccinium angustifolium, Andropogon spp., and Carex Pennsylvania)* of any of the openings.

The East West opening was a 0.9 ha linear shaped opening with a 30° south-facing slope running the entirety of the opening. Trees were sporadic around the margins of the opening (*Quercus alba, Quercus velutina, and Pinus banksiana)* and the center of the opening was a mosaic of open ground colonized by lichens and patches of *Andropogon spp*.

The Gravel Pit opening, similar to the TB opening, was a 0.7 ha oval shaped opening with a bowl-like topography. Vegetation in the GP is considerably lower in abundance compared to the TB and the vegetation that is present is considered invasive in the state of Michigan (*Centaurea maculosa, Hypericum perforatum, Verbascum thapus*). This opening was the most heavily modified of the study openings and is considered highly disturbed due to its frequent use by the public for recreational off-road vehicles.

Nest-site selection

Each June from 2013 to 2016 I conducted visual encounter surveys beginning at approximately 1900 h at each of my study sites in MNF to locate nesting females. Nesting females were monitored until egg deposition, upon which time I temporarily covered the nest using a predator-proof exclosure until the following morning. Within 24 hours of egg deposition I collected microhabitat data from within a 1-m × 1-m quadrat placed around the nest. Microhabitat data included percent bare soil, percent understory vegetation, slope angle, slope aspect, canopy cover density, distance from nest to nearest tree within the forest opening, and distance from nest to nearest forest edge. I visually estimated slope to the nearest 5 degrees and measured the aspect of the slope using a standard field compass. I visually estimated percent bare soil and percent understory vegetation within the 1-m × 1-m quadrat. The quadrat was centered over the nest and I considered any vegetation under 1m in height to be understory vegetation. However since percent bare soil and percent understory vegetation were highly correlated ($r = -0.92$), in my statistical analysis percent bare soil was used in favor of understory vegetation.

I measured canopy cover density using a spherical convex densiometer held at breast height. I took four densiometer readings from the center of the nest while facing north, south, east, and west then averaged the four readings to obtain average canopy cover density. I used a Trimble Geo 7 series GPS to mark the location of each nest to an accuracy within 25 cm. Using a Trimble Geo 7 series GPS, I walked the edge of each opening to create polygons of each of the study sites in ArcGIS (version 10.3). Then using ArcGIS (version 10.3) I measured the distance in meters from each nest to the forest edge closest to the nest. Once I had recorded the microhabitat variables from the Box Turtle nests, I used ArcGIS (version 10.3) to select one random point associated with each Box Turtle nest. To create the random points a constraining layer of each opening ($n = 4$) was constructed, then within each constraining layer random single feature points were created. I then replicated the methods used to collect microhabitat data from actual nests to gather microhabitat data at each random point generated in ArcGIS.

Nest Success

After all microhabitat data were recorded from the Box Turtle nests I installed predator-proof exclosure boxes made of wood and 1/4in. mesh steel hardware cloth by digging roughly 20cm into the ground and burying the exclosures to assure larger predators could not access the nest for the duration of incubation. Additionally, after the predator-proof exclosures were installed, beginning in June of 2014 I placed temperature dataloggers (iButton DS1922L-F5 thermochrons, Maxim Integrated) within each nest programmed to record nest temperatures at hourly intervals for the duration of the incubation period. I carefully buried the temperature loggers immediately adjacent to the nest chamber at the depth of the center of the clutch within the nest. This ensured the temperature logger would collect accurate nest temperatures while not disturbing the nest itself (Jaffé et al., 2008; Morrison et al., 2009). Fifty-five days after the eggs were laid I began monitoring the nests for emerged neonates.

Neonate Morphometric Data and Radio-Telemetry

Once an emergence had occurred I collected morphometric data on the neonates including carapace length, width, and height as well as plastron length and width using calipers. The mass for all neonates was collected using a digital scale. In order to accurately monitor neonate survival during the first year of life, each was fitted with a .35g or .5g R1614 radio-transmitter (Advanced Telemetry Systems®) to the right rear side of the carapace using clear two-part epoxy (Gorilla Epoxy adhesive®). Radio-transmitter and epoxy weight did not surpass 8% of the neonate's total body mass and did not impede neonate movements in anyway (Beaupre et al., 2004). I released all hatchling Box Turtles on the same day as the observed emergence at the nest site following data collection. If an emergence did not occur at a nest during the fall the predator-proof exclosure was left installed till the following spring and was again monitored daily for possible spring emergences. Once all of the live hatchlings had emerged from a nest I excavated each nest to look for any eggs that may have failed to develop or any neonates that had failed to make it to the surface. I categorized a nest as successful if at least one hatchling emerged from the nest on its own and was found at the surface (Kipp, 2003).

During the fall activity season (from nest emergence to overwintering) I located each neonate two to three times per week using radio-telemetry. Once located, I used a Trimble® Geo 7x Global Positioning System unit to mark the location of each neonate with an accuracy of ± 25cm. If a signal could not be detected for a particular neonate I would visually inspect the last recorded location for signs of life or depredation. If no evidence of activity or depredation were found at the last known location I would scan the surrounding area for 1-2 hours in an attempt to pick up a signal.

Once the neonates ceased movement for roughly two weeks signaling the beginning of overwintering, the predator-proof exclosures were reinstalled surrounding each neonate and were monitored daily beginning each spring for reemergence. All neonates that survived through overwintering were fitted with a new .35g or .5g R1614 radio-transmitter using clear two-part epoxy and morphometric data (neonate weight, CL, CW, CH, PL, and PW) were again collected to record any changes in growth that may have occurred since emergence. Neonates were then located three to four times a week until radio contact was lost, a mortality event occurred, or the individual was depredated.

STATISICAL ANALYSIS

Nest-site Selection Statistical Analysis

I used logistic regression to model the factors affecting nest-site selection and designed 15 models using microhabitat data collected at nest sites selected by Box Turtles and the randomly selected sites (Table 1). Models were constructed using microhabitat variables that would primarily affect nest temperature and all variables had been found to characterize nest-sites in previous turtle nesting studies. Hughes and Brooks (2006) found that painted turtles (*Chrysemys picta*) preferentially selected nest-sites free of vegetation. Nest sites with low vegetation and higher percent bare soil are likely to receive increased solar radiation and could remain warmer through out the incubation period. Because Box Turtles typically nest in forest openings and previous studies have found canopy cover to be a reliable descriptor of turtle nest sites in other species the percent canopy cover was also included in models (Janzen and Morjan, 2001; Hughes and Brooks, 2006). The slope and aspect of the ground surrounding a nest site would greatly affect the intensity and duration of exposure a nest could have to the warming effects of solar radiation and as such both were selected as likely important variables to include in the models. Further previous studies on multiple turtle species have found significant support for females preferentially selecting nest sites based on slope aspect (Schwarzkopf, 1984; Garmestani et al., 2000). Predation pressure is known to influence female turtle behavior related to nest-site selection, where younger females will select nest-sites with suboptimal microhabitat characteristic for egg development that are closer to ecological edges to reduce the females risk of predation (Harms, 2005; Spencer and Thompson, 2003). Although I did not measure predation pressure at the nest sites, I included distance from forest edge and distance to nearest tree in the suit of variables used in model construction as they also likely impact the thermal characteristics within the nest by affecting the intensity of solar radiation reaching the nest-site.

I used logistic regression to explore the relationship between the microhabitat surrounding nests and microhabitat surrounding random sites. The binary dependent variable was nest site (1) or random site (0). Additionally, since the study sites are spread across MNF, study site was also included as a variable in an attempt to detect how each site might affect nest-site selection. Akaike's information criterion corrected for small sample sizes (AIC_c) was used to rank all models. Important values included the number of parameters in each model, including an intercept (K), the delta AIC_c or the difference between the highest ranked model and the model of interest, the AICc weight (ω_i), the cumulative weight, the log likelihood where smaller values indicate better model fits, and the evidence ratio between the highest ranked model and the model of interest. All analyses for nest-site selection were conducted in program R Studio (version 0.99).

Nest Success Statistical Analysis

Since nests in my study were protected from depredation the next likely factor to influence nest success were variables that influenced the thermal properties of the nest site. I used the same models from the nest-site selection analysis to predict nest success with the addition of a "thermal squeeze" model (Table 2). The thermal squeeze model was used by Hughes and Brooks (2006) to predict survival to hatch in Midland Painted Turtles (*Chrysemys picta*) as a function of the date of oviposition. The Thermal Squeeze model was originally recommended by Compton (1999) in response to the constraint shorter growing seasons placed on the development of embryos of northern populations of Painted Turtles, resulting in nests laid later in the season not having enough time to develop before winter. In addition to the "thermal squeeze" model, I used the duration of incubation in days as an additional model. Clutch size will likely influence the thermal makeup inside the nest with some eggs from larger clutches potentially being deeper and thus cooler (Schwarzkopf and Brooks, 1987). Additionally the clutch size will also affect the probability of a nest being successful as a larger clutch size has a higher probability of an egg to hatch regardless of environmental characteristics. Because I are unable to separate this probability from the environmental characteristics selected to predict nest success I included clutch size as a covariate in all models related to nest success. Due to this study spanning multiple breeding seasons within the same population, some females nested more than once across years. I included female identity as a variable in an additional series of models for nest success, as there could be an underlying effect on nest success by particular females that the microhabitat data alone could not address. The nest success analysis included 32 models with a binary dependent variable where successful nests were assigned a (1) and unsuccessful nests were assigned a (0). I used logistic regression for my analysis between microhabitat data and Box Turtle nest success. I ranked my nest success models using AIC_c and important values included the number of parameters in each model including an intercept (K), the delta $AIC_{c,}$ the AIC_c weight (ω_i), the cumulative weight, the log likelihood, and the evidence ratio of the AIC_c weight (ω_i) between the highest ranked model and the model of interest. All analyses for nest success were conducted in program R Studio (version 0.99).

Nest Temperature and Nest Success

Although temperature loggers were implanted in all nests monitored from 2013-2016, I was only able to collect temperature data spanning the entire duration of incubation from 43 of the total 58 nests. Due to the smaller sample size, I did not include temperature in my primary models, but rather my models for nest-temperature were run as a separate exploratory analysis. I used logistic regression for my analysis between nest temperature data and Box Turtle nest success with a binary dependent variable where successful nests were assigned a (1) and unsuccessful nests were assigned a (0). I constructed 4 models in an attempt to explore the relationship between direct nest temperatures in the field during incubation and nest success and ranked the models using AIC_c (Table 3). Important values included the number of parameters in each model including an intercept (K), the delta $AIC_{c,}$ the AIC_c weight (ω_i), the cumulative weight, the log likelihood, and the evidence ratio. Models included the average nest temperature over the incubation period, the minimum temperature of the nest during the incubation period, the maximum temperature reached by the nest during the incubation period, and the number of hours a nest was above 22.5 °C. The hours above 22.5 °C model was created in response to a Ewert and Nelson (1991) study which found that the minimum constant egg temperatures that permitted embryonic development in Eastern Box Turtles was 22.5°C. Thus females should select nest-sites that have a suite of microhabitat variables that facilitate nest temperatures above 22.5°C. Further exploratory analysis included the use of Wilcoxon Signed Rank tests to directly compare temperature parameters between successful and unsuccessful nests. I compared all four variable used in the logistic models including, average nest temperature over the incubation period, the number of hours a nest was above 22.5 °C, and minimum and maximum temperatures experienced by the nest during incubation. All analyses for nest success and nest temperature were conducted in program R Studio (version 0.99).

Kaplan-Meier Survival Estimates

I used the data collected through radio-telemetry tracking of the neonate box turtles to estimate annual neonate survival probability using the nonparametric Kaplan-Meier survival estimator (Kaplan and Meier, 1958). Because the emergence time of each nest as well as the emergence time of each individual within the nests often varied, I utilized a modified version of the Kaplan-Meier procedure that allowed for new individuals to be added after the study period had begun with the emergence of the first neonate (Pollock et al., 1989). This staggered-entry method also allowed for the censoring of individuals whose fates were unknown due to radio failure or loss of signal possibly due to large-scale movements. All neonates were considered at risk until a death occurred and was confirmed by the retrieval of a carcass or radio communication was lost in which case the individual was censored. Survival curves were constructed to examine annual neonate survival across all years of the study (2012-2015) as well as for each year. Additional survival curve models included estimated annual survival by month of emergence, opening (TB, SV, EW, GP), and by neonate weight (g) at emergence. Because the Kaplan-Meier procedure does not support the use of continuous variables I constructed three weight classes: small (5.5 - 7.0 g), medium (7.1 - 8.6 g), and large (8.7 - 10.2 g) to separate neonates for the survival analysis. The weight classes were constructed using the 1st and 3rd quartiles as well as the median of the range of weights measured from the neonates from 2013-2015. All analyses of neonate survival were conducted using the Survival package (Therneau et al., 2015) for program R version 2.15.1 (R Development Core Team, 2012).

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