HABITAT SELECTION, MOVEMENT, AND SURVIVAL OF HATCHLING WOOD TURTLES (GLYPTEMYS INSCULPTA) IN AN ATYPICAL HABITAT

by

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DEDICATION

This thesis is dedicated to my family and my grandparents. I owe my passion and pursuit of knowledge about the natural world to them. Without their love and support this work would not have been possible.

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ABSTRACT

HABITAT SELECTION, MOVEMENT, AND SURVIVAL OF HATCHLING WOOD TURTLES (GLYPTEMYS INSCULPTA) IN AN ATYPICAL HABITAT

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George Mason University, 2014

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Hatchling turtles are the least understood life stage of North American turtles because they are cryptic and difficult to follow in their environment. Although few field studies have been conducted, data suggest that hatchling turtles have higher mortality rates than adults due to their smaller size, more potential predator species, and an increased vulnerability to fluctuating environmental conditions. Therefore, understanding the factors that influence survival in this critical life stage is a crucial step for turtle conservation. The North American wood turtle is considered endangered by the IUCN due to habitat loss, poaching for illegal trade, increased abundance of predators, and climate change. Nevertheless, little is known about the first two months post hatching in this species as few studies have examined hatchling behavior, and only one study has followed hatchling wood turtles from emergence until hibernation. This study

site, at the southern end of the range, is unlike typical wood turtle habitat in that it lacks natural sand beaches along the stream; therefore it is probable that hatchling turtles emerge in habitats less conducive to survival. I used paired logistic regression to investigate whether hatchlings were selecting micro habitats during migration and once they reached the stream. I also investigated hatchling movement by looking at weather patterns and comparing the route the hatchling took to reach the stream. A Cox proportional hazards model was used to test a series of hypothesis about survival, and estimate the mortality rate for hatchling wood turtles. I found that hatchlings move in relatively straight directions along the contour of the landscape rather than the shortest path from the nest to the stream, and are more likely to migrate when the weather is overcast or raining, thus preventing desiccation. Once the hatchlings reached the stream they selected micro-habitats that offered lots of cover along the bank of the stream. Survival varied greatly by year, and was influenced by the nest patch the hatchling emerged from. Habitat surrounding the nest patches and at the stream is likely playing a role on survival. The results indicate that the interplay of weather, habitat and behavior all play important roles in the early stage survivorship of wood turtles.

PREFACE

Biodiversity provides the world with goods and services valued at over a trillion dollars annually (Costanza et al. 1997). Despite the importance of these resources, humans are responsible for a biodiversity crisis with species' extinction rates of 1000 times that normally found in the fossil record (Pimm et al. 1995). Should these rates continue, within 240 years we could be looking at the earth's sixth mass extinction (Barnosky et al. 2011). Humans directly impact approximately 83% of the earth's terrestrial surface (Sanderson et al. 2002), and 100% of the oceans (Halpern et al. 2008). Therefore, it is not surprising that these impacts are driving many species extinct (Baillie et al. 2004, Hails 2008). Species loss from specific ecosystems is responsible for altering processes necessary for the productivity and sustainably of the earth's ecosystems (Loreau et al. 2002; Hooper et al. 2005; Tilman 1999; Wardle et al. 2011). Current analyses demonstrate that species loss could rank as one of the greatest drivers of ecosystem change in the 21st century (Hooper et al. 2012).

Of all the groups of animals affected by the biodiversity crisis, none may be as negatively impacted as turtles. Turtles are at a greater risk of extinction than birds, mammals, amphibians, and sharks (Turtle Taxonomy Working Group 2010). Nearly half of the world's 300 species of turtles are threatened with extinction (Turtle Taxonomy Working Group 2010; www.iucnredlist.org; Hoffmann et al. 2010). Declines in turtle

species around the world are linked to overharvesting, habitat loss and degradation, invasive species, pollution, disease, and climate change (Gibbons et al. 2000; Buhlmann et al. 2009; Turtle Conservation Coalition 2011; Ernst and Lovich 2009). These declines are due in part to the turtle's natural history coupled with human impact. Adaptions that were once successful such as delayed sexual maturity, high fecundity combined with high juvenile mortality, and high adult survivorship are now resulting in vulnerability due to the impact of humans and development (Turtle Conservation Coalition 2011). The loss of turtles is important to ecosystems around the world because of the contribution in services they provide (Ernst and Lovich 2009; Turtle Conservation Coalition 2011). Turtles provide services such as mineral cycling, indicators of pollution, seed dispersal, impacts on soil processes, as well as being of cultural importance to humans (Ernst and Lovich 2009; Turtle Conservation Coalition 2011). Should turtles disappear from these ecosystems, biodiversity will decline, as will the ecosystem services that turtles provide (Turtle Conservation Coalition 2011).

Many species of freshwater turtle are facing declines in North America; however one particularly vulnerable species is the wood turtle (Glyptemys insculpta). The wood turtle is a medium- sized turtle (straight carapace length (SCL), 20.4cm- 23.4cm) whose populations range from Virginia to Nova Scotia, Canada, with disjunct populations to the west in the Great Lakes region of Michigan and Minnesota (Ernst and Lovich 2009). Wood turtles are amphibious and occupy a variety of riparian habitats (Harding and Bloomer 1979). During the late spring and summer months, wood turtles are primarily terrestrial; however, when air temperatures drop in the fall, they congregate in cool, clear

streams where they overwinter in hibernacula such as pools, burrows, beneath over hanging roots, and in leaf litter (Ernst and Lovich 2009). Like most turtle species, wood turtles mature slowly, reaching sexual maturity around 14 years of age and living an estimated 70 years in the wild (Ernst and Lovich 2009) (Table 1.1). Compared to other freshwater turtle species, wood turtles have large home ranges with an average of 28.3 ha (Arvisais et al. 2002), and females have been known to travel up to 4 four kilometers to find suitable nesting habitat (Walde et al. 2007) (Table 1.2). Due to the wood turtle's ecology and life history, the species is experiencing declines across its entire range in the presence of increased human development (Jones et al. 2014). Because the wood turtle is equally dependent on aquatic and terrestrial habitats, a change to either environment will have adverse effects on the species (Akre and Ernst 2006).

Despite progressive and stringent protection such as the Endangered Species Act (ESA), National Environmental Policy Act (NEPA), Clean Air Act (CAA), and Clean Water Act (CWA), turtles are still at risk in North America (Ernst and Lovich 2009). Out of the 56 species native to North America, 35 (63%) require conservation action or attention (Ernst and Lovich 2009). Turtle decline in North America can be greatly attributed to increased development and rapid expansion of road networks (Steen and Gibbs 2004; National Research Council 1997). A number of turtle species such as bog (*Glyptemys muhlenbergii*), spotted (*Clemmys guttata*), wood, blandings (Emydoidea blandingii), and box (Terrapene carolina carolina) are considered amphibious or terrestrial; therefore a large portion of their life is spent on land foraging, thermoregulating, and seeking shelter (Carter et al. 1999; Ernst and Lovich 2009; Joyal et

al. 2001; Litzgus and Mousseau 2004). In particular, specific life cycles require considerable movements on land such as females seeking nesting sites, hatchlings migrating from nests to water, movement from unfavorable habitat conditions, and males searching for mates (Gibbons 1986). These terrestrial movements combined with high road density result in high mortality (Steen and Gibbs 2004). Long pre-reproductive periods, high mortality of juvenile stages, and the resultant long generation times make populations unsustainable (Brooks et al. 1991).

The wood turtle is listed as endangered by the IUCN due to habitat loss and degradation, poaching for the illegal pet trade, direct road and agriculture mortality, increases in predators, and climate change (van Dijk and Harding 2011). The preferred habitat for wood turtles occurs along broad, level valleys which have been converted into agriculture and development at high rates throughout the species' range (Jones et al. 2014). While fragmentation and degradation are their greatest threats (Vogt 1981), direct mortality as a result of land conversion occurs from automobiles and machinery (Akre and Ernst 2006; Saumure et al. 2007; Jones et al. 2014). A study in Southern Quebec tracked wood turtles in an agriculture landscape and found that 20% of their sampled population died as a direct result of farm machinery (Saumure et al. 2007). Populations of wood turtles are especially vulnerable to human-induced, premature loss of adults due to their slow growth rate and late maturity (Ernst and Lovich 2009). A study of two wood turtle populations in Connecticut documented 100% loss within 10 years when their habitat was opened to human recreation (Garber and Burger 1995). Aside from the direct

and indirect effects of habitat alteration, wood turtles face the serious threat of commercial collection for the pet trade (Levell 2000). Adult wood turtles can sell for as much as $350, while juveniles bring around $125 (Jones et al. 2014). Instances of commercial collection have occurred in almost every state the wood turtle occupies, and evidence demonstrates that poachers can collect over 100 turtles in a span of a few days (Jones et al. 2014; Hollowell 2011; McCollough 1997).

While numerous studies have documented the ecology, life history, and conservation of adult wood turtle populations throughout their range, very little is known about the hatchling stage (Daigle and Jutras 2005, Ernst and Lovich 2009, Garber and Burger 1995, Harding and Bloomer 1979, Kaufmann 1992, Saumure et al. 2007, Walde et al. 2007). Hatchlings have been considered the least understood life stage in North American turtles (Morafka 1994). Hatchling turtle behavior, movement, and habitat selection remain under studied due to the difficulty in finding and tracking individuals. However, understanding this stage is important because it is predicted to be the stage with the greatest vulnerability to mortality (Frazer et al. 1990; Ernst and Lovich 2009). Therefore, even small changes in nestling survivorship will be magnified in importance due to few hatchlings surviving in a given year (Frazer et al. 1990; Congdon et al. 1994). Hatchling turtles have higher rates of mortality relative to adults because of their low body mass, greater host of potential predators, and an increased vulnerability to fluctuating environmental factors (Ernst and Lovich 2009, Frazer et al. 1990, Greene 1988, Tamplin 2009). Due to these increased risks, it is likely that hatchlings will behave differently from adults.

Temperature affects almost all aspects of turtle ecology and plays an important role in determining fitness (Blouin-Demers and Weatherhead 2001). Moreover, environmental and physiological interactions are especially important for hatchling turtles due to their proportionately greater surface area that absorbs heat faster than adults (Boyer 1965; Stevenson et al. 1985). Increases in body temperature leads to moisture loss, which in turn can result in death due to desiccation (Kolbe and Janzen 2002). Desiccation and predation are the two most likely causes of mortality of hatchling turtles migrating from nest to water (Finkler 2001; Kolbe and Janzen 2002). Studies have demonstrated that survival rates for hatchling turtles can vary greatly when relative humidity (RH) is manipulated. At a constant temperature few hatchlings survived at 70% RH; however most survived at 90% RH (Finkler 2001). In a manipulated study where predators were controlled for, recapture rates more than doubled when predators were excluded from hatchling migration (Janzen et al. 2000). Therefore microhabitat selection is likely important for lowering the risk of the both predation dehydration (Finkler et al. 2000).

Few studies have investigated the hatchling stage in freshwater turtle species beyond the migration from nest to water (Castellano et al. 2008; Mcneil et al. 2000; Paterson et al. 2012; Rosenberg and Swift 2010; Standing et al. 1997). Of the studies that have examined post-emergence behavior, there are patterns across species. Many species do not make direct routes to a water source, but rather delay their journey by utilizing microhabitats that provide them with lots of dense ground cover (Burger 1975; Kolbe and Janzen 2002; Mcneil et al. 2000; Rosenberg and Swift 2010; Tuttle and Carroll 2005;

Standing et al. 1997) (Table 1.3). During migration there is likely a tradeoff between increased risk of dehydration and amount of time exposed to predators (Kolbe and Janzen 2002). Any movement could make hatchlings more conspicuous and prone to predation (Huey 1982). Selection of micro-habitats can allow hatchlings to improve their performance and fitness by reducing the associated risks of migration (Huey 1991).

Few studies have investigated the post emergence behavior of hatchling wood turtles (Castellano et al. 2008; Tuttle and Carroll 2005), and only one tracked them from emergence until hibernation (Paterson et al. 2012). Tuttle and Carroll (2005) studied post emergence behavior of hatchling wood turtles in New Hampshire by dusting them with fluorescent powder. Their study showed 12 hatchlings moved from nests to a stream, but only one turtle made a direct line. Most turtles in their study took an average of 6.5 days (range of 1-24) to reach a stream (Tuttle and Carroll 2005). In New Jersey, Castellano et al. (2008), radio tracked hatchling wood turtles which also did not move directly to the nearest stream. Their hatchlings took an average of 43 days to reach a stream, with 4 hatchlings still on land at the end of the study (62 days). Paterson et al. (2012) examined survival and habitat selection of hatchling wood turtles from emergence until hibernation in Ontario, Canada. They found hatchlings selected microhabitats with less leaf litter than random sites. They also demonstrated low first year survival (S=0.11), equivalent to survival rates for other freshwater species (Congdon et al. 1993; Frazer et al. 1990, Frazer et al. 1991, Iverson 1991, Mitchell 1988, Paterson et al. 2012) (Table 1.4).

For this thesis, I have investigated post emergent behavior, microhabitat selection, and survival of hatchling wood turtles from emergence until hibernation. My study took

place within the George Washington National Forest in Virginia at the southern extent of the wood turtle's range. In Virginia, the wood turtle is listed as threatened (VWAP) and in critical conservation need due to urbanization, siltation of streams, pollution, increase in predators, and vehicular mortality (Mitchell and Pague 1987; Mitchell 1994). Wood turtles were once found in watersheds within Fairfax, Loudon, Arlington, Warren, Shenandoah, Frederick, Clarke, and Rockingham counties (Figure 1.1). However, they are now primarily restricted to west of the Shenandoah River with only one population remaining in Fairfax County (Akre and Ernst 2006). Nearly all remaining populations in Virginia exist in agricultural or forest habitat. Much of the forested habitat is managed by the George Washington National Forest (GWNF), but wood turtles are found at low density in most of the forest. In order to examine the demographics of wood turtle populations within the GWNF Forest, a five year study investigated nest site selection, nest success, population demographics, and hatchling survival (Akre et al. unpublished). The focus of my study was the hatchling movement and survival portion of this study.

Tables and Figures

Table 1.1 Life history and survivorship of North American turtle species compared to the wood turtle.

Species	Sympatric	Age at Maturity	Adult Annual Survival	Life Span	Citation
Emydoidea blandingii	yes	14	0.96	-	Congdon et al. 1993
Chelydra serpentina	yes	11-14	0.96	-	Congdon et al. 1994
Kinosternon subrubrum	yes	4	0.876	-	Frazer et al. 1991
Glyptemys insculpta	-	14	0.88	70	Ernst and Lovich 2009; Jones 2009
Chrysemys picta	yes	6-10	.76-.96	35-40	Harding 1997; Mitchell 1988; Tinkle et al. 1981
Kinosternon sonoriense	no	7	0.86	-	Heppell 1998; Shine and Iverson 1995

Table 1.2 Home range size comparing the wood turtle with other North American species.

Species	Habitat	Home range (ha)	Sympatric	Citation
Glyptemys insculpta	Amphibious	28.3	-	Arvisais, et al. 2002
Glyptemys muhlenbergii	Amphibious	0.52	yes	Carter et al. 1999
Terrapene carolina	Terrestrial	2.26	yes	Donaldson and Echternacht 2005
Graptemys flavimaculata	Aquatic	1.28-5.75	no	Jones 1996
Clemmys guttata	Amphibious	5-14	yes	Litzgus and Mousseau 2004
Chelydra serpentina	Aquatic	3.44	yes	Obbard and Brooks 1981

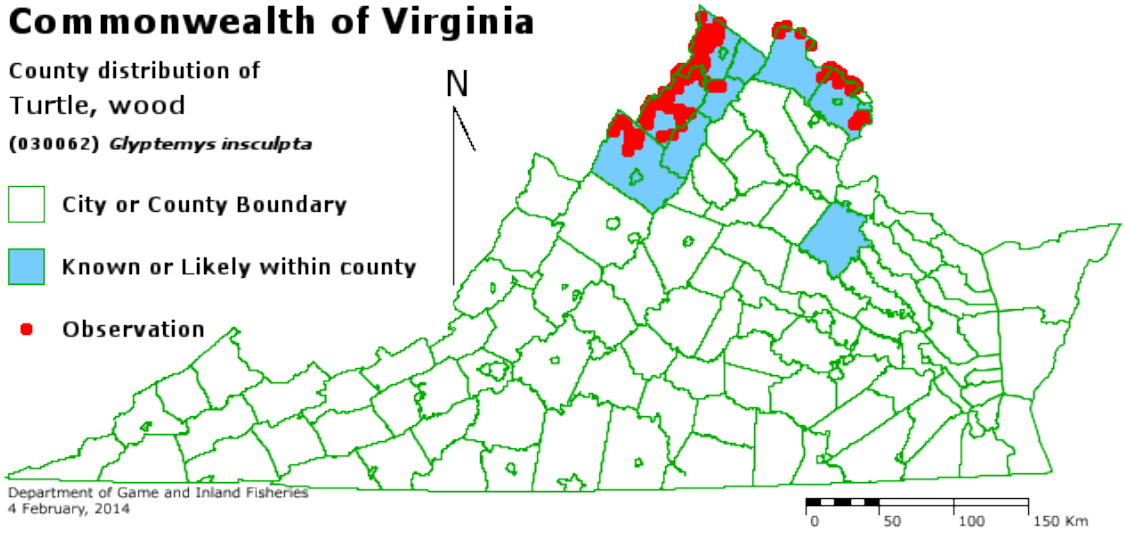
Table 1.3 Hatchling studies focused on migration.

Species	Focus of Study	Results	Citaion
Diamond back terrapin (Malaclemys terrapin	Post emergence behavior	Hatchlings moved towards vegetation rather than water source.	Burger 1975
Wood turtle (Glyptemys insculpta)	Terrestrial movements following emergence	Hatchlings did not move directly to water, but rather remained terrestrial for weeks	Castellano et al. 2008
Snapping turtle (Chelydra serpentina)	Water loss and migration	Hatchlings migrated during periods of rainfall and intermediate temperatures	Kolbe and Janzen 2002
Blandings (Emydoidea blandingii)	Movement towards water	Hatchlings were more likely to enter vegetation than water.	McNeil et al. 2000
Western pond turtle (Actinemys marmorata)	Post emergence behavior	Hatchlings delayed their journey to water by selecting microhabitats with dense ground cover	Rosenberg and Swift 2010
Blandings (Emydoidea blandingii)	Post emergence behavior	Hatchling movement was random in respect to water. Trails did not follow a straight line	Standing et al. 1997
Wood turtle (Glyptemys insculpta)	Movements and behavior	Hatchlings took on average a week to reach water and selected microhabitats with dense ground cover	Tuttle and Carroll 2005

Table 1.4 Survivorship for hatchling freshwater turtle species.

Species	Survivorship	Site	Citation
Blandings turtle	0.261	E.S. George Reserve, Michigan	Congdon et al. 1993
Slider	0.105	-	Frazer et al. 1990
Common mud turtle	0.261	Savanah River, South Carolina	Frazer et al. 1991
Yellow mud turtle	0.191	Crescent Lake National Wildife Refuge, Nebraska	Iverson 1991
Painted turtle	0.193	Laurel Lake, Virginia	Mitchell 1988
Wood turtle	0.11	Algonquin Provincial Park, Ontario Canada	Paterson et al. 2012

Figure 1.1 Distribution of wood turtles in the state of Virginia.



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CHAPTER ONE: HABITAT SELECTION AND MOVEMENT OF HATCHLING WOOD TURTLES (*GLYPTEMYS INSCULPTA)* FROM EMERGENCE TO HIBERNATION

Abstract

Hatchling turtles are the least understood life stage of North American turtles because they are cryptic and difficult to follow in their environment. While few field studies have been conducted on this life stage, data suggest that hatchlings may behave differently from adults in both their habitat choices and movement. The wood turtle is listed as endangered (IUCN 2013). The study site selected is atypical for wood turtles because nests sites are located distant from the stream. I tested the hypotheses that hatchling wood turtles select microhabitats as they migrate from their nests, and that their movements are correlated with specific weather events. I hypothesized that both microhabitat selection and movement rate were selected to prevent predation and desiccation. I radio tracked 68 hatchling wood turtles from emergence until mortality over a period of two years (2012-2013). I examined microhabitat selection during two separate migration phases: migration from the nest bank to the stream, and once the hatchling reached the stream. I also investigated what abiotic factors influenced the likelihood of movement, and the path that hatchlings took to reach the stream. I used paired logistic regression analysis to test microhabitat selection. During the migration period hatchlings demonstrated a slight preference for micro-habitats with more open canopy than paired random sites. Once at the stream, hatchlings demonstrated a stronger preference for habitat selection than during the migration period. The streamside model suggests hatchlings select gravel stream banks with deep leaf litter, more vegetation, and a closer proximity to logs when compared to paired random sites. Using logistic generalized linear models, the likelihood of movement was paired with weather and spatial data. Hatchling movements were influenced by daily weather events, the day

prior to their movement, and two days prior to their movement. Cloud cover, rainfall, temperature, and nest patch are all significant predictors of movement. Hatchlings appear to be more concerned with reaching the stream than utilizing micro-habitats between the nest and stream. Movement from the nest to the stream was strategic and relied on weather events, as well as following the contour of the landscape to reach the stream. The results of this study contradict the findings from a similar study at the wood turtle's northern extent; therefore providing evidence of a shift in hatchling behavior from the northern to southern extent of the range. This study provides further insight to a portion of wood turtle life history that until recently little was known about.

Introduction

To understand animal behavior at the appropriate spatial and temporal scale, it is important to know how their environment influences their life history such as movement, thermoregulation, growth, and survival (Morrison, et al. 2006). Habitat selection, defined as the use of habitats disproportionate to their availability, allows animals to meet the requirements needed for survival and reproduction (Jenkins et al. 2013; Johnson 1980). The most common examination of habitat selection is to compare habitat use with its availability across an appropriate scale (Thomas and Taylor 2006). In order to quantify habitat use and availability, it is important to identify key biotic and abiotic factors to the focus species. For ectotherms, such as turtles, temperature is a key factor because body temperature plays an important role for survival and reproduction (Huey 1991). Body Temperature interacts with the turtle's physiology to influence its immediate ability to perform important behaviors such as feeding and predator avoidance. In turn, these

behaviors ultimately affect its ability to grow, survive, and reproduce (Huey and Stevenson 1979; Heinrich 1981; Huey 1982; Knapp and Casey 1986; Dunham et al. 1989). Habitat selection in adult turtles is predicted based on temperature differences between microhabitats (Carter et al. 1999, Compton et al. 2002, Arvisais et al. 2004); however few studies have examined the juvenile (i.e. hatchling) stage (Forsythe et al. 2004, Paterson et al. 2012). Hatchling turtles have higher rates of mortality relative to adults because of their lower body mass, which results in a greater suite of potential predators, and an increased vulnerability to fluctuating environmental conditions (Tamplin 2009). Behavior, such as basking to thermoregulate, may involve movements that make hatchling turtles more conspicuous and prone to predation. Therefore, a dynamic tradeoff exists between physiological capacity and avoidance of predation (Huey 1982). Due to the influence of predation, it is likely that habitat selected by hatchling turtles will differ from adults of the same species.

The period for hatchling turtles from their nest site to their arrival at post-emergent habitat is hypothesized to be a period of high mortality due to the lack of orientation to its environment and landscape, as well as thermoregulation, predators, and naiveté to food (Janzen et al. 2000). However, little information exists for the majority of freshwater turtles about this life stage, and information that does exist is often anecdotal (McNeil et al. 2000). The assumption for aquatic turtle species is that hatchlings should move directly to a water source to prevent predation and desiccation (Ultsch 2007). Contrary to this assumption, a number of studies have demonstrated that hatchlings will not head directly for water, despite the need to hibernate under water in blandings and

wood turtles (Mcneil et al. 2000, Standing et al. 1997, Castellano et al. 2008, Tuttle and Carroll 2005). Kolbe and Janzen (2002) considered the indirect movement of hatchlings as a trade-off between moisture loss and predation. They concluded that some hatchlings may make fast movements to water and risk moisture loss while minimizing exposure to predators. Alternatively, hatchlings may conserve water resources during times of unfavorable environmental conditions, thereby prolonging the journey but increasing predation risk (Kolbe and Janzen 2002). These theoretical considerations of migration strategies have not been tested on wild populations for most species.

I investigated microhabitat selection and movement patterns for hatchling wood turtles (Glyptemys insculpta) from nest emergence until in-water hibernation. I considered two phases to this movement; the first being the migration from the nest to the stream, and the second being the habitat used once the hatchling reached the stream until it entered hibernation. I tested the hypotheses that hatchling turtles select habitats disproportionate to their availability on the landscape, and move during times of favorable environmental conditions. I also compared their movement path with straight line migration to the closest stream as a way to understand their migration strategy. I predicted that hatchling movements would reflect their dual concerns of desiccation and predation risk and would select micro-habitats that were cooler, more humid, and had more cover to conceal them from predators. I also predicted that hatchlings would move during the day of, or the day following rain events and cooler temperatures, and that they would follow the contour toward the closest stream.

Study Site Description

The study was located within the George Washington National Forest in Shenandoah and Frederick Counties, Virginia and encompassed approximately 1800 ha. Exact location is withheld due to the threat of poaching for the illegal pet trade (Compton et al. 2002). The study site is composed of two first order streams that feed into each other and drain out of the site. A U.S. Forest Service gravel road runs the length of the study site and parallels both streams (Fig 2.1). Other human disturbances in the watershed include a power line right-of-way and six cutting units that were logged in the past four to sixty years. Predominant canopy species consist of oak (*Quercus alba, Q. cocinna, Q. prinus, Q. rubra, and Q. velutina*), maple (*Acer rubrum and A. saccharum*), and several species of pine (*Pinus rigida, P. strobus, and P. virginiana*). Sub-canopy species include hophornbeam (*Ostrya virginiana*), shadbush (*Amelanchier* spp.), dogwood (*Cornus florida*), and witch-hazel (*Hamamelis virginiana*). The shrub and woody understory is composed of winterberry (*Llex verticilata*), viburnum (*Viburnum* spp.), spicebush (*Lindera benzoin*), Vaccinium (*Vaccinium* spp), huckleberry (*Gaylusuchia* spp.), blackberry (*Rubus* spp.), greenbriar (*Smilax* spp.), lyonia (*Lyonia* spp.), mountain laurel (*Kalmia latifolia*), and virginia creeper (*Parthenocissus quinquefolia*). This study site differs from most wood turtle landscapes in that both streams lack sandy banks commonly utilized by nesting female turtles (Bulhlmann and Osborn 2011; Hughes et al. 2009). Over a series of five years a total of 185 nests have been recorded and monitored at this site. To date female turtles have nested in roadside banks (56%), an abandoned barrow pit (15%), a power line (12%), a dirt road (.06%), cutting units (.05%), stream bank (.02%), and a collapsed beaver dam (.01%) (Akre et al.

unpublished data, personal observation). Only 4 of 185 nests have been in a naturally created nest bank. This study is part of a larger project to determine nest site selection, nest success, hatchling survival, and recruitment of wood turtles within this study area (Akre et al. unpublished).

Methods

Hatchling Capture and Measurement

Nests were monitored daily in 2012 and 2013 for emergence from 7:30 A.M. to 12:00 P.M. beginning August 1 and continuing until the first in-ground frost in late September to mid-October. Emerged hatchlings were collected and partitioned by clutch. Individual emerged hatchlings were weighed (±0.1g) using an electronic scale (Ohaus Corporation), carapace and plastron lengths were measured (0.01cm) using 50 cm calipers (Swiss Precision Instruments, Inc). Prior to emergence, four nest patches were selected to sample hatchlings for this study. These patches were selected to be representative of the spatial and environmental variation found within the study site. In pre-selected nests, hatchlings weighing ≥ 8.90g were outfitted with radio transmitters (Blackburn Transmitters, Nacogdoches, TX; 0.50g). Transmitters were attached with fast drying epoxy gel (Devcon, Inc. 5 minute epoxy gel) (Figure 2.2). Transmitters with epoxy weighed 0.60g, and did not exceed 7% of the mass of the hatchling. Hatchlings were marked with a unique clutch identifying code using visual implant elastomer (Northwest Marine Technology Inc. Shaw Island, WA). Hatchlings were then released in the nearest shaded area of the nest from which they emerged. Radio-tagged hatchlings

were located every other day beginning the day after release and continuing until death, hibernation, or loss of signal. Upon radio location, the hatchling was recorded as observed or unobserved (under cover), and, if observed, whether active or inactive. If the hatchling was active its behavior was recorded (inactive, alert, walking, basking, swimming, and feeding). All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

Field

Microhabitat variables known to be associated with habitat selection and survival were measured within a $1m^2$ Daubemire frame centered on the hatchling (Huey 1991, Kolbe and Janzen 2002, Patterson et al. 2012). These included the following: ground cover and count of woody and herbaceous stems, and leaf litter depth (cm). I also recorded soil moisture (%) within 10 cm of the turtle, temperature (°C) and relative humidity (%) under the cover used by the hatchling (if applicable) (0-3 cm below ground surface), at the ground surface (0 cm), and directly above (100 cm) the location, soil saturation (dry, saturated, standing water), canopy cover at the hatchling's level and at 1 m above the hatchling using a spherical densiometer the shortest distance from the hatchling to a set of features (m) (stream, intermittent stream, ephemeral standing water, seep, road, nest, log, and edge). Features greater than 20m were not included. All measures were also recorded at a randomly selected location within 30m, used as a paired control. Random parings were created using a random number from 1-30, and then by selecting a random compass bearing. When a random number was chosen, I walked that many meters at the random bearing chosen by the compass. The maximum of 30 m was

chosen because it is in the range of average daily distances travelled by hatchlings of this species (Tuttle and Carroll 2005, Castellano et al. 2008). Only soil moisture, temperature, humidity, and soil saturation were recorded at the time of capture in order to avoid disturbance. The remainder was recorded in the following 14 days after the turtle had moved on. I obtained the geolocation of each new hatchling location with a Trimble Geo XT and Tempest antenna attachment (Trimble Navigation, Ltd. Sunnyvale, CA). Post processing of point was performed with Pathfinder Office software and achieved an accuracy of ±2m using differential correction (Trimble Navigation, Ltd. Sunnyvale, CA).

Each hatchling location was later paired with local weather data collected from the nearest National Oceanic and Atmospheric Administration (NOAA) station located 4.5km away. The following variables were collected: precipitation (to nearest 0.1 mm), minimum and maximum daily temperature (˚Celsius), and average daily temperature. In order to estimate average daily temperature during the active period of the day (7:00 A.M. to 7:00 P.M.) I used a thermochron ibutton data logger (Maxim Integrated, San Jose, CA) placed at the top of a 1m wooden stake and coated in plasti dip (Plasti Dip, St. Loius Park, MN). Three stakes were placed across the study site in the shaded forest. Daily cloud cover was recorded at the end of each day by the researcher as either no, partial, or full cloud cover.

Data Analyses

Habitat Selection

In order to reliably answer whether an animal is selecting one habitat over another, consideration of the animal's mobility is important. Pairing the selected habitat

with a non-selected control compares use with availability at the same place and time, thus making it more appropriate for hatchling turtles with limited mobility (Breslow and Day 1980; Compton et al. 2002; Paterson et al. 2012). Logistic regression is a robust technique that does not require multivariate normality, and it allows the use of categorical predictor variables (North and Reynolds 1996). Logistic regression models are based on a binary response (1 if the habitat used, 0 if not selected), and are interpreted by relative changes in habitat variables between the binary responses. Paired logistic regression is interpreted by coefficients in the form of odds ratios. Odds ratios greater than one indicate the probability of selection increases with a variable, and odds ratios less than one indicate the probability of selection decreases with a variable (Breslow and Day 1980, Hosmer and Lemeshow 1989, Ormsbee and McComb 1998).

Paired logistic regression analysis was performed using the survival package in program R to determine what habitat features hatchling turtles selected from emergence to hibernation (R Core Team, 2013). Data from both years of the study were combined for analyses. Two separate models were run corresponding to migration and streamside habitat. The migration model contained all data points until the hatchling was within 10m of the stream, at which point all locations were included in the streamside model.

A covariance matrix was used in initial data exploration to eliminate covariates with an $r > 0.6$ (Li-tze and Bentlerb 1999). The following variables were used in both microhabitat selection models: temperature and humidity at the turtle's location, canopy cover, woody stem count, distance to log, leaf litter depth, percentage of ground cover that was: woody, herbaceous, litter, coarse woody debris, rock, moss, and gravel (Table

2.1) . Through initial data exploration and preliminary analysis, 13 candidate models were selected including a null model and a full model containing all covariates (Table 2.2 & Table 2.3). Candidate models were developed to test different hypothesis, and were based on which covariate showed stronger influences on the response variable through various data exploration and plotting techniques. I used an information-theoretic approach for evaluating potential candidate models that explain microhabitat selection (Burnham and Anderson 2002). I used Akaike's information criterion (AIC) to rank each competing model, and the model with the lowest AIC score was considered to be the most supported (Burnham and Anderson 2002). When models were within two AIC of one another, the model containing only significant covariates was considered to be the most supported. Prior to model selection, I measured the variance between individuals and nest patches to investigate whether they had random effects.

Movement

Generalized linear models are an effective way to model linear relationships as they do not assume a normal distribution and allow for the use of a binomial dependent variable (Bolker 2008). A logistic generalized linear model was used to test whether movement, defined as a 1 if the hatchling moved or a 0 if it did not, was influenced by weather, spatial location, and movement history. The following variables from the day of the movement, the day prior to movement, and two days prior to movement were used: cloud cover, precipitation (.01mm), minimum daily temperature (˚C), maximum daily temperature (˚C), and average daily day time temperature (˚C). The nest patch the hatchling emerged from, and whether the hatchling moved the day prior and two days

prior were also included in the models (Table 2.4). A total of 10 candidate models were tested including a null model and a model containing all covariates. Candidate model selection was developed to test various hypotheses, and was based on which covariates had stronger relationships with the response variable through data exploration and plotting. Goodness of fit for each model was assessed using McFadden's p^2, analogous to the multiple correlation coefficient (r^2) used in linear regression (McFadden 1974).

Visual inspection of serial hatchling locations in GIS suggested that hatchlings moved in a relatively straight path from the nest bank to the stream. It is known that hatchling freshwater turtles go directly to water upon emergence (Ehrenfeld 1979); therefore I investigated whether hatchlings were homing to the stream and moving over the landscape or following the line of least resistance, such in the way water would travel if poured from the nest bank. A path was drawn in ArcGIS representing the shortest path from each nest bank to the steam. A second path was drawn using ArcGIS's hydrology tool to measure the path water would flow if poured from the nest bank and drained to the stream (contour line). I investigated this by comparing each hatchling location to both lines using a paired samples t-test.

Results

General

In 2012, 41 turtles were tracked for an average of 18 ± 17 days (range 1-58). I radio tagged hatchlings from four nest sites which included a total of eight clutches.

Daily tracking began on August 7 and continued until October 4. One turtle was successfully tracked through the hibernation period and was monitored until early June 2013, at which time its transmitter became detached. In 2013, 27 turtles were tracked for an average of 40 ± 24 days (range 3-64). These hatchlings were sampled from four clutches and the same nest sites as the previous year. Continuous tracking began on August 21 and continued until October 16. Ten hatchlings had their transmitters replaced at the onset of hibernation to be tracked through the winter.

Habitat Selection during Migration

The migration model was constructed using112 paired locations with an average of five locations per individual. The best model predicting microhabitat selection during migration contained one significant covariate, percentage of closed canopy (Table 2.5). The model suggests that for each increase in percent of overhead canopy cover the likelihood of a hatchling selecting the habitat decreased by 0.01 (Table 2.6). Although there is some supportive evidence that hatchlings select microhabitats with less canopy cover, there seems to be a lack of preference for microhabitat selection when migrating to the stream.

Habitat selection streamside

The streamside model was constructed using 242 paired locations with an average of eight per individual. Prior to data analyses, all covariates were tested for collinearity, and those with an r value greater than 0.6 were removed. The best model for streamside habitat, defined by the lowest AIC score contained seven significant covariates (Table 2.7). The model suggests hatchlings select habitats that are warmer, composed of mostly

gravel and rock ground cover, and with deeper leaf litter, contain more vegetation, and are closer to a log. For each cm increase in leaf litter depth, the odds of a hatchling selecting the habitat increased by 20% (Table 2.8 & 2.9).

Movement

Following nest emergence, hatchlings took an average of 9.0 ±6.3 days to migrate from the nest to a stream. Hatchling movements averaged 61.26 ±69.46 meters; however when incorporating days of dormancy, their average daily movement was 15.77 ±44.24 meters (Table 2.10). Hatchling locations (N=146) were significantly closer to the contour line (M=11.11, SD=13.02) than the straight line path (M=15.72, SD=18.02) (t= -3.10, P value= 0.0023) (Fig 2.3 & 2.4, 2.5, 2.6) (Table 2.11). The best model describing the likelihood of movement, selected by the lowest AIC score, contained six significant covariates, and one significant interaction term (p^2=.16) (Table 2.12) (Figure 2.7). The model suggests hatchlings are more likely to move when it is partially cloudy or a mix of rain and sun on the day of the movement. Hatchlings moved more when it rained two days prior and had a warmer minimum temperature two days prior (Table 2.13 & 2.14). Also, hatchlings were less likely to move if they had moved the day prior and more likely if they had moved two days prior. The nest patch the hatchling emerged from also played a role in the likelihood of movement.

Discussion

Hatchling wood turtles did not select specific microhabitats during their migration to the stream. Hatchlings rather migrated along the contour of the landscape to reach the stream instead of following the straightest possible path from nest to stream. They

moved during times of favorable weather conditions such as after rain events and during periods of intermittent clouds, taking an average of 9 days to reach the stream. When hatchlings did move, they travelled relatively large distances averaging 61.26m each move. Once at the stream, hatchlings selected microhabitats that were closer to logs, had more vegetation, deeper packs of leaf litter, warmer temperatures, and more gravel than surrounding available habitats. These microhabitats were often in the form of debris jams where leaf litter collected against a log along the banks of the creek.

Despite the associated risks with migration, previous studies on hatchling wood turtles and sympatric species demonstrated that hatchlings did not migrate directly to water, but rather remained terrestrial for periods of time (Castellano et al. 2008, McNeil et al. 2000; Standing et al. 1997; Tuttle and Carroll 2005). It has been hypothesize that in habitats with a longer active season there may be no pressure to reach the stream directly upon emergence (Castellano et al. 2008). However, the results of this study contradict this hypothesis as my study took place at the southern extent of the wood turtle's range, and yet they only spent an average of 9 days migrating compared to an average of 43 days observed in New Jersey (Castellano et al. 2008).

Once hatchlings made it to the stream and begun selecting microhabitats, they chose features that provided more cover, potentially decreasing the risk of predation. Paterson et al. (2012) demonstrated that hatchling wood turtles at their northern extent selected microhabitats with less leaf litter and cooler temperatures; however, in my study hatchlings selected microhabitats with deeper leaf litter and warmer temperatures. It is possible that this difference in selection can be attributed to the hatchlings experiencing

longer active seasons in the south. Hatchlings emerge later and hibernate earlier in the north; therefore pressure to find hibernacula may be far greater. Hatchlings that do not need to immediately prepare for overwintering will be faced with the risks of remaining terrestrial longer. More cover and warmer temperatures may help hatchlings avoid predation during the longer active season.

This study provides new insight into a portion of turtle life history we know little about. The results of this study contradict what is known about hatchling wood turtles in their first year of life; thereby demonstrating the importance of conducting multiple studies and in a variation of range and habitat to truly understand animal behavior. The fact these results are the opposite of a similar study at the other end of the animal's range suggests there is a shift in behavior throughout their range. Future studies should investigate habitat selection between the southern and northern extents where the active season would be more average to further explain this behavior. Due to this study site representing atypical wood turtle habitat, some of the behaviors may be contributed to the greater migration distance in this study. Future studies should investigate how hatchling wood turtles behave in more typical habitat where they emerge in close proximity to the stream. Migration distance likely plays a factor in behavior; therefore understanding how they behave when they emerge at the stream will provide a better understanding of the variation observed in different studies.

Tables and Figures

Table 2.1 List and description of covariates used in habitat selection models ($1m^2$).

Covariate	Description
Temp (C˚)	Temperature at turtle location or control
Hum (RH %)	% Humidity at turtle location or control
Canopy (%)	Percentage of open canopy
Woody Stem	Count of woody stems within the plot
Log	Distance (m) to the nearest log (>5cm in diameter and at least .5 meter long)
Herbaceous	Percentage of ground cover that is herbaceous
Woody Sapling	Percentage of ground cover that is woody seedlings
Litter	Percentage of groundcover that is leaf and twig (<1cm in diameter) litter
CWD	Percentage of ground cover that is coarse woody debris (≥1cm in diameter)
Rock	Percentage of ground cover that is rock
Moss	Percentage of ground cover that is moss
Leaf Litter Depth	Depth (cm) of leaf litter in plot

Table 2.2 Mean and range of covariates used in migration habitat selection analysis.

Covariate	**Turtle** (N=112)	**Control** (N=112)
Temp	24.50 (7.4-42.5) ± 5.09	24.43 (7.3-46.5) ± 5.47
Humidity	67.69 (28-98) ± 15.60	66.6 (4-98) ± 17.01
Canopy	67.35 (0-100) ± 33.77	69.89 (0-100) ± 33.30
Woody Stem	3.30 (0-25) ± 5.70	3.32 (0-75) ± 8.63
Log	6.02 (0-20) ± 7.94	6.59 (.04-20) ± 8.09
Herbaceous	43.93 (0-100) ± 41.47	41.71 (0-100) ± 40.59
Woody	4.55 (0-70) ± 10.13	3.34 (0-40) ± 7.66
Litter	27.86 (0-99) ± 34.05	28.88 (0-100) ± 32.18
CWD	1.16 (0-40) ± 5.15	1.60 (0-55) ± 7.08
Rock	5.04 (0-90) ± 15.33	7.61 (0-85) ± 18.89
Moss	2.22 (0-74) ± 8.95	2.32 (0-45) ± 7.71
Gravel	6.79 (0-100) ± 20.31	4.88 (0-95) ± 16.21
Litter Depth	2.35 (0-12) ± 2.52	2.13 (0-10) ± 2.20

Table 2.3 Mean and range of covariates used in streamside habitat selection model.

Covariate	**Turtle** (N= 242)	**Control** (N=242)
Temp	23.79 (6.3-44.1) ± 4.50	23.35 (5-51.8) ± 5.02
Humidity	65.87 (22-98) ± 16.07	66.67 (28-98) ± 15.06
Canopy	77.33 (0-100) ± 29.95	76.84 (0-100) ± 31.67
Woody Stem	3.32 (0-33) ± 5.69	2.94 (0-75) ± 6.71
Log	3.51 (0-20) ± 6.03	3.75 (0-25) ± 6.37
Herbaceous	29.46 (0-100) ± 35.65	29.05 (0-100) ± 36.12
Woody	4.40 (0-50) ± 8.69	3.24 (0-40) ± 7.29
Litter	31.39 (0-98) ± 32.20	34.37 (0-100) ± 34.68
CWD	2.77 (0-70) ± 8.24	2.57 (0-50) ± 7.24
Rock	12.35 (0-90) ± 21.67	9.74 (0-95) ± 20.46
Moss	2.99 (0-80) ± 10.27	3.02 (0-80) ± 10.88
Gravel	7.81 (0-100) ± 17.68	5.38 (0-100) ± 16.70
Litter Depth	2.50 (0-12) ± 2.29	2.28 (0-10) ± 2.13

Table 2.4 Description of weather and spatial related covariates used in movement models.

Covariate	**Description**
Cover	Daily cloud cover as full clouds, partial clouds, or full sun
Cover1	Daily cloud cover the day prior to movement
Cover2	Daily cloud cover two days prior to movement
Temp	Average temperature for the active period of the day (7:00 A.M. – 7:00 P.M.)
Temp1	Average daily temperature day prior to movement
Temp2	Average daily temperature two days prior to movement
TMIN	Minimum daily temperature
TMIN1	Minimum daily temperature day prior to movement
TMIN2	Minimum daily temperature two days prior to movement
TMAX	Maximum daily temperature
TMAX1	Maximum daily temperature day prior to movement
TMAX2	Maximum daily temperature two days prior to movement
Precip	Amount of daily rainfall in .01mm
Precip1	Daily rainfall prior to movement
Precip2	Daily rainfall two days prior to movement
Move1	Binomial covariate of whether the hatchling moved the day prior
Move2	Binomial covariate of whether the hatchling moved two days prior

Table 2.5 Candidate model selection process for habitat selection during migration from the nest bank to the stream. The model with the lowest AIC score and containing only significant covariates was considered to be the most supported model.

Model	K	AIC	Delta AIC	AIC wt.
Canopy	1	199.74	0	0.21
Canopy + Herbaceous	2	199.76	0.01	0.21
Canopy*Herbaceous	3	200.85	1.11	0.12
Canopy + Humidity	2	200.96	1.22	0.12
Canopy + Leaf Litter Depth	2	201.62	1.88	0.08
Canopy + Herbaceous + Litter Depth	3	201.7	1.96	0.06
Canopy*Humidity	3	202.14	2.39	0.06
Canopy*Leaf Litter Depth	3	202.45	2.71	0.05
Humidity + Canopy + Herbaceous + Litter Depth	4	203.41	3.67	0.03
Canopy + Humidity + Litter Depth	4	204.69	4.95	0.02
Temp*Humidity + Canopy + Herbaceous + Litter + Litter Depth	7	208.18	8.44	0
Temp + Humidity + Canopy + Log + Herbaceous +Woody Stem + Woody Sapling% + Litter + CWD + Rock + Moss + Litter Depth	12	213.29	13.54	0
Null Model	1	667.06		

+ = Add covariate
* = Interaction between covariates

Table 2.6 Fitted parameter estimates from the most supported model predicting habitat selection during migration using paired logistic regression.

Covariate	Odds Ratio	Sig. Value	Lower .95	Upper .95
Canopy Cover	0.9904	0.05	0.9834	0.9975

Table 2.7 Candidate model selection process for habitat selection once the hatchling reached the stream. The model with the lowest AIC score and containing only significant covariates was considered to be the most supported model.

Model	K	AIC	Delta AIC	AIC wt.
Temp + Log + Herbaceous + Rock + Woody + Gravel + Litter Depth	7	265.11	0	0.62
Temp*Litter Depth + Log	4	269.83	4.72	0.06
Temp + Humidity + Canopy + Log + Herbaceous + Woody + Litter + CWD + Rock + Moss + Gravel + Litter Depth	12	270.07	4.96	0.05
Temp + Log + Litter Depth	3	272.3	7.19	0.02
Herbaceous + Log + Woody + Gravel + Litter Depth	5	272.78	7.67	0.01
Herbaceous + Woody + Litter Depth + Log	4	274.93	9.83	0
Temp + Gravel % + Litter Depth	3	279.31	14.2	0
Temp*Litter Depth	3	280.06	14.89	0
Litter Depth	1	281.42	16.31	0
Temp*Gravel	3	281.52	16.41	0
Herbaceous+ Gravel + Litter Depth	3	281.55	16.44	0
Herbaceous + Woody	2	283.68	18.57	0
Null Model	1	546.73		0

Table 2.8 Fitted parameter estimates from the best supported model predicting streamside habitat selection using paired logistic regression.

Covariate	Odds Ratio	Sig. Value	Lower .95	Upper .95
Leaf Litter Depth	1.2	0.001	1.0556	1.3681
Temp	1.09	0.05	0.9972	1.1824
Log	0.93	0.001	0.8868	0.9754
Rock	1.016	0.001	1.0049	1.0266
Herbaceous	1.008	0.01	1.0004	1.0158
Woody	1.03	0.01	1.0021	1.0577
Gravel	1.015	0.01	1.0008	1.0288

Table 2.9 Odds of a hatchling selecting a microhabitat with a change in a habitat variable.

Covariate	Odds Ratio	Magnification	Odds of Selection
Leaf Litter	1.2	3cm depth	60.00%
Temp	1.09	3˚C increase	2.70%
Log	0.93	1.5m away	-1.05%
Rock	1.016	50% increase	8.00%
Herbaceous	1.008	50% increase	0.04%
Woody	1.03	50% increase	1.50%
Gravel	1.015	50% increase	7.50%

Table 2.10 Results of paired t-test demonstrate hatchlings migrate significantly closer to the contour line than the straightest possible path.

Summary Stat	Contour line	Straight line
Mean	11.11	15.72
Variance	169.57	324.58
N	146	146
t Stat	-3.0973	
P value	0.0023	

Table 2.11 Summary statistics of the average distance hatchlings travelled.

	Daily average distance*	Average movement distance	Min	Max
Patch 1	13.12 ± 36.84	50.23 ± 57.94	0	140.53
Patch 2	27.53 ± 65.22	89.91 ± 91.52	0	201.1
Patch 3	31.61 ± 53.34	70.81 ± 60.24	0	104.75
Patch 4	12.57 ± 39.06	55.86 ± 66.23	0	244.84
Combined	15.77 ± 44.24	61.26 ± 69.46	0	244.84

***Daily average distance accounts for days of dormancy.**

Table 2.12 Candidate model selection for predicting the likelihood of movement based on the weather, patch, and movement history. The model with the lowest AIC score in considered the best supported model.

Model	K	AIC	Delta AIC	AIC wt.
cover*Precipitation + patch + move1 + move2 + Precip2+ TMIN2	13	1054.49	0	0.94
cover+ cover1 + cover2 + patch + move1 +move2 + Precip + Precip1 + Precip2 + TMIN +TMIN2 + TMAX	18	1061.27	6.78	0.03
patch+ move1 + move2+ Precip2+ TMIN2	8	1063.46	8.97	0.01
patch + move1 + move2+ Precip2+ TMIN + TMIN2 +TMAX	10	1063.52	9.03	0.01
cover + cover2 + patch+ move1 + move2 + Precip1 + Precip2 + TMIN + TMIN2 +TMAX	15	1064.13	9.65	0.01
cover1*Precip1 + patch+ move1+ move2 + Precip2 + TMIN2	13	1066.51	12.02	0
cover*Precipitation + patch+ move1 + move2 + Precip1 + TMIN2	13	1068.16	13.67	0
patch + move1+ move2 + Precip2	7	1089.58	35.09	0
patch+ Precip2 + TMIN2	6	1181.23	126.74	0
Null Model	2	1267.86	213.37	0

Table 2.13 Fitted parameter estimates for the best supported model predicting the likelihood of movement using logistic regression.

Covariate	Odds Ratio	Sig. Value	Lower .95	Upper .95
Cover: Partly	2.11	0.05	1.0041	4.447
Cover: Sunny	0.77	-	0.5328	1.1088
Patch 2	1.24	-	0.7664	2.0093
Patch 3	2.33	0.05	1.1677	4.654
Patch 4	0.76	-	0.5324	1.0957
Move1	0.1	0.001	0.0528	0.1919
Move2	1.88	0.001	1.3556	2.6147
Precip2	1.006	0.001	1.0034	1.0087
TMIN2	1.08	0.001	1.0461	1.1211
Cover: Partly*Precip	0.98	-	0.9541	1.0173
Cover: Sunny*Precip	1.009	0.01	1.0027	1.0157

*Interaction term

Table 2.14 Odds of moving with a change in covariates.

Covariate	Odds Ratio	Magnification	Odds of Moving
Partly Cloudy	2.11	Vs. Full Clouds	111.00%
Sunny	0.77	Vs. Full Clouds	-33.00%
Patch 2	1.24	Vs. Patch 1	24.00%
Patch 3	2.33	Vs. Patch 1	133.00%
Patch 4	0.76	Vs. Patch 1	-24.00%
Move1	0.1	Vs. Didn't move day prior	-90.00%
Move2	1.88	Vs. Didn't move two days prior	88.00%
Precip2	1.006	1mm	0.06%
TMIN2	1.08	3˚C	2.40%
Partly*Precip	0.98	Over Cloudy and no rain	-0.02%
Sunny*Precip	1.009	Over Cloudy and no rain	0.01%

Figure 2.1 Study site in the George Washington National Forest, VA. The site is approximately 500ha. and consists of two streams paralleled by a gravel road.

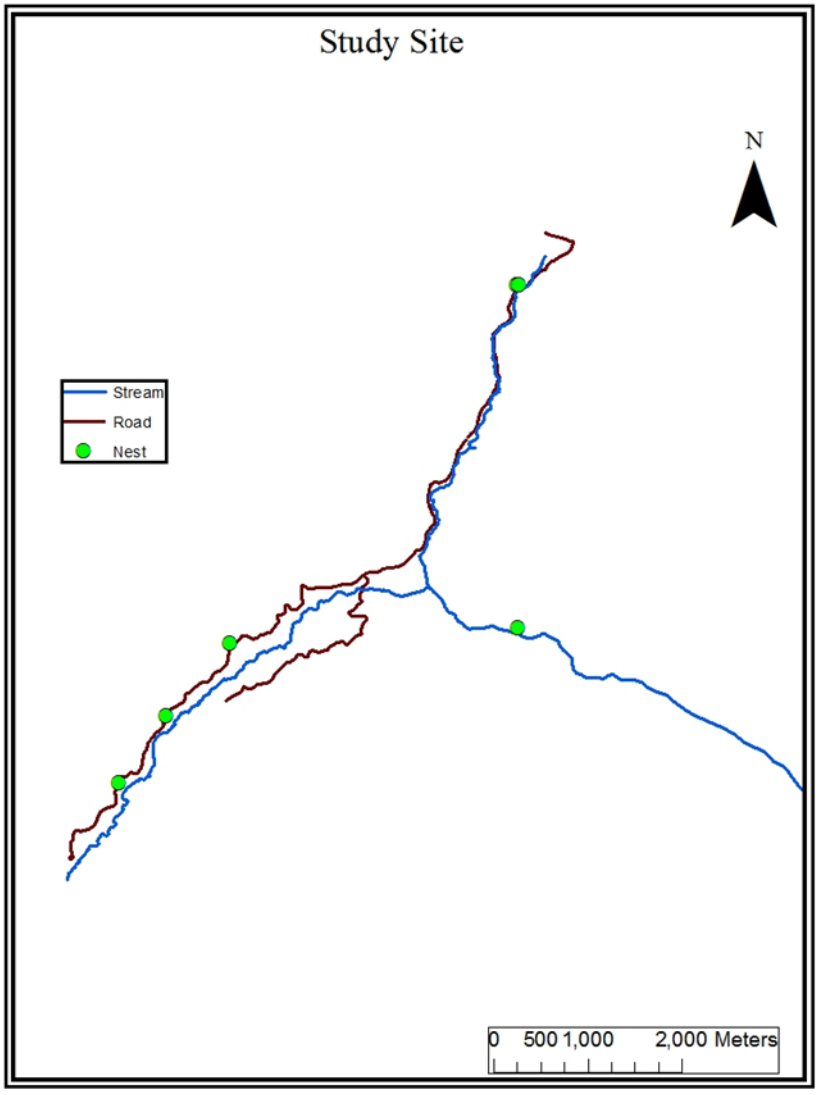


Figure 3.2 Hatchling wood turtle fitted with radio transmitter.

Figure 2.3 Location of migrating hatchlings compared with the straightest possible path and the path representing the contour of the landscape (Patch 1).

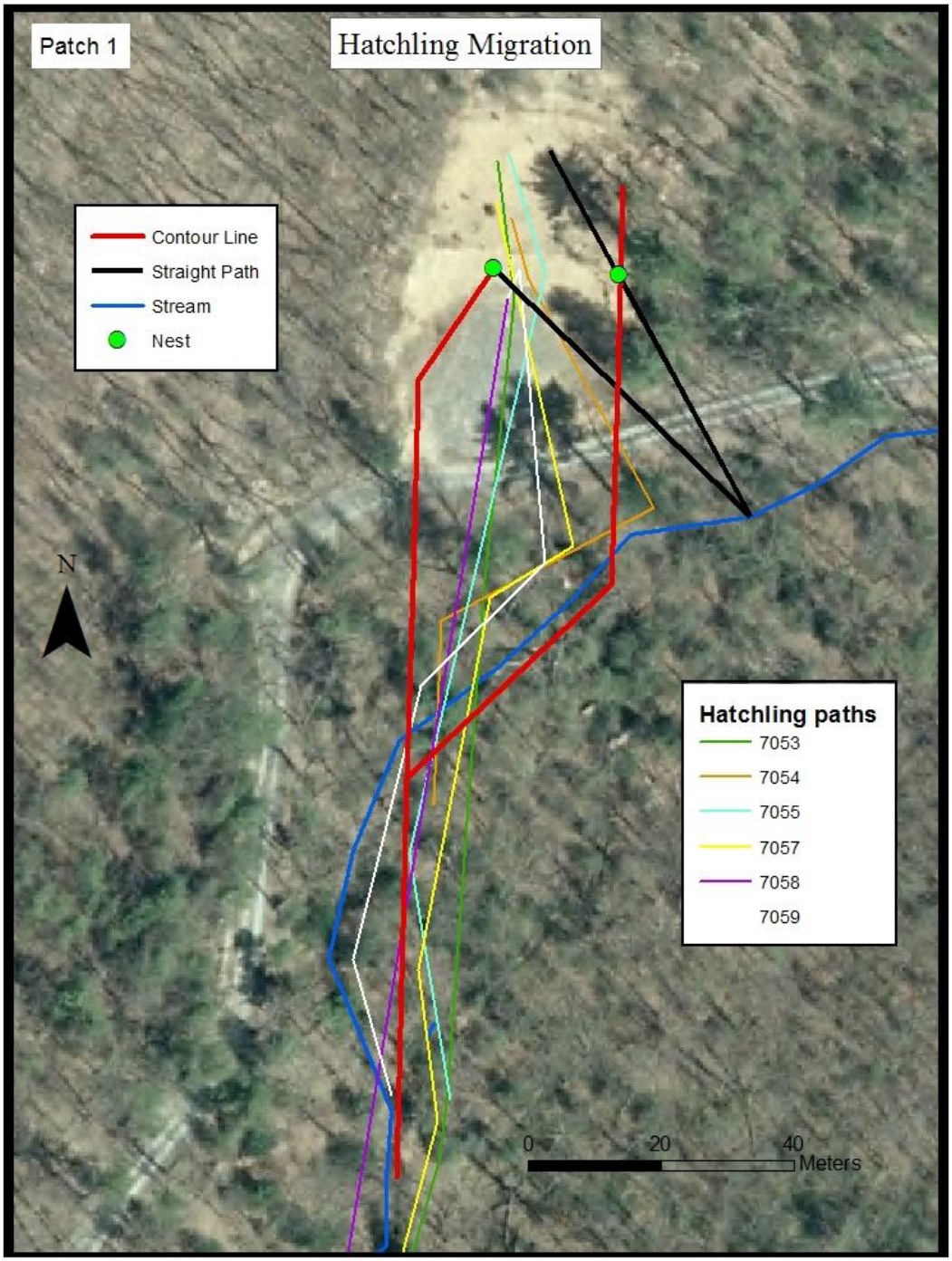


Figure 2.4 Location of migrating hatchlings compared with the straightest possible path and the path representing the contour of the landscape (Patch 2). At this patch hatchlings appeared to follow a man-made path that mimicked the decline in slope.

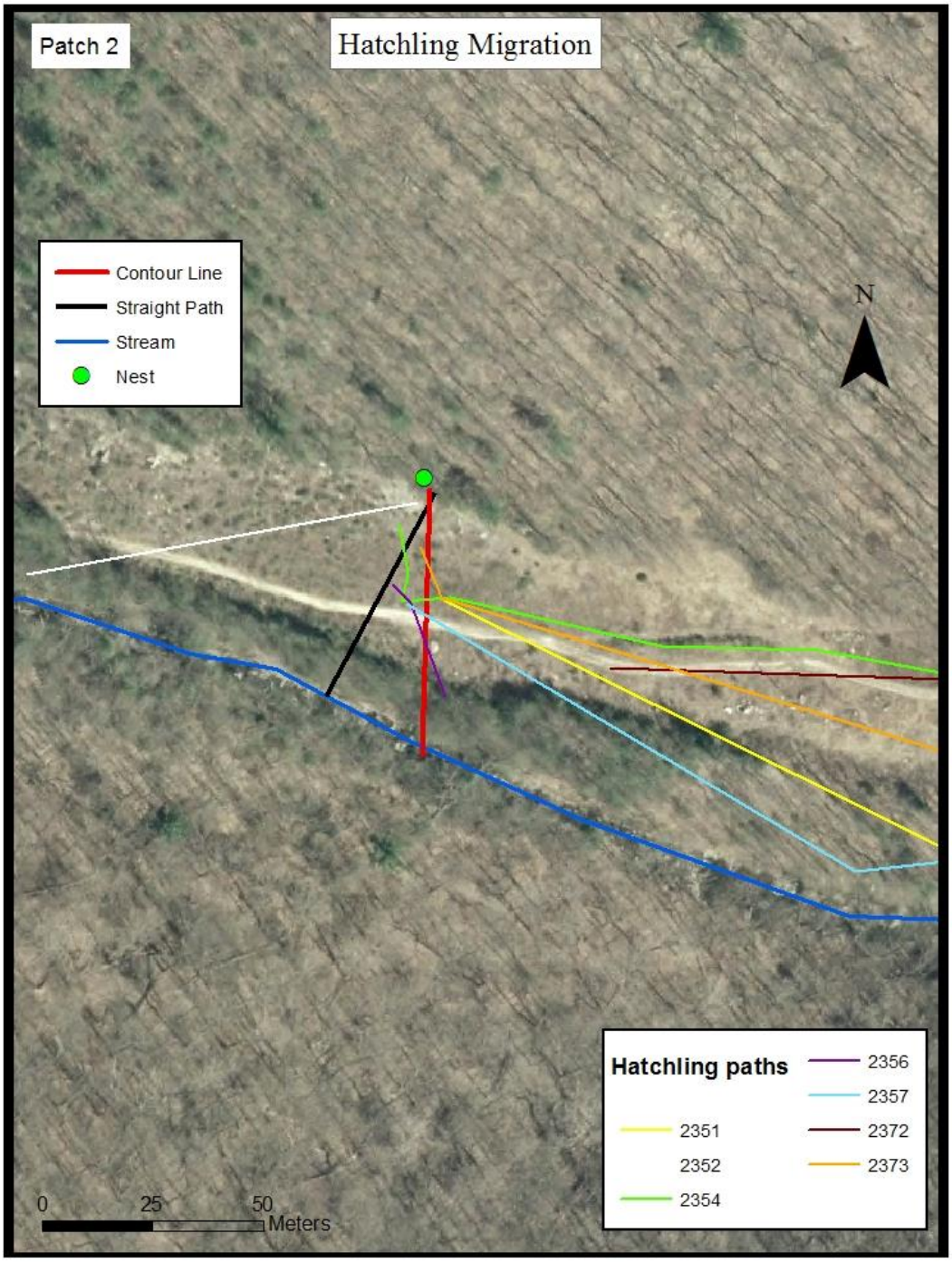


Figure 2.5 Location of migrating hatchlings compared with the straightest possible path and the path representing the contour of the landscape (Patch 3).

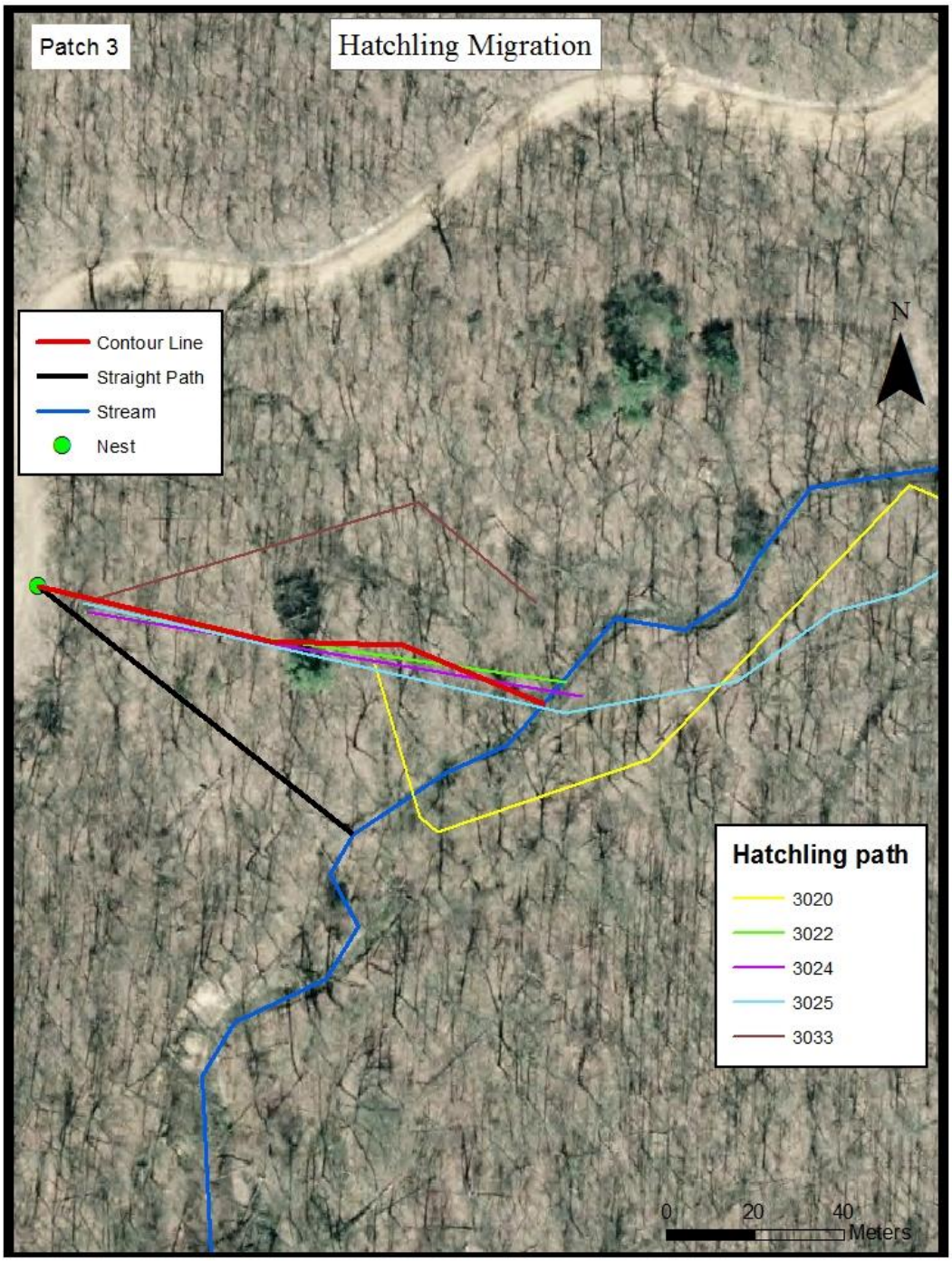


Figure 2.6 Location of migrating hatchlings compared with the straightest possible path and the path representing the contour of the landscape (Patch 4).

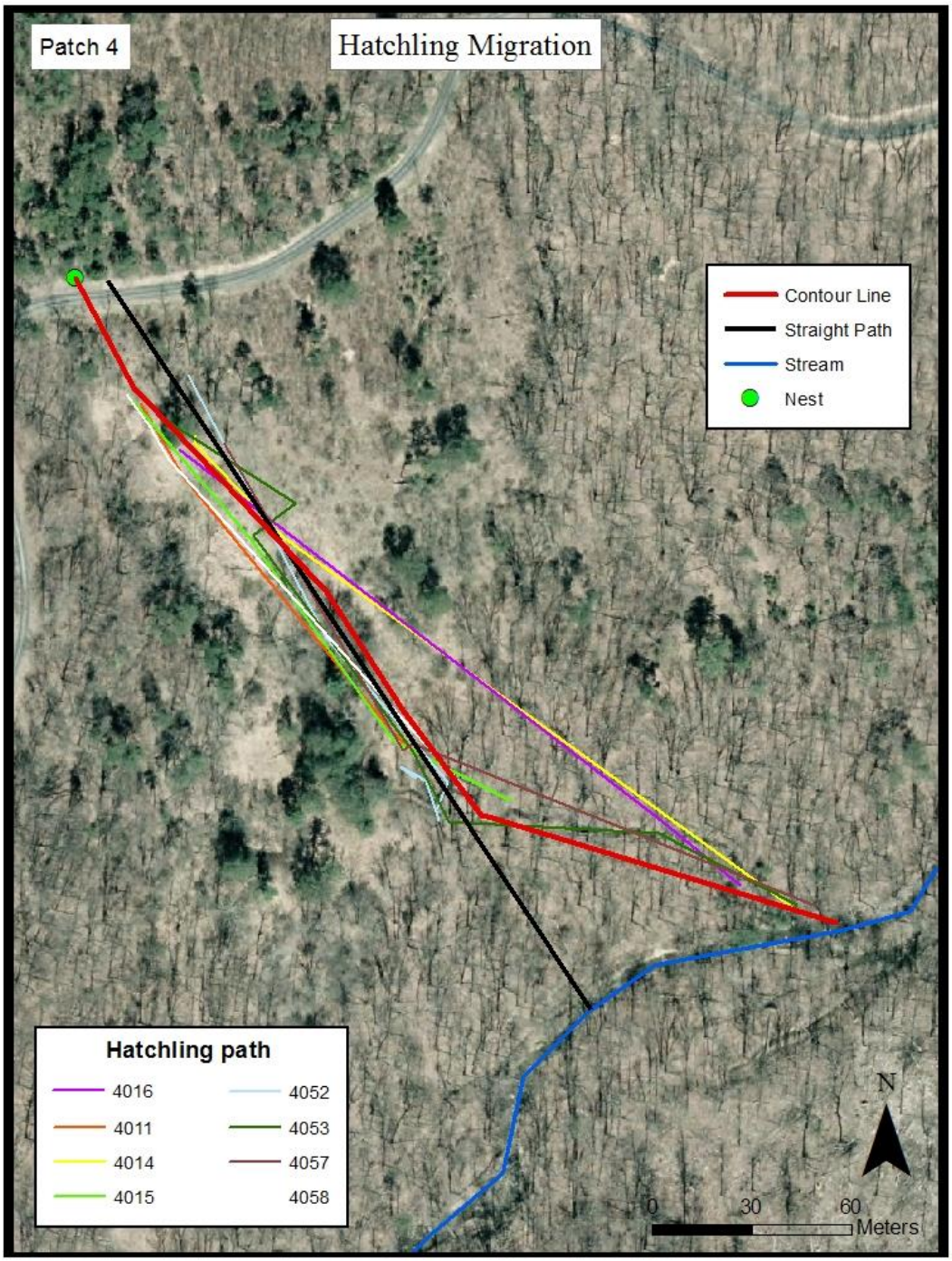
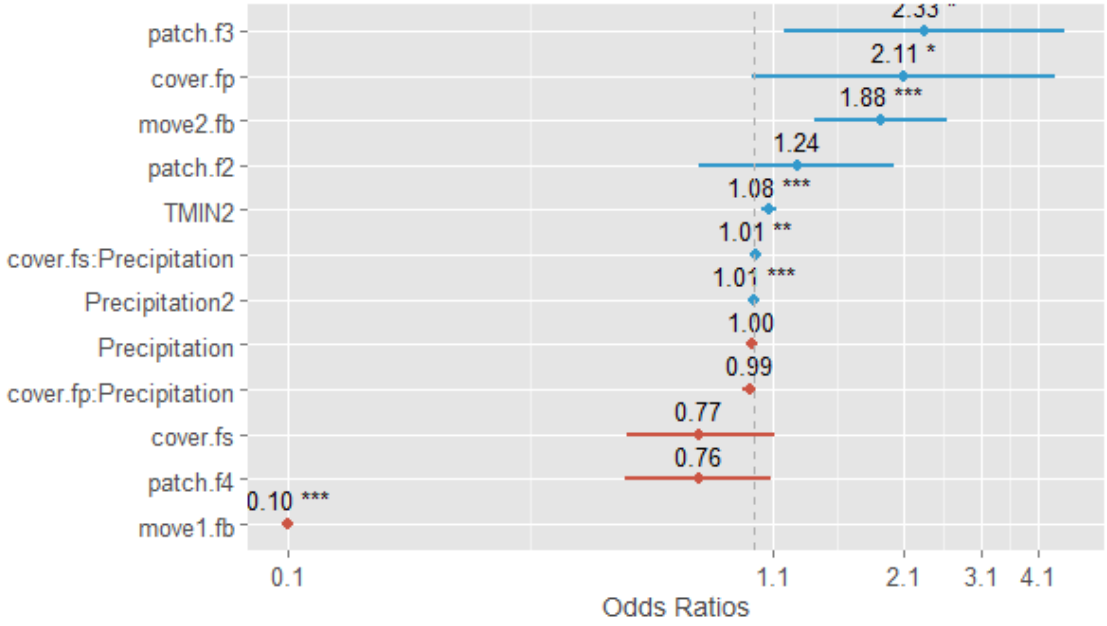


Figure 2.7 Odds ratios for the best supported model predicting movement. Odds ratios are displayed with confidence bars and significance ratings.



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CHAPTER TWO: SURVIVAL OF HATCHING WOOD TURTLES (GLYPTEMYS INSCULPTA) FROM EMERGENCE TO HIBERNATION

Abstract

In turtles, the hatchling stage is a period of relatively low survivorship due to small body mass, greater host of potential predators, and an increased vulnerability to fluctuating environmental conditions relative to adults. However, few field studies have examined what factors influence the survival rate in turtles during their first year of life due to the difficulty in tracking them. It has been hypothesized that the migration from nest to water is a period of especially high mortality for freshwater turtles due to the vulnerability associated with long overland movements. I used the cox proportional hazards model to investigate what factors influence survival in hatchling wood turtles, a threatened amphibious species, from nest emergence until hibernation. I found a distinct annual variation in survival rates, as well as variation among the nest patches the hatchlings emerged from. Migration distance does not appear to be linked to survival, as mortality was more likely to occur once the hatchling reached the stream.

Introduction

For turtles, the hatchling stage is a critical time period characterized by low survivorship (Frazer et al. 1990; Ernst and Lovich 2009). Life history theory and empirical data show survival rates as low as 0.06 for hatchling freshwater turtle species in their first year of life (Heppell 1998), as oppose to adult survival ranging from 0.76-0.96 (Harding 1997; Congdon et al. 1994). Low survival rates such as these are specific to the hatchling stage due to their smaller body mass, greater host of potential predators, and an increased vulnerability to fluctuating environmental conditions (Frazer et al. 1990; Ernst and Lovich 2009; Tamplin 2009).

Despite the importance of the hatchling life history stage, little research has been conducted to understand the factors that influence survivorship in hatchling freshwater turtles, and these only investigated the effect of size (Janzen 1993; Janzen et al. 2000). Only with recent improvements in radio transmitter size and battery duration has it become possible to track hatchling turtles for sufficient time (Castellano et al. 2008; Paterson et al. 2012). Several hypotheses about what life history, behavioral, and environmental factors affect survival have emerged, but remain mostly untested for the majority of freshwater species (Tamplin 2009, Janzen 1993).

Previous research has demonstrated that the migration from nest to water is a crucial period for hatchling turtles (Finkler 2001; Kolbe and Janzen 2002; Janzen et al. 2000; Tucker 2000). The energy required for reptiles to move is dictated by body temperatures, and ultimately the temperature of their environment (Huey 1982). In order to increase body temperature needed for migration, hatchlings may need to utilize thermoregulatory behaviors such as basking. However, basking behaviors may increase conspicuousness of the hatchling and thereby increase predation rates (Huey 1982). In addition to increasing conspicuousness, hatchlings must worry about the duration of time spent basking as they are able to absorb heat relatively quickly compared to adults due to their proportionately greater surface area (Boyer 1965). Increases in body temperature can lead to increased moisture loss, which in turn leads to desiccation (Kolbe and Janzen 2002). Therefore, hatchlings may be required to migrate during or after times of rainfall or high humidity. Alternatively, hatchlings may ignore environmental conditions and migrate via fast bursts to minimize exposure to predators (Kolbe and Janzen 2002). With

evidence suggesting migration as a period of high mortality for hatchling turtles, I predict hatchlings that emerge further from the water will have lower survival rates due to the increased distance needed to migrate.

The wood turtle, a semi-aquatic North American species, is known to nest in sandy beaches along creeks and rivers (Bulhlmann and Osborn 2011; Hughes et al. 2009) (Figure 3.1). Their nesting requirements are areas of sand or well drained soils, open canopy, southern exposure, and absence of vegetation (Ernst and Lovich 2009; Harding and Bloomer 1979). Previous studies describing nest site selection demonstrate wood turtles prefer areas closer to the stream such as sand and gravel bars (Table 3.0)

In Virginia, at the southern extent of their range, the densest populations are found in the lower reaches of watersheds where sandy beaches for nesting exist (Kleopfer et al. 2009). While wood turtles can be found in high elevation sites, their populations may be limited by lack of suitable nesting habitat (Kleopfer et al. 2009; Ernst and Akre 2006). Without traditional nesting habitat, wood turtles may be required to nest further away from the stream, therefore increasing the risks associated with migration by the emerging hatchlings.

I investigated survival of hatchling wood turtles from nest emergence until hibernation in an atypical landscape lacking traditional nest beaches along the stream. The study site, in the upper reaches of its watershed, consists primarily of human-created nesting habitat such as roadside banks, an abandoned borrow pit, and a power line right-of-way (Figure 3.2). These nesting sites are 54-276 meters from the hibernating stream, and may be functioning as ecological traps (Schlaepfer et al. 2002) for emerging

hatchlings, due to the dangers associated with extended migration. I tested the hypothesis that survival would vary based on nest patch due to the variation in distance from the stream as well as adjacent habitat. I also tested the "bigger is better" hypothesis proposed by previous studies that larger hatchlings have a higher rate of survival (Janzen 1993; Janzen et al. 2000). Lastly, I investigated whether survival would vary by year due to seasonal fluctuations in weather.

Methods

Hatchling Capture and Measurement

Nests were monitored in 2012 and 2013 for emergence daily from 7:30 A.M. to 12:00 P.M. beginning August 1 and continuing until the first in-ground frost in late September to mid-October. Emerged hatchlings were collected and partitioned by clutch. Individual emerged hatchlings were weighed (±0.1g) using an electronic scale (Ohaus Corporation), carapace and plastron lengths were measured (0.01cm) using 50 cm calipers (Swiss Precision Instruments, Inc). Prior to emergence, four nest patches were selected to sample hatchlings for this study. These patches were selected to be representative of the spatial and environmental variation found within the study site (Table 3.1). In pre-selected nests, hatchlings weighing ≥ 8.90g were outfitted with radio transmitters (Blackburn Transmitters, Nacogdoches, TX; 0.50g). Transmitters were attached with fast drying epoxy gel (Devcon, Inc. 5 minute epoxy gel) (Figure 3.3). Transmitters with epoxy weighed 0.60g, and did not exceed 7% of the mass of the hatchling. Hatchlings were marked with a unique clutch identifying code using visual implant elastomer (Northwest Marine Technology Inc. Shaw Island, WA) (Figure 3.4).

Hatchlings were then released in the nearest shaded area to the nest. Radio-tagged hatchlings were located every other day beginning the day after release and continuing until death, hibernation, or loss of signal. Upon radio location, the hatchling was recorded as observed or unobserved, and, if observed, whether active or inactive. If the hatchling was active its behavior was recorded (inactive, alert, walking, basking, swimming, and feeding). All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

Field

Radio-tagged Hatchlings were assigned to the following categories: survived study period, killed by predator, drowned, desiccated, killed by vehicle, or unknown fate (Table 3.2). Hatchlings were recorded as preyed upon if partial remains were found, or a transmitter was recovered showing obvious signs of damage. The hatchling was recorded as drowned if intact remains were recovered underwater or the transmitter was irretrievable under a rock in the stream. Hatchling remains found crushed on the gravel road was recorded as killed by vehicle. Intact remains found on a gravel nest bank during a sunny day were recorded as desiccated. Signal loss prior to the end of the batteries' duration was recorded as unknown; however premature loss could have been attributed to a predator destroying the transmitter.

The location of each new hatchling was recorded with a Trimble Geo XT and Tempest antenna attachment (Trimble Navigation, Ltd. Sunnyvale, CA). Post processing of GPS points was performed with Pathfinder Office software and achieved an accuracy of ±2m using differential correction (Trimble Navigation, Ltd. Sunnyvale, CA).

Movement, environmental variables, and mortality locations were measured and plotted using ArcGIS (ESRI 2012) with the extension Geospatial Modeling Environment (Beyer 2012).

Data Analysis

I used a Cox proportional hazards model (Cox 1972) to relate survival to a suite of explanatory variables. The Cox proportional hazards model allows covariates to be distribution free and for the use of multiple covariates (Finkelstein 1986). Previous studies that modeled survival in fledgling birds were used as format to determine survival rate of hatchling turtles (Nur et al. 2004; Suedkamp et al. 2007; Berkeley et al. 2007). The Cox model measures the hazard rate or mortality rate, defined as daily chance of mortality, and interpretation is analogous to the odds ratio in logistic regression (Hosmer, et al. 2013). Hazard rates >1 indicate that the probability of mortality increases with a variable, and hazard rates <1 indicate the probability of mortality decrease with that variable. Survival analysis was performed using the survival package in program R (R Core Team 2013) with the following covariates: year of the study, emergence nest patch, size at emergence, and weight at emergence (Table 3.3).

Prior to data analysis a covariance matrix was used in initial data exploration to eliminate covariates with an $r > 0.6$ (Li-tze and Bentlerb 1999). Through initial data exploration and analysis, 9 candidate models were tested including a null model and a model containing all covariates. I used an information-theoretic approach for evaluating potential candidate models that explain survival (Burnham and Anderson 2002). I used Akaike's information criterion adjusted for small sample sizes (AICc) to rank each

competing model (Burnham and Anderson 2002). The model with the lowest AICc score was considered to be the most supported, unless two or more models were within two AICc of one another. In the case of multiple competing models, the Cox and Snell pseudo r^2 value was used to assess goodness of fit (Cox and Snell 1989). The model that provided the greatest goodness of fit was considered the most supported.

The cox proportional hazards model assumes that the hazard rate for any individual is a fixed proportion of the hazard for any other individual (Cox 1972). When this assumption is violated it leads to over-dispersion of the data, and underestimates of variance (Tsia et al. 1999). The assumption can be tested by plotting the Schoenfeld residuals (Schoenfeld 1982), and using a chi-squared test to detect significant for any of the covariates and their residuals. A chi-squared test showed no significance for any of the covariates, therefore the assumption was met (Grambsch and Therneau 1995).

In order to further explain the variation between years of the study, weather data was collected from the nearest National Oceanic and Atmospheric Administration (NOAA) station located 4.5km away. The following variables were collected: precipitation (to nearest 0.1 mm), minimum daily temperature, and maximum daily temperature (˚Celsius). Weather from the date of the first nest oviposition until the hatchlings ceased movement was pooled together for each year. A t-test was performed to test if the weather was statistically significant between years of the study.

Results

I tracked 41 hatchling turtles from four patches in 2012 and 27 turtles from 3 patches in 2013. Fates were assigned to all 68 hatchlings. During the monitoring period,

35 (51%) hatchlings were listed as preyed upon if either remains were found (N=21), or a recovered transmitter showing signs of damage (N=14). Six hatchlings (9%) were found drowned as a recovered corpse in the stream or irretrievable under rocks in stream. One (1%) hatchling was killed by a vehicle travelling on a gravel road. One (1%) hatchling was found desiccated on a nest bank close to where it emerged. Ten (15%) were recorded as fate unknown. Fifteen (22%) hatchlings survived the study period and overwintered in a stream. The mean survival time was 18.3 ± 17.38 days over a period of 66 days for 2012, and 40.2 ± 23.82 days over a period of 66 days for 2013. The majority (72%) of deaths occurred within twenty meters of a stream (Figure 3.5).

A Cox proportional hazards model was created using the number of days alive, and censored by whether the animal died (1) or survived within the study period (0). The model contained 68 hatchlings with 50 censored (death) events. The best model, defined by the lowest AICc score and highest pseudo r^2 value, contained three significant covariates (Table 3.4). The model suggests mortality was influenced by year of the study, the nest patch the hatchling emerged from, and weight at emergence (Table 3.5) (Figure 3.6). The mortality rate for hatchlings in 2012 was 152% greater than in 2013. For each gram heavier the hatchling was at emergence the mortality rate increased 31%. Mortality rate for nest patch the hatchling emerged from varied greatly from 133% greater chance of mortality (Patch 3) to 138% less chance of mortality (Patch 4).

In order to explain the variation in survival between the two years of the study a t-test was used to investigate whether temperature and precipitation were significantly different between the years. Precipitation was greater on average in 2012 (M= 35.20,

SD= 86.52) than 2013 (M= 33.98, SD= 106.33), however the difference was not statistically significant (t= 0.0855, P value= .9319). Daily minimum temperature was higher in 2012 (M= 13.59, SD= 4.96) than 2013 (M= 12.66, SD= 5.08), however the difference was not significantly different (t= 1.2606, P value= .2090). Daily maximum temperature was significantly warmer (t= 2.6540, P value= .0086) in 2012 (M= 28.02, SD= 4.29) than 2013 (M= 26.62, SD= 3.84). Overall, 2012 was a warmer year with slightly more average rainfall.

Discussion

The proportion of hatchlings surviving in the first year of the study (0.09) was slightly higher than worst case predictions (0.01-0.06) made by previous studies (Frazer et al. 1990; Frazer et al. 1991). However, in the second year of the study the proportion surviving (0.48) was higher than the best case predictions (0.275-0.360) (Frazer et al. 1990; Frazer et al. 1991), as well as observed values (.193-.261) (Congdon et al. 1993; Mitchell 1988). Paterson et al. (2012) demonstrated that 0.11 of a sampled population of hatchling wood turtles survived in their study. The results of my study demonstrate great variation from one year to another (Figure 3.6), with survivorship both below, and well above previous studies. The mortality rate predicted by the Cox proportional hazards model demonstrated a 152% greater chance of mortality for hatchlings in 2012 when compared with 2013. Weather between the two years varied little between temperature and precipitation, with the exception of the first year reaching significantly higher daily temperatures. Perhaps the hotter weather had an impact on survival. Predator abundance

could also vary between years, thus observing oscillating predator-prey relationships in this study.

In addition to year, the nest patch the hatchling emerged from also had a significant impact on survival (Figure 3.7). Each nest patch selected for the study varied in its distance from the stream, adjacent habitat, and stream habitat. I predicted that the greater migration distance would have a negative impact on survival; however the nest patch with the greatest distance from the stream exhibited the highest survival (Table 3.7). The majority of mortality events (72%) occurred within 20m of the stream, and not during the migration period. Therefore, it seems likely that stream and streamside habitat at the different patches is having an effect on survival.

The best model predicting survival suggested that smaller hatchlings had greater survival rates. This evidence goes against the bigger is better hypothesis suggested by previous studies (Kolbe and Janzen 2002). However, this may be attributed more to patch than individual weight. Despite patch and weight not being collinear due to greater variation of weight within patches than among patches, the nest patch with the highest survival had smaller hatchlings (Figure 3.9). With an addition of more sampled patches and a third year of data, this trend will likely become weaker, as a better average develops. In order to better explain the variation in patch, year, and whether weight is impacting survival, a third year of the study is planned.

Future analyses will attempt to explain the differences in survival during migration and once the hatchling has reached the stream. To date, it appears survival is much higher during the migration period than at the stream, however due to the small

sample size; the cox proportional hazards model cannot be split between these time periods. Future studies should further investigate the role of migration in survival. These data suggest that migration distance does not affect survival, but rather something occurring at the stream, such as predators, is contributing to mortality. Delaying migration to the stream may increase the rate of survival by hatchlings spending less time in an area with higher predation. Agencies focusing on increasing wood turtle hatchling recruitment should focus on increasing survival along the stream, rather than reducing the migration distance.

Tables and Figures

Table 3.1 Nest Site selection and preference for wood turtles across their range.

Nest Location	Author
Cut bank along river	Buech et al. 1997
Sandy Beaches	Buech et al. 1997
High meadows	Harding and Bloomer 1979
Elevated railroad beds	Harding and Bloomer 1979
Unimproved woodland roads	Harding and Bloomer 1979
Forest openings (logging or fire)	Harding and Bloomer 1979
Banks bordering streams and rivers	Harding and Bloomer 1979
Sandy Beaches	Hughes et al. 2009
Sandy Beaches	Jones and Sievert 2009
Sandy Beaches	Parren 2013
Sandy Beaches	Saumure and Bider 1998
Sand/Gravel Pit (10-100m from river)	Walde et al. 2007

Table 3.2 Description of the different patches hatchlings were sampled from. Patch number corresponds to all analyses.

Patch	Patch Type	Distance from Stream (m)	Surrounding habitat	Stream Habitat
1	Gravel Borrow Pit	54	Open seepy field	Headwater Stream
2	Power Line Roadside	54	Maintained early succession	2nd order stream with riffle and small waterfalls
3	Cut Bank	127	Mixed hardwood forest	Headwater Stream
4	Roadside Cut Bank	276	Herbaceous Seep and hardwood forest	Blown out beaver dam complex

Table 3.3 Fate of all radio tracked hatchlings by year. Hatchlings that made it to winter (~70 days of the study) were counted as surviving the duration of the study.

	Survived	Predated	Drowned	Desiccated	Killed by vehicle	Unknown
Year 2013	13	8	3	1	0	2
Year 2012	4	26	3	0	1	8
Total	17	34	6	1	1	9
Percent	25.00%	50.00%	9.00%	1.47%	1.47%	13.24%

Table 3.3 Description of covariates used in the survival analysis.

Covariate	Description
Year	The year of the study (2012 and 2013).
Patch	Nest patch the hatchlings emerged from (Four different sample locations).
Size	Carapace length of the hatchling (mm).
Weight	Weight of hatchling at emergence (g).

Table 3.5 Candidate model selection process for predicting survival using the Cox Proportional Hazards test. The most supported model is the one with the lowest AICc score and highest pseudo r^2.

Model	K	AICc	Delta AICc	AICc Wt.	Cum. Wt.	LL	Pseudo R^2
Patch + Weight	4	372.47	0	0.25	0.25	-181.92	0.287
Patch	3	372.92	0.45	0.2	0.45	-183.27	0.259
Year + Patch + Weight	**5**	**373.22**	**0.75**	**0.17**	**0.63**	**-181.13**	**0.304**
Patch + year	4	373.33	0.86	0.16	0.79	-182.35	0.278
Year + Patch + Size	5	373.73	1.26	0.13	0.92	-181.38	0.299
Year + Patch + Size + Weight	6	375.59	3.11	0.05	0.98	-181.1	
Year + Weight	2	378.51	6.04	0.01	0.99	-187.16	
Year	1	379.03	6.56	0.01	1	-188.49	
Null	0	386.89	14.42	0	1	-193.44	

Table 3.6 Fitted parameter estimates for Cox proportional hazards test. Hazard rate is interpreted the same way odds ratios are interpreted for logistic regression.

Covariate	Mortality Rate	Sig. Value	Lower .95	Upper .95
Year 2	0.66	-	0.3400	1.2718
Patch 2	1.47	-	0.7329	2.9724
Patch 3	2.33	0.1	1.0000	5.4297
Patch 4	0.42	0.05	0.1798	0.9703
Weight	1.31	-	0.9363	1.8328

Table 3.7 Migration strategy related to migration distance and survival.

Patch	Days taken to reach stream (avg)	Distance emerged from stream (m)	Life Span (avg)
1	10.56 ± 5.93	54	25.95 ± 19.03
2	8.77 ± 7.19	54	16.06 ± 14.43
3	4.56 ± 4.22	127	11.1 ± 6.03
4	11.93 ± 5.85	276	44.85 ± 25.87

Figure 3.1 Range map for wood turtles in North America.

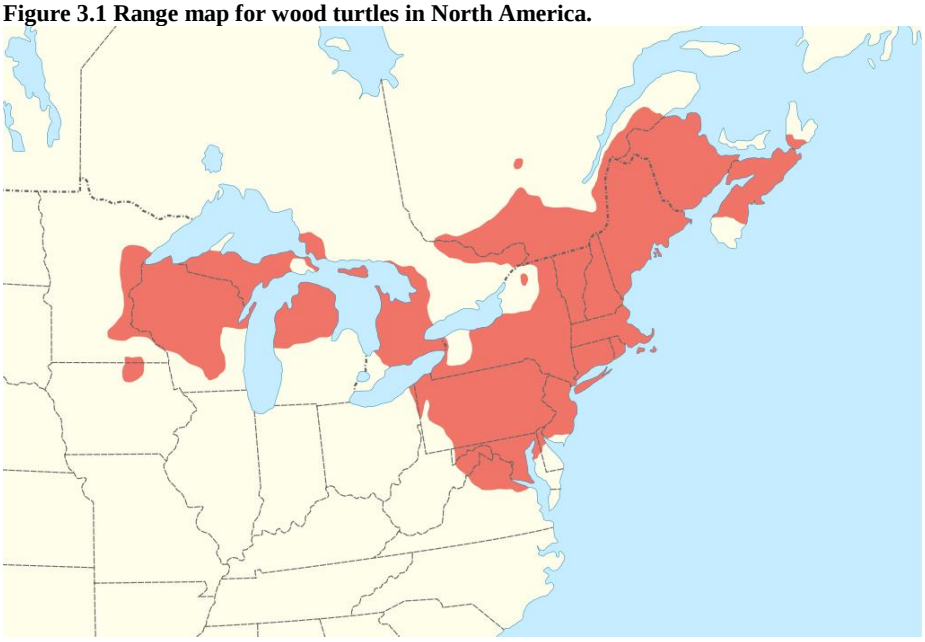


Figure 3.2 Example of the nesting habitat created non-purposefully through road construction by the U.S. Forest Service.

Figure 3.3 Hatchling wood turtle fitted with radio transmitter. Transmitters were attached to the rear of the carapace and best attempted to sit flush with the depth of the shell.

Figure 3.4 Visual implant elastomer was used to assign a unique code to each clutch of hatchlings. Each clutch was assigned a location on the plastron along with a color that fluoresces under UV light. Should hatchlings be captured in the future we will know which clutch they emerged from.

Figure 3.5 Location of dead hatchlings in relation to their distance from the nest and stream. Hatchlings that survived the study period are also displayed.

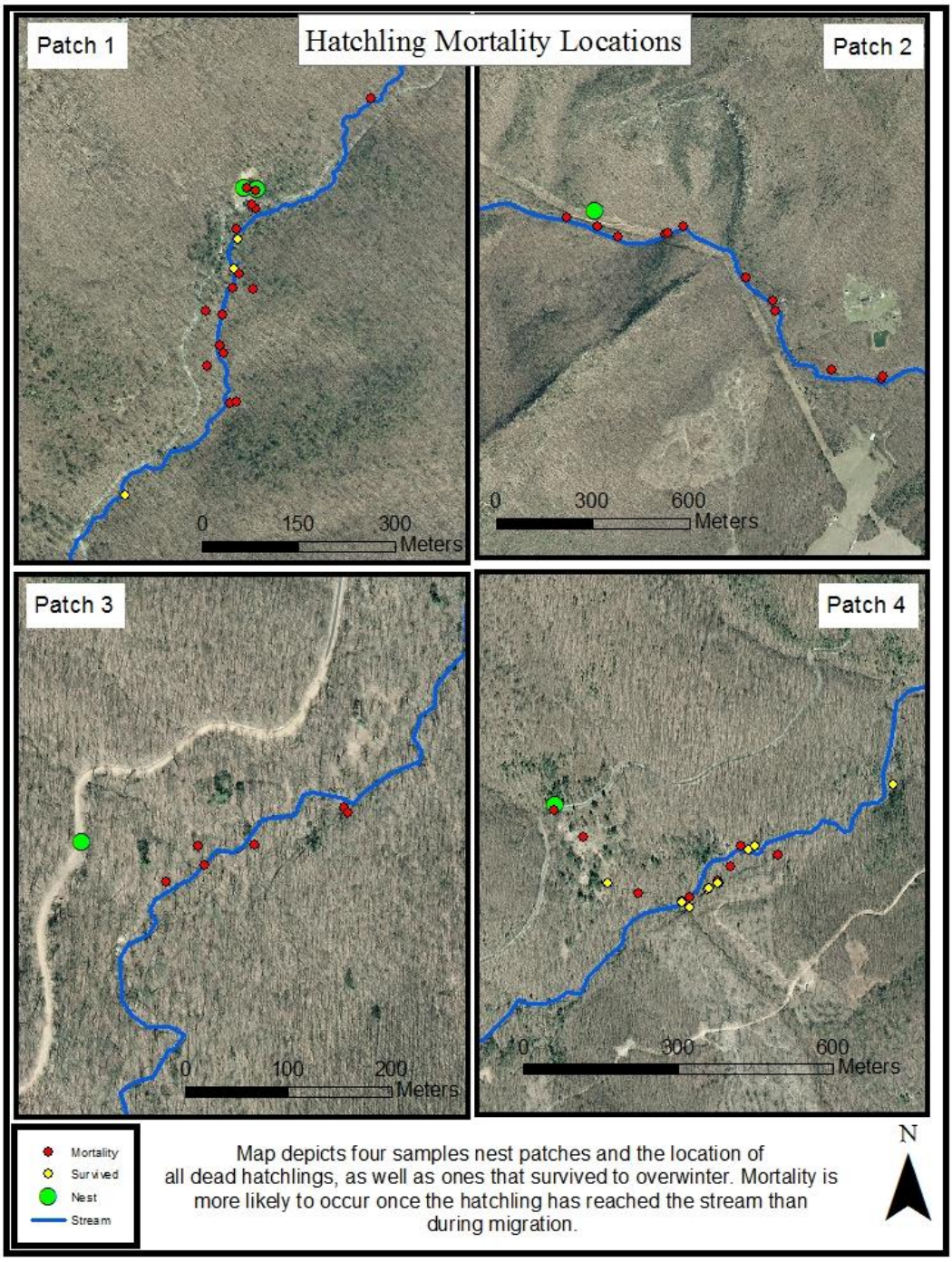


Figure 3.6 Hazard rate with 95% confidence intervals for the best supported model based on AICc score. The best model included covariates year, patch, and weight.

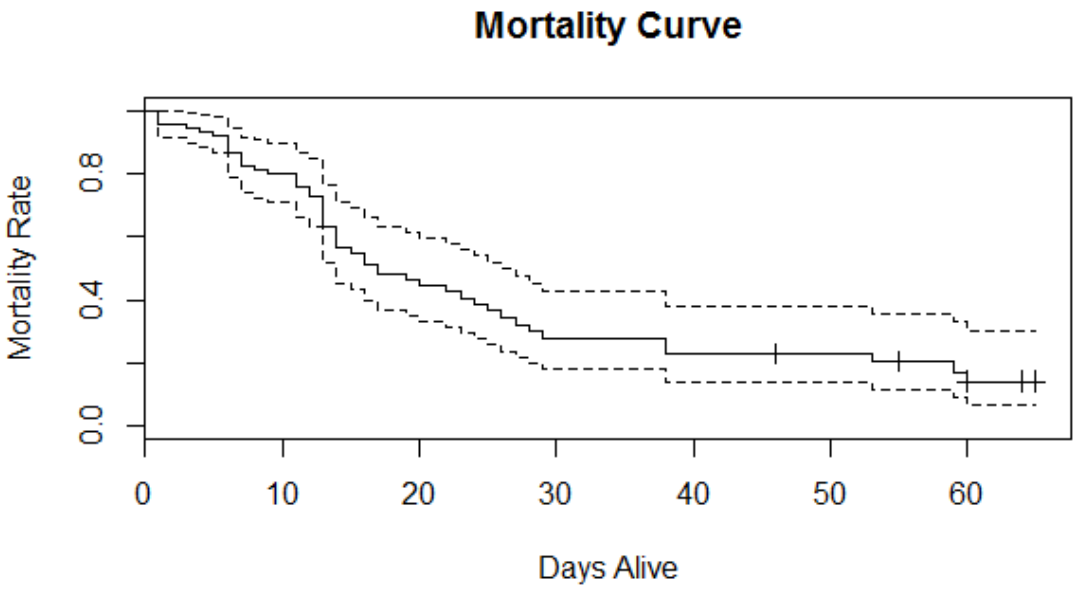


Figure 3.7 Survival (measured in days alive) varied greatly between the two years of the study. Higher survivorship was observed in the second year.

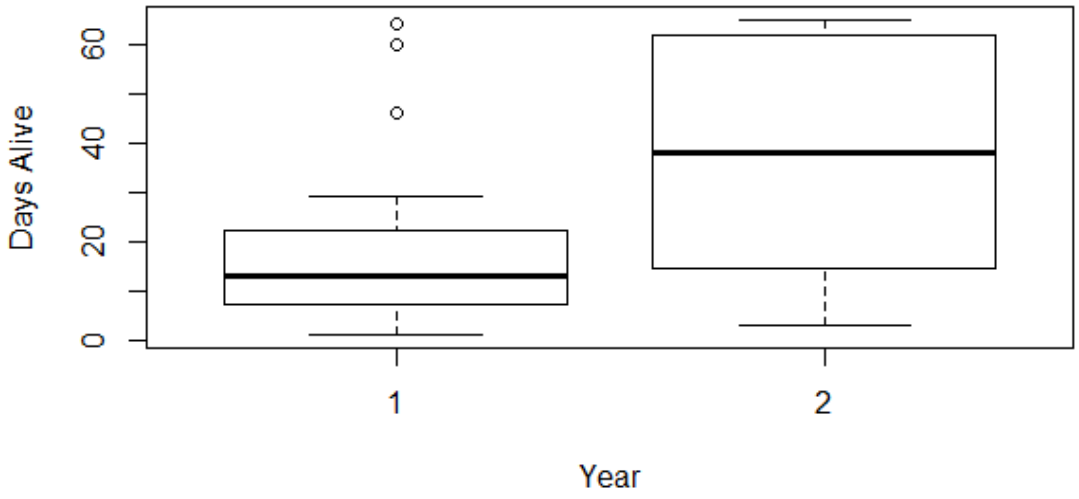


Figure 3.8 Four nest patches, each differing in habitat structure and distance to stream, were chosen to investigate patterns of survival. Survival (measured in days alive) varied greatly among the four patches.

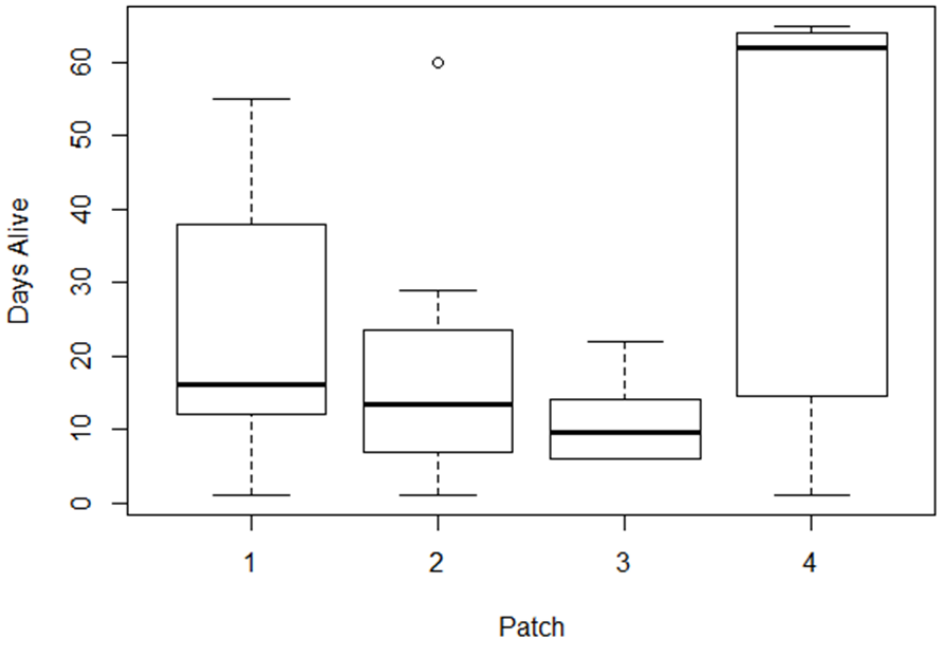
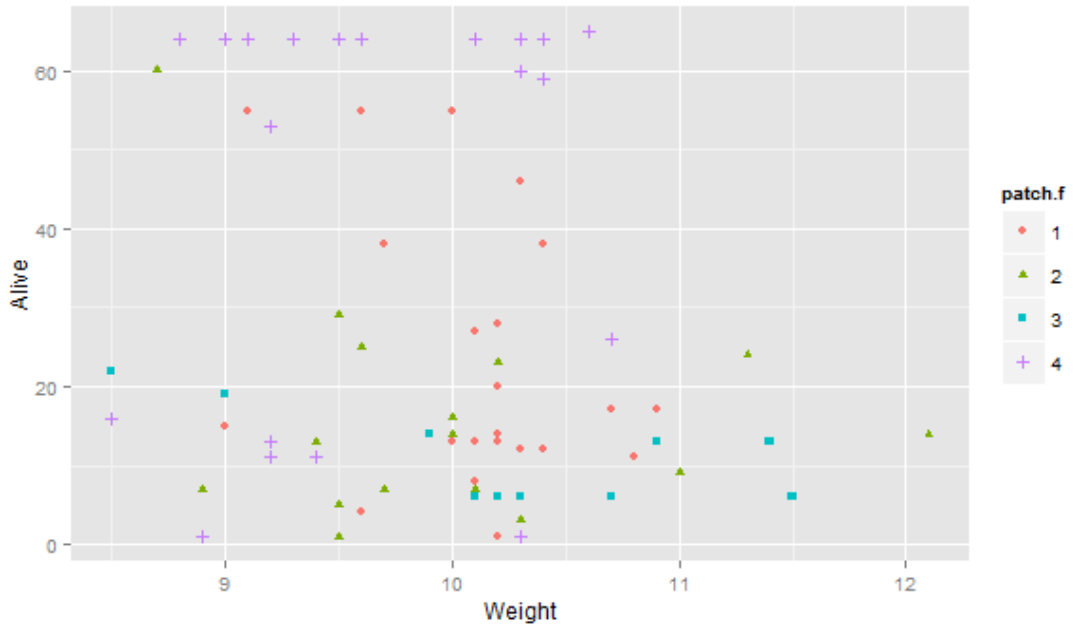


Figure 3.9 Relationship between survival (days alive) and weight. The Cox model demonstrated a survival advantage to smaller hatchlings; however the pattern is not clear due to one patch having much higher survival than the rest.



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BIOGRAPHY

My fascination with turtles started at a young age while rescuing box turtles succumbing to the effects of urban sprawl in southern New Jersey. My parents would help turtles cross roads and relocate them to areas where forests were still intact. As I grew older I realized the amount of box turtles I would encounter in a summer was significantly declining. I knew something was wrong when the animal that defined my childhood disappeared at such rapid rates. The box turtle became a symbol of the "garden state", full of farm land and forests that I remembered from my childhood. When it came time for college it was no question that I would chose environmental studies at my local university. I studied at Rowan University near my home town for four years. During this period, I enrolled in the Smithsonian-Mason semester program located in Front Royal, VA at the Smithsonian Conservation Biology Institute. It was at the SCBI that my passion for turtles and conservation started to develop into a career and future. I began working with Dr. Thomas Akre on a five year study of the wood turtle. Little did I know at the beginning, I would stick around for all five years of the study. I ended up carving out a piece of this study for my master's thesis seen in this paper. Writing my thesis on the wood turtles was a dream come true, and a culmination of my life's experiences and passions.