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Examining the Effects of Penning on Juvenile Eastern Box Turtles (Terrapene carolina carolina)

Nicolas Frederick
Virginia Commonwealth University

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Examining the Effects of Penning on the Site Fidelity of Juvenile Eastern Box Turtles (Terrapene carolina carolina)

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

By

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Abstract

Box turtle (*Terrapene carolina*) populations have been declining over the last several decades, and one major cause is increasing urbanization. As a result of habitat fragmentation, wildlife managers are frequently turning to new and alternative management strategies. Traditional box turtle management has included relocation, which has been met with limited success. This study aims to combine these strategies with another less-studied one: forcing turtles to overwinter on site by penning them in an outdoor enclosure. Two sets of juvenile box turtles were released at the Virginia Commonwealth University Rice Center: one penned on site in a pen for one year, the other allowed to move freely. Our objective was to compare a variety of factors between these two groups to see if penning was as effective as traditional approaches. Movement and location patterns were tracked using radio transmitters for two years and analyzed using GPS technology. Body condition and health status of all turtles were measured and compared over time as well. Finally, a life history model was developed to determine the effectiveness of management programs. While the penning treatment significantly reduced activity areas, it appears that all juvenile turtles had high site fidelity (87.5%) regardless of treatment. The eastern box turtle seems to be a prime candidate for penning used in conjunction with other management options.
Chapter 1.1

Introduction

Eastern box turtle ecology

The eastern box turtle (Terrapene carolina carolina) is a reptile in the order Testudines. Their distribution covers the majority of the eastern seaboard from Florida to Massachusetts, where only fragmented populations exist, and go as far west as the Mississippi River (Dodd 1991). They predominantly reside in mature forests and prefer a dense understory where they are provided cover from predators and resources such as food, nesting sites and hibernacula (Hester et al., 2008). The lifespan of the eastern box turtle is generally thought to be about 80 years and it can be reproductively active for 30-50 years (Henry 2003). Box turtles are omnivorous, which allows them to influence several levels of terrestrial and aquatic food webs (Jones et al., 2007; Braun and Brooks, Jr. 1987; Dodd 2001). Box turtles are mostly a terrestrial species, but they still can be found in or near large bodies of water. They have been shown to be facultative mycovores and are an integral part of a wide variety of fungal life cycles. This suggests that the turtles may play a more intrinsic role in fungal life cycles than other species (Jones et al. 2007). Box turtles also have value as seed dispersing agents, even increasing germination rates of certain fruits (Braun and Brooks, Jr. 1987).
Anthropogenic environmental change

Eastern box turtle populations are declining (Dodd 2001; Cook 2004; Henry 2003; Hall et al., 1998). Currently, the International Union for the Conservation of Nature (IUCN) has the eastern box turtle listed as “Near Threatened;” however, the last known census was done in 1996 (IUCN 2009). While certain areas can still retain high populations of box turtles, these populations have been shifting to an older dominant age class with low recruitment (Henry 2003, Hall et al., 1998). This may be because box turtle population growth is affected by a wide range of environmental factors and human influenced stressors (Henry 2003).

Climate change is a growing concern for many reptile species worldwide. Box turtles, as with most other reptiles, rely on their eggs being incubated at certain temperatures and even a 2% increase in global temperature can drastically affect sex ratios, reduce fecundity and shorten growing seasons (McCallum et al., 2009). At the current rate of climate change, reptiles will not be able to evolve fast enough to accommodate the change (Janzen 1994; McCallum et al., 2009). In theory, climate cooling could be more deleterious than global warming because species in warm climates can disperse to new areas; however, dispersal often leads to mortality (Arau et al, 2006). Box turtles are also impacted by the global wildlife trade as well (Karesh, 2005). Between 1995 and 2000 about 30,000 box turtles were shipped from Louisiana alone as part of the pet trade industry (Gibbons et al., 2000).

In spite of these population pressures, the biggest reason for box turtle decline is urbanization and associated anthropogenic environmental change (Hall et al., 1998; Dodd
2001; Cook 2004; Bradley and Altzier, 2006). In 2000, approximately 3.1% of the contiguous United States was classified as urban (1000 people per square mile in the core census block and at least 500 ppsm in surrounding blocks) (Vickery et al., 2009). By 2050, that number will be as high as 8.1% (Vickery et al., 2009). Currently, the two most urban regions in the country are the northeast at 9.7% and the southeast at 7.5%, which consist of the majority of the eastern box turtle’s habitat (Vickery et al., 2009).

Landscape alteration can have impacts on areas protected from development, as changes to an urban landscape can affect hydrology and flooding of these protected areas (Hall et al., 1998; Ehrenfeld 2008). Flooding can alter or eradicate box turtle population structure, leading to low recruitment or increased adult mortality (Hall et al., 1998). In areas where the eastern box turtle overlaps with high human densities, populations of turtles are only maintained in small patchwork communities in suburban areas and effectively extirpated from city limits (Cook 2004; McKinney 2006). The impact of these fragmented landscapes on biodiversity and population dynamics is contingent on the size of the fragment (Markovchick-Nicholls et al, 2008), but species dependent on large forested areas for recruitment are affected by any amount of fragmentation (Ferraz et al., 2007; Nitschke 2008). The eastern box turtle occurs in approximately 40 of the 100 fastest growing counties in the country, which span the range of the species (Christie 2006). As a result of habitat fragmentation, *T. carolina* is often seen attempting to cross roads, which leads to high rates of mortality (Cook 2004). Concerned individuals then relocate these turtles and that may affect the distribution of the eastern box turtle (Belzer 1999). Many turtle species have higher road mortality for females because they tend to
cross roads in search of adequate nesting sites and this could partially explain male biased sex ratios in studies showing an increase in urbanization (Steen et al., 2006). While box turtles gain some benefit in food resource allocation as a direct result of urbanization, mortality increases drastically in urban landscapes (Budischak 2006).

**Relocation as a management tool**

To address the threat of human conflict, wildlife managers have increasingly used methods of relocation. Using definitions proposed by Dodd (1991) on the conservation strategies of reptiles and amphibians, relocations involve moving an animal from an area where they are immediately threatened to an area where they would be less likely to incur habitat loss. Relocations that entail releasing organisms into areas either formerly or currently occupied by the target species are called repatriations, and those that require the release of individuals into areas not previously occupied by the target species are called translocations (Dodd 1991). Collectively, these are referred to as RRT programs (Cook 2004). RRTs are stressful and traumatic to the organisms and can significantly reduce reproductive output immediately following release (Lance et al., 2004). Stress is also cumulative, which is why handling RRT animals should be kept to a minimum, and can contribute to the death of many organisms immediately following their release (Teixeria et al., 2007).

Another major problem for reptiles is the tendency for these organisms to return to their original habitats. In a study focused on translocated gopher tortoises, any organism translocated under 1000 m was able to return to the previous habitat. Those that
were translocated over 1000 m were unable to return to their homes but exhibited high rates of daily movement and were at high risk of both leaving the translocation site and predation (Field et al., 2007). Box turtles have exhibited similar responses when relocated (Cook 2004). Though turtles moved in excess of 10km from their original habitat may not exhibit homing behaviors, site fidelity may not be maintained (Tuberville et al., 2005; Cook 2004; Dodd 2001).

There have been few studies focusing on long-term survivability of relocated box turtles (Cook 2004). Relocation in regions with a high level of habitat fragmentation will obviously intensify these concerns as turtles attempt to navigate the landscape (Nazdrowicz et al., 2008; Rittenhouse et al., 2007), especially considering the box turtle’s preference for woodland areas (Williams and Parker, 1987).

Box turtles generally have the same home range for their entire lives, so any alteration of that home range is significant (Dodd 2001; Henry 2003; Hester et al., 2008). Relocated turtles become unaware of their surroundings and can have ranges in excess of three times the resident individuals, as well as an increase in their energy output and exposure to environmental hazards (Hester et al., 2008). Winterkill can be a major source of first year mortality as relocated box turtles are more likely to succumb to winterkill because of their determination to return to their original homes (Dolbeer 1971). Site fidelity, the ability to retain relocated animals on the original release site, is also difficult to maintain with relocated adult individuals, especially in the first year of release (Hester et al., 2008; Tuberville et al., 2005; Field et al., 2007). Failure to remain in the release area leads to higher rates of mortality (Rittenhouse et al., 2007). Straight line movement
from release (or the furthest maximum distance an individual travels from the release point) can be used as a measure of site fidelity by determining which individuals have effectively moved beyond the study site without establishing a home range (Tuberville et al., 2005). Site selection should involve finding large continuous habitat, which may not always be available (Rittenhouse et al., 2007). Cook (2004) suggested an area of at least 500 ha when considering any eastern box turtle relocation, a suggestion that is not always feasible.

Holding individuals over-winter in a pen located in the relocation area may increase their survivability by keeping them on site (Tuberville et al., 2005) as well as reducing energy expenditure and providing additional protection during the winter months. Another possible solution for this may be releasing juvenile turtles because they generally have smaller home ranges than adults and may exhibit less initial movement (Dodd 2001). Juveniles may not have established a connection to their original habitat and therefore may not attempt to home.

While RRT studies have gained notoriety among reptile managers, the success of these studies has become difficult to determine (Belzer 1999). Prior to 1991, herpetology relocations were only 19% effective, while mammalian and avian relocation efforts in 1987 were 44% (Wolf et al., 1996). Since then, successful herpetological relocations have risen to 42% (Germano et al., 2008). Some of the current successes for reptiles can be explained by a shift from using eggs and hatchlings in relocations to juveniles and adults (Germano et al., 2008). Determining long term success of these projects is still difficult because of the scope required to truly analyze these relocation efforts (Dodd 2001).
general, relocations to solve human/animal conflicts fail because too much emphasis is placed on removal of the organism and not enough is placed on conservation (Fischer and Lindenmayer, 2000).

To enhance the strength of using relocation as a conservation effort, more baseline data for a particular situation needs to be documented. In addition, rigorous release and monitoring guidelines need to be established for the sake of continuity to truly determine success. Better post-project monitoring and willingness to publish even unsuccessful attempts at relocation should strengthen relocations as a management strategy (Fischer and Lindenmayer, 2000).

Penning as a management option

The most consistent data on RRT programs come from a series of studies published on the Mojave gopher tortoise (*Gopherus agassizii*) (Field et al., 2007). This species is threatened and has had to undergo several translocations in an effort to increase population numbers (Field et al., 2007). Early attempts at relocation were highly unsuccessful due to a poor maintained site fidelity (Tuberville et al., 2005) and disease introduction from captive individuals (Jacobson 1993). The most notable successes have incorporated an on site containment area in an attempt to force the tortoises to acclimate to their surroundings and therefore reduce their initial movement patterns (Tuberville et al., 2005). This process is known as penning. When considering long-term survivability
of the species, reducing movement after release may increase the success of relocation efforts (Field et al., 2007).

Initial penning results of the gopher tortoise were inconclusive because of brief penning durations (less than 30 days) (Tuberville et al., 2005). Subsequently, a long term penning experiment was conducted by Tuberville (2005) and the 12 month penning treatment greatly increased site fidelity (from 23% to 91%) and significantly reduced the activity area (total area used by an individual for one field season) of the animals. These results were strengthened when it was recently determined that tortoises given water a few months leading up to release had a higher survivability than those that didn’t receive any water prior to release as individuals not receiving water had difficulty of locating water in confinement (Field et al., 2007).

Penning is not without reservation, however. Disease transmission to resident populations of tortoise is still an issue as captive individuals are potential sources of introduced disease and stress because of their association with other tortoises in a confined space (Field et al., 2007). Once a disease is spread to resident populations it becomes nearly impossible to reverse (Jacobson 1993). In addition, the stress of confinement has shown to produce varying levels of testosterone and corticosterone not seen in wild populations (Lance et al., 2004).

Another potential drawback to penning is that the competency of these penned individuals in the wild may be reduced because of an abundance of available resources (food, shelter, etc) that are easily accessible in captivity but must be earned in the wild (Cook 1983). Using juvenile individuals may address these concerns. Juvenile reptiles
may be more practical for captive releases than other organisms because they easily mimic wild behaviors and are naturally more instinctive than mammals or birds (Alberts, 2007).

Relocations and disease transmission

The emergence of current infectious diseases in wildlife is predominantly a direct result of human anthropogenic change (Dazak 2001). Deforestation and subsequent land use changes have increased the morbidity and mortality associated with emergent parasitic disease in wildlife generally as a result of increased soil temperatures and a shift to neutral pH levels (Patz et al., 2000; Bradley and Altzier, 2006). One fear of using RRTs as management strategies is the possibility of disease transmission (Bertolero et al., 2007). Coupled with increased disease prevalence in locations where turtles are in need of relocation, alien pathogens can be introduced into the release site, and this risk increases as turtles are brought from greater distances to the release site (Cunningham, 1996).

Due to the threat of disease transmission, the success or failure of a relocation project can be determined by how healthy the organisms are in the study area, including prior residents. A strong health screening process to catch these infections is not available for many reptiles, but remains necessary (Jacobson 1993). Extensive behavior and disease analysis exists for the gopher tortoise, where individuals were closely examined
for infection or oddities in behavior, as well as quarantined before being released onto a new location (Berry 2001).

Box turtles are affected by a wide array of infections (Brown et al., 2003). The incidence of disease in some turtle populations has increased, given recent pressures from habitat loss and exposure to pollutants (Schumacher 2006). There are also case studies of turtles contracting Myobacterium (Noyes et al., 2007) and Iridoviral infections (Allender et al., 2006; Johnson et al., 2007) that can lead to death. Chelonids are even capable of contracting mycotic diseases, which mostly affect the integumentary system (Jacobson et al., 2000). It has been postulated that diseases such as TV3, a Ranavirus, originally thought to use turtles as a disease reservoir are now also causing serious infection among many chelonian populations (Allender et al., 2006; De Voe et al., 2004). Box turtles are also susceptible to secondary pathogens such as Mycoplasma, which is associated with upper respiratory tract infections (Feldman 2006).

In Virginia, aural abscesses (ear infections) are common, which can lead to a larger number of bacterial microflora capable of further infection in the turtle (Joyner et al., 2006). Maintaining healthy turtle populations may influence the survival of many amphibians and reptiles (Blaustein and Kiesecker, 2002), so having a disease profile and consistent monitoring of wild and captive individuals is essential, but these profiles are limited and need to be expanded (Homer et al., 1998).
Objectives

The objectives of this study were to compare the mean movement, activity areas, habitat use, body condition, and overall health between:

(1) immediately released juvenile Eastern box turtles, (2) year 2 juvenile turtles and (3) over-winter confined juvenile Eastern box turtles.

By these comparisons we hoped to devise a system of relocation that could maintain long-term survivability of the species and reduce the mortality of introduced turtles, especially over winter and during initial release.
Chapter 1.2

Methods

Study site

The study site, located ~37 kilometers outside of Richmond, Virginia is composed of two adjacent forests. These sites form a semi-contiguous habitat separated by an infrequently traveled unpaved road. The resident turtle population density can only be estimated based on observed frequency of individuals while in the field. The method is not reliable and true density will require several years of observation before it can be determined. Since 2006, 49 resident turtles have been marked and released. During that time 17 have been recaptured. The combined study site is about 350ha and our turtles used about 50ha of the available space. This puts the adult population density somewhere around 1 turtle/ha. Further research may produce higher densities, but this population does not currently compare with high density populations that are found elsewhere in the turtle’s range (Penick et al, 2002).

The main study site is known as the VCU Inger and Walter Rice Center (37° 20’N, 77° 12’W) and incorporates the surrounding private property: The Rice Center is approximately 138 ha and is located along the James River in Charles City County (See Appendix). When combined with the adjacent property, the entire research site is approximately 350 ha. It consists of both mixed deciduous and pine forests as well as tidal and non-tidal wetlands. The forest is primarily composed mostly of loblolly pine
(35% of study area) followed by deciduous (26%) and mixed forest (16%) habitat. Most of the site surveillance occurred within both the pine and deciduous forest, although a few turtles were found consistently in non-tidal palustrine wetlands (10% of the study area). The approximate surrounding population density is 14 persons/km² with relatively minimal human impact and no known environmental contaminants (EPA 2008).

**Juvenile turtles**

In order to have the most reliably successful introduction, several key factors of box turtle biology were considered. The offspring of turtles rescued from a development site were donated by Stephanie Foertermyer. These turtles had passed the point of high mortality (generally after year one) but were young enough at 3 years of age to not have established home ranges (before maturity at 6 – 10 years old). Their parents came from a development site that was only 48 km from the release site. The juveniles were raised in an outdoor penned facility that mimicked their preferred habitat as much as possible; the turtles were held overwinter at this site much like they would in the wild; and they were fed a high protein diet for the first three years of their lives. The turtles had outsized their age class. At age three we would expect the turtles to have an average carapace length between 50 – 75 mm (Budischak et al, 2006). Our turtles had carapace lengths of 89 – 111 mm, which is important because box turtle maturity is determined more by size than it is by age. Therefore, these turtles were closer to subadults (75 – 125 mm) than juveniles in size. The turtles were detained 13km from the release point at an outdoor facility for about one month prior to release in order to monitor their health status. The
month-long waiting period allowed the health of the turtles to be assessed and any infected animals to be quarantined in an attempt to reduce possible disease transmission to the Rice Center.

**Study design**

The turtle movements and activity areas were broken into two treatment groups each with 10 individuals: a pen treatment and a no pen treatment. After a one month confinement for a health screen, the turtles were chosen at random, using a random number generator, marked on their scutes, given radio transmitters (obtained from Advanced Radio Telemetry Systems) and placed in the field. The release site was located between both the pine and mixed deciduous forests (See habitat map in Appendix A) to give the turtles the most available habitat types to use. Each group was placed in the field on the June 9, 2008, with the pen group placed inside their enclosure and the no pen group released approximately 15 m from the center of the pen. At this point the no pen turtles were allowed to move throughout the site and monitoring could begin.

The ten pen treatment turtles were housed on site at the Rice Center (See Appendix A) for one year prior to their release. A fence was erected by trenching the area around where the turtles were housed, placing a tarp-like material into the trench, and driving stakes into the ground to support the fence (Appendix B). Box turtles are natural burrowers, so it was imperative that we dug deep enough to prevent them from escaping their enclosure. The penned turtles were kept on site in a 736 m² enclosure that was
approximately six inches into the ground and was about a foot above the ground. The pen
was examined briefly on each trip to ensure no structural damage had occurred. Water
and hibernacula were supplemented for the pen turtles because they had no direct access
to either; however, food resources had to be found within the containment area. After the
pen turtles over-wintered on site for one year, the fence was removed June 5, 2009.

*Field methods*

Field surveys were conducted in 2008 and 2009. These turtles were monitored
with radio transmitters and their location was recorded with a global positioning system
(GPS Garmin 250). Their locations were documented on each visit to the Rice Center,
every day for the first two weeks of release (Fig 1) and about 4 to 5 times a week during
the active season (June – October) until the turtles found hibernacula. The turtles were
tracked during random hours of the day on random days of the week, though no night
monitoring was done for safety reasons. The majority of our monitoring involved
walking non-linear paths throughout the forest.

When a turtle was located, we would make a visual assessment of the turtle to
determine unusual behavior or severe infection. We would then record the GPS
coordinates and move to the next turtle. During each trip, if resident turtles were found
they were marked, given a health assessment, and released. Each month a more thorough
assessment of juvenile health was made. The infrequent contact was essential to reduce
observer influence on the individuals. Because increases in size do not scale with
increases in weight (e.g. a turtle with proportionally larger carapace length than another will not have the same proportional change in weight), measuring the residuals of a linear regression of morphometric measurements by weight gave us a better indication of the turtle’s overall health (Budischak et al., 2006). These residuals are commonly referred to as body condition (Schulte-Hostedde et al., 2005). During the monthly examinations, their body condition was reevaluated and they were checked for evidence of infection, as box turtle energetics do not vary by sex but they do vary by season (Penick et al., 2002). Throughout the study, observations of naturally occurring behavior were recorded, which included evidence of mating behavior, obtaining food, and predator avoidance. These observations are merely anecdotal, but could speak to an acclimation to the environment, which is essential for RRT programs (Alberts 2007).

Movement analysis

The individual capture locations for each turtle were plotted using a geographical information system (Arc GIS, ver. 9.1, ESRI, Redlands, CA). The data points were imported into Arc GIS so they could be plotted on a physical map of the Rice Center for habitat and daily movement analysis. Each turtle point indicated a movement from one tracking event to the next. When those tracks were ordered by date, the distance between one point and another could be calculated using Hawth’s Tools (Beyer 2004) to convert locations into paths. These distances, when compiled over the length of the active season allowed us to make inferences on the movement patterns of individuals. The compiled
data points placed on the Rice Center map helped determine site fidelity. Any individual turtle crossing Route 5 (a 55mph two lane road) or otherwise leaving the research area (approximately 1 km from release with no settlement) and any turtle moving beyond transmitter range (~500m from one track to the next) were deemed off site. Activity areas, while not indicative of home range in the first year of release (Tuberville et al., 2005), were calculated as well by using multiple convex polygons. These polygons included all of the points where the turtle was located, including straight-line dispersal events. In addition to these procedures, maximum straight line distance from release was calculated for each individual turtle. Any turtle traveling further than 1km was considered to have “left the site” even though the total research area held all turtles throughout the duration of the study.

A habitat analysis was conducted based on coverage data which delineated the habitat of the Rice Center into 30x30 m pixels. Turtle points were categorized by their location in a habitat pixel (Fig 4). If a point was not on a clear habitat line, the ecotone was randomly selected. If points were outside of the Rice Center habitat delineation (~10% of total points), they were not counted. A contingency table was created to compare available habitat to habitat use for each turtle treatment and for each year. A chi square analysis was used to determine any differences. Cramer’s V test was used to compare variable association in chi square analyses. If a high variable association was found, differences could not be described because variation in one variable could be described by another.
Health and disease analysis

Prior to release or relocation at the Rice Center, the health status of all 20 of the juvenile eastern box turtles was evaluated by physical examination, taking weights, morphometric measurements, blood sampling using the venipuncture technique (Fig 5), and swabbing the mouth and cloaca of the turtles to test for Ranavirus infection by PCR. Weights, shell height, carapace and plastron width and length were all calculated initially and throughout the study with a caliper and digital scale (Appendix B). Cloacal and mouth samples were taken using fine tip sterile swabs that were immediately cooled and stored until they could be analyzed in the lab (Appendix B).

The overall health status of each individual was determined by checking for lesions, swelling, body fluid discharge, external parasites, coloration, malformations, and behavior. A similar health assessment for gopher tortoises is detailed in Berry (2001). Growth rates of our juveniles can be compared by size and by age to juvenile growth rates in other populations to determine their similarity to wild juveniles (Budischak et al., 2006). Blood was taken from the subcarapacial vein using either a 25 or 27 gauge precision glide needle, depending on the size of the individual. The vessel can be located where the carapace and the neck meet in the middle of the shell. Initially, this was done with the help of the wildlife veterinarian Dr. Jonathan Sleeman. Subsequently, blood was collected in the field on three separate occasions without a veterinarian. The blood was smeared onto slides for further analysis and placed on Flinders Technical Associates (FTA®) cards to start a genetic database for the Rice Center turtle population. The
smears were done approximately every six months, with the FTA® cards only being used during the first day of blood collection.

The blood was analyzed for various components. Each smear was dyed using the Diff-Quick technique. This involved placing the smears in a clearing solution for 3-5 minutes, after which they were individually dipped 25 times in each of the next two staining solutions. Immediately following that procedure, the slides were left to dry. They were subsequently placed in xylene solution for 5 minutes. Afterwards, they were permanently sealed to preserve the stain and were now available for direct microscopic analysis. Each smear was strenuously scanned for any evidence of parasitic or viral infections. Anything suspicious was recorded.

Prior to the PCR analysis, the DNA collected from cloacal and mouth swabs was broken down using standard DNA extraction procedures. Each swab was placed in a SETS tube and 50 μl of sterile PBS was added. Then they were centrifuged for three and a half minutes. The supernatant was discarded, leaving the cell fragments, to which we added 50 μl of Prep Man Ultra. These were vortexed for 30 seconds each then heated for 10 minutes at 100 ºC. They were spun again for an additional 3 minutes and subsequently placed in a -20º freezer until needed for PCR testing.

The DNA isolated from the swabs was used, through PCR, to analyze Turtle Virus 3 (TV3), which shares 100% sequence identity with Frog Virus 3 (FV3) and has been found in box turtles in North Carolina, Maryland, and Virginia (Allender et al., 2006; De Voe et al., 2004). To assay for the 417 bp fragment, the PCR comprised of a 25 μl solution that held a 3 μl of 2.5 mM forward and reverse primer IE (Galli et al., 2006),
2.5 μl 10X HotMaster Taq Buffer, 0.5 μl HotMaster Taq DNA polymerase, 2.5 μl of 2.5 mM dNTP, 16 μl water, and 0.5 μl DNA template. These were processed as part of the thermocycler protocol at 90°C for 1 min, 30 cycles of 94°C for 30 sec, 45°C for 40 sec, and 72°C for 40 sec, followed by extension at 72°C for 5 min. Subsequently, then were held at 4°C until they were run. We used a 1.2% gel product to run the PCR that required a 2 minute warm up followed by each well receiving 20 μl of solution. There was a positive and negative control in each gel, and a ladder made up of 3 μl of ladder and 17 μl of water. The FV3 strain, ordered from ATCC, was diluted 1/100 and used as the positive control. The remaining wells were filled with 12 μl of water and 8 μl of the sample.

Statistical analysis

The movements of our turtles were compared by year and by treatment: no pen turtles released in 2008 (NP08) were compared to no pen turtles released in 2009 (NP09); and the pen turtles emerging from their hibernacula in 2009 were compared to the initial release of the no pen turtles (NP08). Since each group could not be tracked on exactly the same day, path lengths were converted to movements per day instead of by track. The groups were compared by their morphometric characteristics using one way ANOVA to ensure there were no significant differences between the groups (p = 0.342). Independent t-tests were used to determine differences between treatments. The no pen treatment turtles were compared by year using a paired t-test because the second year turtles may
be more acclimated to their surroundings and may not exhibit the same movements as
they did in year one. This method of analysis has been utilized in the literature as
differences in movement by year are of particular interest in RRT programs (Tuberville et
al, 2005; Cook, 2004). Area covered was determined using multiple convex polygons and
similarly analyzed to path lengths. Site fidelity was tested using Pearson’s goodness of fit
test based on the proportion of individuals leaving the site. We expect first year no pen
turtles to have significantly higher daily movement and area covered than year two
turtles. We expect pen turtles to have significantly lower daily movement and area
covered than year one no pen turtles, and therefore have more similar patterns in
movement and area covered to year two no pen turtles.

To calculate body condition, we first calculated a curve estimation regression to
confirm carapace length as the strongest predictor of weight. Each of the five
morphometric measurements were compared by weight using a linear regression to
determine which was the strongest predictor of weight. Carapace length was the strongest
indicator, which conforms to previous research (Wilson and Earnst, 2005). We used the
residuals from a linear regression of mass on a measure of carapace length to serve as an
index of body condition. This method has been used in many vertebrate taxa and while it
is not without controversy, Schulte-Hostedde et al. (2005) found that this method satisfies
critical assumptions of the data. Repeated measures ANOVA were used to test for
differences in body condition by month, treatment, and month by treatment interaction.
All statistical analyses were done using SPSS software, ver. 17.0.

Research Authorization
This study was been approved by VCU IACUC (No. AM10209).

Chapter 1.3

Results

Movement parameters and habitat use

Released turtle step-length movements did not vary significantly by treatment ($F(2, 18) = 0.010, (p = 0.882)$), or between the activity areas of the pen treatment and NP09 turtles ($F(2, 18) = 0.512, (p = 0.711)$) (Fig 1). Pen treatment activity areas were significantly smaller than no pen turtles in 2008 ($F(2, 18) = 1.476, (p = 0.017)$). There were no significant differences in movement by year ($t = 0.633, n = 20, p = 0.544$) but NP09 turtles had significantly smaller activity areas than NP08 turtles ($t = -3.826, n = 20, p = 0.004$). Site fidelity did not vary by treatment ($X^2 = 1.065, df = 18, p = 0.595$) or by year ($X^2 = 1.458, df = 18, p = 0.349$). Over the first two weeks, one penned turtle moved 643 m from its release point, but the next furthest dispersal was only 258 m. By the end of the study, one pen turtle did disperse over 1 km from release, but all other pen turtles had dispersed less than 305 m from release (Table 1). The no pen turtles dispersed an average of 472 m (125 – 625).

A chi square analysis revealed that there are differences in habitat use that are unlikely to occur by chance ($X^2 = 227.695, df = 10, p < 0.000$) (Table 2). The Cramer’s V test statistic is not close to 1 (0.261), which indicates that our variables are not closely associated with each other. Therefore, turtles assigned to different treatments show differences in habitat use (Fig 2).
Figure 1: The total mean movement of juvenile box turtles across the active season shows no significant differences by either year or treatment.

Table 1: Average area covered (m²) for juvenile turtles over two time intervals calculated using multiple convex polygons. Estimates of furthest distance are based on individual turtles and not an average. Shortest distance is the shortest maximum distance observed by each group. No pen 09 distances are shown for anecdotal reference only.

<table>
<thead>
<tr>
<th>Turtle Group</th>
<th>2 wk</th>
<th>Largest area</th>
<th>Smallest area</th>
<th>Furthest distance</th>
<th>Shortest distance</th>
<th>Average Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Pen 08</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Pen 09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Error bars: 95% CI
Table 2: Contingency table showing expected versus observed frequencies of individuals by habitat. Expected values are based on habitat delineation of the VCU Rice Center. Observed values are based on GPS points inside the Rice Center.

<table>
<thead>
<tr>
<th></th>
<th>Evergreen Forest</th>
<th>Deciduous Forest</th>
<th>Mixed Forest</th>
<th>Palustrine Forested Wetland</th>
<th>Palustrine Emergent Wetland</th>
<th>Palustrine Shrub Wetland</th>
<th>Palustrine Shrub</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No Pen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>11,904</td>
<td>31,178</td>
<td>52,367</td>
<td>12,319</td>
<td>625</td>
<td>125</td>
<td>472</td>
</tr>
<tr>
<td>2009</td>
<td>7,627</td>
<td>9,001</td>
<td>17,298</td>
<td>4,233</td>
<td>566</td>
<td>210</td>
<td>501</td>
</tr>
<tr>
<td>Pen treatment</td>
<td>10,854</td>
<td>14,563</td>
<td>39,232</td>
<td>4,983</td>
<td>1,212</td>
<td>147</td>
<td>395</td>
</tr>
<tr>
<td>Pen w/o anomaly</td>
<td>3,727</td>
<td>6,171</td>
<td>19,745</td>
<td>4,983</td>
<td>305</td>
<td>147</td>
<td>278</td>
</tr>
</tbody>
</table>

Table 2: Contingency table showing expected versus observed frequencies of individuals by habitat. Expected values are based on habitat delineation of the VCU Rice Center. Observed values are based on GPS points inside the Rice Center.
Figure 2: Habitat allocation based on the number of location points in each habitat type showing a difference in habitat use between treatments and by year. Pen turtles use more mixed habitats while no pen turtles use more pine habitat.

**Health screens and body condition**

Parasite loads for all turtles at the Rice Center have been minimal. Mosquitoes were seen biting several turtles (3 pen; 4 no pen) on multiple occasions, while ticks (3) and leeches (1) were only found sporadically on the no pen turtles. No evidence of blood parasites for any turtle group was found. Although Ranavirus had been detected across from the Rice Center at the Harrison Lake Fish Hatchery in the summer of 2007 and 2008, no turtle DNA showed evidence of infection. All turtles survived their first winter
and were seen emerging in the spring of 2009. Three transmitter whips (tails) were disconnected shortly after emergence causing us to lose one turtle to transmitter failure. During a one week period in August 2009, six more individuals were lost to transmitter failure before the replacement transmitters could be attached; only two of those were recovered. In total, five of twenty turtles are no longer being monitored because of transmitter failure. Mating behaviors were observed on five occasions. In 2008, one no pen turtle was observed mounting a resident female (Appendix B). Several resident males were observed mounting our juvenile turtles in both 2008 (3) and 2009 (1), although these could be displays of dominance in some instances (Cook 2001). One individual in the no pen group of juveniles has developed a debilitating eye infection that has reduced its ability to forage (Appendix B). As a result, the turtle has had extreme weight loss and is not expected to survive the winter.

Since the start of the study, our turtles have grown an average of 1.6 mm/yr in carapace length. Our turtles are larger than expected for their age class. The carapace length of our turtles from initial release ranged from 88mm to 111mm, which are sizes more indicative of the 5-9 age class than the 0 – 4 they are actually in (Fig 3). As if to corroborate this, a five year old resident juvenile turtle, appropriately aged by growth rings (Wilson et al., 2003), was discovered in July of 2008 with a carapace length of 89 mm and a three year old was found in July of 2009 with a carapace length of 66 mm.

Assumptions of sphericity were not met for body condition using the repeated measures ANOVA (p = 0.001), so the Huynh-Feldt test was used to adjust the degrees of freedom for a more reliable F statistic. Month had no influence in body condition (F(2,96,
The influence of release treatment on body condition does not appear to be dependent on month ($F_{(5.919, 65.111)} = 1.931, p = 0.090$). The treatment itself also seems to have no influence on body condition ($F_{(2, 22)} = 0.768, p = 0.476$).

Descriptive statistics for body condition can be found in Tables 5 and 6. Individual months appear to have differences, but unequal variances and low sample size appear to reduce the effectiveness of the analysis.

Figure 3: Graph displaying the expected ranges of size and age for the eastern box turtle taken from Budischak et al, 2006. Of note, our juvenile turtles fall into the 0 – 4 age group but the 5 – 9 age group in terms of size.
Table 3: Within subject effects (Huynh-Feldt) show no significant difference in body condition within month or month by treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>1.234</td>
<td>2.960</td>
<td>0.417</td>
<td>0.423</td>
<td>0.735</td>
</tr>
<tr>
<td>Month*</td>
<td>11.266</td>
<td>5.919</td>
<td>1.903</td>
<td>1.931</td>
<td>0.090</td>
</tr>
<tr>
<td>Error(Month)</td>
<td>64.177</td>
<td>65.111</td>
<td>0.986</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Body conditions by treatment indicate no significant difference between treatments.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.083</td>
<td>1</td>
<td>0.083</td>
<td>0.430</td>
<td>0.519</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.296</td>
<td>2</td>
<td>0.148</td>
<td>0.768</td>
<td>0.476</td>
</tr>
<tr>
<td>Error</td>
<td>4.247</td>
<td>22</td>
<td>0.193</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 1.4

Discussion

The main findings of this headstart project are as follows: 1) all turtles maintained site fidelity for the first year of release, 2) penning the eastern box turtle, at least for juvenile headstart turtles, does not affect the overall movement of the turtles but it does affect area covered, and habitat use was significantly different between treatments, 3) all turtles survived the first winter of the project and no mortality events were witnessed, with only one instance of serious infection and no significant differences in body condition.

1) Site Fidelity

No turtles definitively left the study site and dispersal rates for each release period (June 2008 for no pen turtles, June 2009 for the pen turtles) were negligible; however, one pen turtle did disperse over 1 km from release. This turtle may be an aberration more than a cause for concern because the rest of the pen turtles were no further than 305 m from the release site. The turtle has subsequently shown movements more indicative of home range than straight-line movement, having reached the 1 km mark in July of 2009 and maintaining that distance the rest of the active season. This is generally thought to be enough to reverse the claim that our pen turtle has not maintained site fidelity (Tuberville et al., 2005). No pen treatments had further straight line movement from release (average distance = 425 m) but none traveled in excess of 650 m from the release site. To
compare, RRT adult turtles have shown straight line movements in excess of 3.2 km over a period of nine months and one adult female traveled 91.8 km over an indeterminate time to reach her initial home range (Dodd 2001). Since both groups of turtles had low straight line distances from release, it does not appear to be an issue for either group. Penning does not appear to affect the distance traveled or furthest distance from release and may be based on individual differences. No turtles crossed a two lane highway, though two no pen turtles were found at the edge of the road and turned back on their own in both situations. One turtle in the pen treatment group crossed a one lane dirt road about 480m from release without incident. Our 20 turtles altogether used about 50ha of available space, 450ha less than what was recommended by Cook in 2004 for a relocation of 25 individuals. Since neither treatment had a significant difference in site fidelity, our study suggests that juvenile box turtles may be released in smaller habitats without the fear of emigration.

Previous RRT studies have shown that site fidelity to the relocation area, especially in the first year of release, can be difficult to ensure (Tuberville et al., 2005). Our juvenile turtles do not exhibit straight line movement and no mortality events were observed. This could be explained by the fact that juvenile box turtles don’t establish a home range until after they reach maturity (Dodd 2001); however, portraying the natural environment accurately during years of captivity can dramatically increase the success of a release program (Alberts 2007). Since our turtles were kept in an outdoor environment and exposed to harsh conditions prior to release, their wintering success rate and subsequent maintenance of site fidelity is not a surprise.
One important distinction to make is that while none of our turtles have verifiably left the research site, five had transmitter failure and their fates are undetermined. These individuals were confined to the center of the research area, but their current locations are unknown, though they are most likely still on site. So, while NP08 site fidelity was 100%, pen fidelity was 80% and year two fidelity is 70% (87.5% cumulative average). These findings are still positively significant as many relocation efforts can have site fidelity as low as 23%, with the majority of first year fidelity falling under 60% (Tuberville et al., 2005). The study is only two years old, which is one year shorter than the majority of RRT studies, so our results are not conclusive but promising.

2) Movement

No overall significance in total movement between treatments or by year can be claimed, but significant differences in activity areas in treatments suggest that penning may benefit relocated juvenile eastern box turtles. Initial release of any RRT program is tenuous, so any reduction in area covered during this period would be beneficial to maintaining site fidelity and thus relocation success of the species (Cook 2004; Tuberville et al., 2005). Given that recent efforts to pen tortoises had shown positive results, perhaps their captivity contributed to a reduction in overall activity areas. For example, wild tortoises exhibited more straight line movement than captives, who remained closer to their original burrows (Bertolero et al., 2007). While using wild individuals for relocations is thought to be more beneficial than using captive individuals, there is still a benefit to using captives (Germano and Bishop, 2008). This is especially
true of juvenile turtles because of the high degree of instinctual behaviors that they retain (Germano and Bishop, 2008).

Since the pen turtles show no statistical difference from the NP09 turtles in either movement or area covered, there may be a difference in climate from one year to the next that could have influenced movement patterns. However, the argument could be made that the NP09 group have adjusted to their surroundings, having significantly lower activity areas for this year versus last. If that were the case, then the pen turtles could exhibit lower activity areas as a direct result of the penning treatment. To strengthen this argument, the pen treatment needs continued monitoring in 2010 to determine if their activity areas decrease on the same order of magnitude seen in the no pen turtles.

Weather patterns for 2009 in Charles City County, VA indicate a slightly wetter and cooler season than in 2008 (Sampson et al., 2009). We would then expect both groups of turtles in 2009 to have higher rates of movement and area covered because box turtle activity in drier and hotter years tends be lower than in cooler, wetter years (Penick et al., 2002). Since that was not the case, weather most likely is not influencing their activity areas and may be a result of the penning treatment. More intense analysis of weather patterns and box turtle movement should be examined in future studies.

Activity areas of the NP09s are significantly lower from their movements in 2008. We would expect to see a drop in movement patterns from year 1 to year 2 since the turtles were released into an unknown environment in year 1 and subsequently could adjust to their surroundings the following year. This is often seen in translocated adult turtles, even in penning treatments (Tuberville et al., 2005). How the pen turtles move the
following year (2010) is the next step in the management process. If the pen treatment activity areas continue to decrease the following year, this could mean that the pen treatment can reduce the dangerous activity period of year 1 release and further increase the validity of the pen treatments.

Habitat use may have some influence on movement. Movement patterns of wild turtles vary considerably by location and by individual (Dodd 2001). Some turtles will simply sit in one location for days and weeks while others will maintain daily movements for the entire season (Cook 2004; Penick et al., 2002). There are differences in habitat use between treatments, so differences in activity areas by treatment may be influenced by habitat. Our juvenile individuals were found mostly in terrestrial forested habitat that resident turtles also occupy. Box turtle movements are not particularly affected by sex (Penick et al., 2002) but adult males have been known to travel great distances in search of mates (generally in September), while females may do the same in search of nesting sites (usually in May or June) (Penick et al., 2002). Our turtles are generally too young to determine sex, though some individuals are showing secondary sex characteristics. Movement of turtles may still be influenced by sex, but we were unable to address this in this study. Future work should create a rigid habitat assessment for each individual turtle in an effort to determine if habitat influences movement.

3) Health assessment

For the duration of the study, no turtles of any group were shown to exhibit any kind of blood parasite or other possible infection. The effect of parasites on box turtle
populations is not known, but small parasite loads are not considered detrimental to the species and are an expected part of their ecology (Dodd 2001). However, parasite abundance is typically determined by the composition of the host community (Johnson et al., 2008). This may suggest that the Rice Center has enough diverse biota to curb parasite transmission. The only major infection observed in our study was an eye infection with possible bacterial origin. These infections are common in individuals coming out of hibernation (Dodd 2001) and while they may clear up under favorable conditions in captivity, conditions in the wild are not always conducive to a rapid recovery. The turtle has remained blind for the duration of the field season and is not expected to survive the winter after a near 35% drop in weight. No inherent differences in body condition may seem promising for our turtles, but small sample sizes and no resident juveniles to compare them to prohibit us from making definitive conclusions.

Ranavirus has been associated with anthropogenic environmental change (St-Armour et al., 2008) and our site has relatively little human impact. This may help explain its absence despite the fact that the disease was found 450 m from the edge of the Rice Center at the Harrison Lake Fish Hatchery (a heavily altered landscape with intensive human interactions with several roads dividing it from the Rice Center) in 2007 and 2008 (Pullen unpublished). The low incidence of disease is indicative of low population densities (Lafferty and Gerber, 2002); however, even though there was no detectable infection, our turtles may still have minute traces of the disease.

In a separate study (Budischak et al., 2006), juvenile eastern box turtles grew an average of 2.76 mm per year. Our turtles have grown an average of 1.6 mm/yr. While
higher rates of growth have been reported for the juvenile age class (Stickel and Bunck, 1989), our turtles are larger than expected for their age. Eastern box turtles mature between the ages of 5 and 9, but maturity in turtles is determined by size. So, while our juvenile turtles may be four years old, their size indicates that they are capable of being sexually mature. The high protein diet and optimal habitat conditions they received while in captivity most likely contributed to their high rates of growth. Mounting behavior was observed on several occasions, with separate individuals. While no confirmed fertility was observed, this does not preclude our turtles from the possibility of reproducing.
Chapter 1.5

Conclusion

Conservation strategies for all species are becoming increasingly important as anthropogenic environmental change increases. The complexities of box turtle ecology present a challenge to wildlife managers. Relocation programs are not without controversy, but may be gaining momentum as our understanding of the target organism’s basic biology improves. Our turtles exhibited natural behaviors expected of wild individuals. Site fidelity was maintained for the first year and all turtles survived their first winter. Penning appears to reduce initial activity areas, but does not appear inherently necessary for juvenile individuals to remain on site. Activity areas may be influenced by habitat use and weather patterns though long term monitoring is needed to confirm this. Relatively minute amounts of habitat were used by our turtles, indicating the possibility of establishing populations in smaller release areas. Our turtles showed low infection rates and appear to be relatively healthy. While determining success is still a few years away, penning the eastern box turtle appears to be a viable option that could be incorporated into conservation strategies for this species.
Chapter 2.1

Introduction

Wildlife management requires a knowledge and understanding of the species in question. Perhaps the most practical way to accomplish this is by studying the life history of the organism and recognizing which moments during the life cycle have the most impact on population growth. As a possible solution, simulation modeling has been implemented in a few chelonid species. The majority of these have focused on endangered sea turtles (Crouse 1987; Crowder 1994; Heppell 1996). These turtles have high egg mortality and relatively long juvenile development and decades long adult stages. While this is certainly true of the eastern box turtle, one key difference is in the amount of eggs produced per season per female. Sea turtles have relatively high fecundity and low juvenile survival, which is the opposite of what we’d expect with the box turtle. The population models that were implemented for the loggerhead sea turtle have determined that juvenile and subadult stage classes as the most important stages to manage, as they had survived the most difficult years of their lives and were nearing contributions to reproduction (Crouse et al., 1987; Crowder et al., 1994).

A population model of the eastern box turtle has not been implemented and could benefit wildlife managers attempting to manage their fragmented populations. While the population dynamics of the box turtle are somewhat understood, no clear analysis of these dynamics has been presented (Dodd, 2001). Using stage based population models may give us a better understanding of the important management stages of the box turtle.
and allow for stronger conservation strategies. For the eastern box turtle, a stage based model is preferable given their long reproductive span (30 – 50 yrs) and relatively slow maturation (about 7 yrs). To accomplish this goal, a Lefkovitch matrix model will be implemented because the length of the stage is incorporated into the model. Our deterministic matrix analysis will be combined with an eigen analysis to: 1) determine the stage specific interactions that contribute the most to the local stochastic growth rate, 2) test the stages for susceptibility to change in growth rates based on changes in fecundity and survival and 3) simulate changes in mortality and fecundity in order to account for varying dynamics across the range of the eastern box turtle. Following similar models created for species that share similar life history characteristics (such as the yellow mud turtle) we would expect the adult stage of *T. carolina* to be the most influential (Heppell et al., 1996).
2.2 Methods

Life Cycle Graph

Box turtles do not have overly complicated life histories. Gestation times average 80 days and eggs are predominantly laid in spring. Hatchlings may not emerge from the nest for a full week while their external yolk sac is being absorbed. They then spend the year growing rapidly before the first winter season. When they reach a size greater than 25mm in carapace length, they are considered juveniles. This generally occurs during the first year of their life (Dodd 2001). As a result, we will assume that turtles emerging from their first winter hibernation are juveniles. The remaining age classes are determined by growth and reproductive ability. Growth rates of eastern box turtles are described in Budischak et al., 2006. Box turtles grow more rapidly in early stages of their life and quickly reduce their growth until it becomes almost negligible. The fastest growth rates range from ages 0 – 5, but growth between 5 and 10 is still high. The difference between these two stages is that maturity is reached in the latter group. This distinction enables us to break the juvenile stage into two parts, juveniles and subadults. Growth slows between 10 and 20 years of age and becomes minute after 20. After year 10, maturity lasts between 30 and 50 years. There is no evidence of reproductive output varying by age after maturity; therefore, they are considered all one stage class. The oldest known gravid female box turtle recorded was 54 years old and reproductive potential may extend beyond 60 years (Henry 2003). Whether or not the reproductive output drops off after this age is uncertain, but probable. Finding turtles that are older than 60 years requires
long term monitoring because there is no reliable way to estimate a turtle’s age after they turn 20. Attempts to age the turtle by growth rings beyond this age is not justifiable because the rings tend to clump together as the turtle ages (Wilson et al., 2003). Even though the literature describes maximum maturity at 50 years and the oldest box turtles have been aged at 75 to 80 years old, we will not consider a fifth class of non-reproductive adults for two reasons: 1) relatively few individuals could live beyond maturity and 2) there is no evidence of *T. carolina* going through senescence. Therefore, our stage based model consists of: 1) eggs/hatchlings, 2) juveniles, 3) subadults, and 4) mature adults (Fig 4). Eggs can only grow into juveniles (G\(_{1,2}\)). Juveniles can survive and stay juveniles (P\(_{2,2}\)) or grow to subadults (G\(_{2,3}\)). Subadults can survive and stay subadults (P\(_{3,3}\)) or become adults (G\(_{3,4}\)), as well as become adults and reproduce (F\(_{3,1}\)). Adults can only stay adults (P\(_{4,4}\)) and reproduce (F\(_{4,1}\)).

Figure 4: Life history diagram of the eastern box turtle.
Eastern box turtle demographic parameters

To parameterize our model, information on fecundity and survival rates for individuals in each stage was gleaned from the literature. From that information, calculations of the probability of either remaining in a stage or moving on to the next was calculated. This information is difficult to determine for long lived individuals and is only the best available, not completely definitive. Relatively few papers have been published on population dynamics of the eastern box turtle. Those that have, report on the survival of adults, not juveniles. The problem with accounting for juveniles has been the difficulty of finding them. So, while a large amount of information exists on adult mortality, movement patterns, and morphology, juvenile records are minimal (Budischak et al., 2006; Nazdrowicz et al., 2008).

Commonly, reptilian species require high juvenile survival to maintain a stable stage distribution; and juvenile turtles have the highest rate of survival among reptiles (Pike 2008). The only verifiable data for juvenile *T. carolina* survival rates has come from a Dodd study in 2006 at Egmont Key in a population of Florida box turtles (*Terrapene carolina bauri*). Egmont had the majority of its undergrowth removed by several tropical storms. This allowed the researchers to find elusive juveniles and determine their survival rates with a much higher degree of accuracy. We understand that there are differences between the two subspecies of turtle, but survival rates of adults appear to be similar for both subspecies, so we assume the same holds true for juveniles.

Another potential issue with the eastern box turtle appears to be that clutch size and reproductive rates vary widely throughout its range. Reproductive output of New
York box turtles is around 2.74 eggs per female (Cook 2004), but Virginia turtles have an output of 1.276 eggs per female per year (Wilson and Ernst, 2005) and Florida box turtles averaged 0.608 eggs per female (Dodd et al., 2006). Choosing the correct metric seems arbitrary, but given that Virginia is not only close to the center of the box turtle home range, but incorporates our research site, these numbers should be sufficient. As a result of this, our model will reflect local population dynamics and will have to be recalculated for differing populations of the eastern box turtle. The majority of our data on reproductive output come from an 11ha study conducted by Wilson and Ernst in 2005, in Lynchburg, VA’s Blackwater Creek refuge. Egg/hatchling mortality is partly based on a Flitz and Mullin 2006 study on nest selection. Egg mortality was estimated based on averaging the analysis of predation-free hatching success rate (47% survival) and extremely high predation rates (12% egg survival). The Blackwater Creek turtles have only 40.5% of their females lay eggs every year, which is used to calculate overall fecundity, increasing this number increases the reproductive output of females. Low egg viability is not unheard of and has been documented in the literature (Dodd 2001). Hatchling survival does not exceed 50% and is predicted based on the amount of eggs reaching maturity (Wilson and Ernst, 2005). The analyses on box turtle population dynamics discussed in this paper are based on the population projections of the wild turtle population described in Wilson and Ernst. Population density for the area is average at around 16 turtles/ha and a 1:1 sex ratio has been calculated. All literature references used to make the models can be found in Table 5.
Table 5: Literature used to determine survival of stage classes for the eastern box turtle.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Survivorship estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs/hatchlings</td>
<td>Wilson and Ernst, 2005</td>
</tr>
<tr>
<td></td>
<td>Flitz and Mullin, 2006</td>
</tr>
<tr>
<td>Juveniles</td>
<td></td>
</tr>
<tr>
<td>Subadults</td>
<td>Dodd et al, 2006</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
</tbody>
</table>

Model

Using data from a well established long term monitoring project conducted in New Castle County, Delaware (Nazdrowicz et al., 2008), density dependence was tested using a count based population viability analysis (PVA). It was determined that eastern box turtles do not exhibit density dependence, which matches the literature (Rittenhouse et al., 2007) (Fig 5). Box turtles often have overlapping home ranges and natural densities range from less than one turtle per hectare to 34 turtles per hectare (Dodd 2001). Therefore, no adjustments for density dependence were made.

Our model design is similar to the loggerhead sea turtle model presented by Crouse (1987) and revised by Crowder (1994). We are using a four stage model more closely resembling Crowder’s model (Table 6). Unlike the loggerhead model, box turtles have relatively high survival rates after year 1. We cannot incorporate all life stages into two classes based on this because differences in fecundity warrant an expansion of the model. Fecundity estimates incorporate eggs produced by females per year, with their duration in the stage class. The caveats and assumptions presented in these models apply to this one as well. Since the data available do not have estimates of population growth or
stage advancement, our parameters will be calculated using the method detailed by Crouse.

We created a population matrix model with stage distribution to allow for stochastic events (Table 7). Our projection matrix allows us to include estimates of reproductive output \( F_i \), the probably of surviving and leaving a stage \( G_i \) and the probability of survival and staying in the same stage \( P_i \). The formula for survival and staying in the same stage is described as:

\[ P_i = \left( \frac{1 - p_i^{d_i - 1}}{1 - p_i^{d_i}} \right) p_i \]

Where calculations are based on the survival of each stage \( p_i \) and the stage duration \( d_i \) (Table 6) (Crouse et al., 1987). Each year the stage classes have the ability to transition to the next stage. The transitions are assumed to be completed by the end of the nesting season, giving rise to the next year’s population census. The proportion of individuals that transition to the next stage is proportional to the survival within the stage (Crouse et al., 1987) and can be calculated by:

\[ G_i = p_i^{d_i}(1 - p_i) \]

Reproductive outputs of adults \( F_4 \) are dependent on their survival from the previous year and the rate of fecundity \( f_i \) described above. Subadults that become mature before transitioning to adults also contribute to reproduction for the year and they are given their own fecundity parameter \( F_3 \). Fecundity is defined by the formula (Heppell 1996):

\[ F_i = (P_i; f_i) + (G_i; f_{i+1}) \]
Woodlot box turtle population growth rate

Figure 5: The count based population viability analysis indicates that the eastern box turtle does not exhibit density dependence as population growth rates are highly variable despite consistently declining populations.
Table 6: Life table for the eastern box turtle based on Budischak et al, 2006.

<table>
<thead>
<tr>
<th>Stage number</th>
<th>Class</th>
<th>Size (mm)*</th>
<th>Approx. age(d_i)</th>
<th>Annual survivorship(p_i)</th>
<th>Fecundity (no. eggs/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eggs/hatchlings</td>
<td>&lt; 25</td>
<td>&lt;1</td>
<td>0.105</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Juveniles</td>
<td>25 – 75</td>
<td>1 – 5</td>
<td>0.877</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Subadults</td>
<td>75 – 100</td>
<td>6 – 10</td>
<td>0.877</td>
<td>0.170</td>
</tr>
<tr>
<td>4</td>
<td>Adults</td>
<td>&gt; 100</td>
<td>11 – 70</td>
<td>0.880</td>
<td>1.123</td>
</tr>
</tbody>
</table>

*Measured by carapace length

Table 7: Format of the stage based population matrix.

<table>
<thead>
<tr>
<th></th>
<th>F_{2,1}</th>
<th>F_{3,1}</th>
<th>F_{4,1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_{1,1}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G_{1,2}</td>
<td>P_{2,2}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>G_{2,3}</td>
<td>P_{3,3}</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>G_{3,4}</td>
<td>P_{4,4}</td>
</tr>
</tbody>
</table>

Every population matrix has a right eigenvector (w) that enables us to estimate intrinsic rates of growth (λ) through the stable stage distribution. The stable stage distribution is obtained from population projections that settle on the same λ for each class. In other words, it is the distribution of each stage available in the natural environment that incorporates the same rate of growth. These growth rates can then be used to analyze the population over the course of several years to determine if it is viable (increasing). The left eigenvector (v) of the matrix represents the reproductive value of each stage, where each stage has the ability to contribute to future population fecundity. Generally, more weight is placed on the stage with the highest rate of fecundity. For example, an egg has the ability to reach maturity and produce eggs of its own, but an adult has a higher probability of producing eggs for any given year than an egg.
Perturbations to the matrix

A population matrix model can test how sensitive a population growth rate is to change. Variations of fecundity, growth or survival can be simulated by each stage successively. $\lambda$ can be recalculated and comparisons to the changes in the different stages can be made. Management of eastern box turtles is a complex process, so knowing which stage has the greatest effect on the rate of $\lambda$ can increase management efficiency. The best way to calculate this is to use elasticity to simulate proportional changes in $\lambda$ by the elements of the matrix ($F_i, G_i, P_i$). This can analyze, for example, whether a minute change in adult survival ($P_a$) has a proportionally large change in the population growth rate. The elasticities allow a comparison of all matrix elements because the elasticities will sum to 1. By comparing these relative changes, we can compare their effect on the different stages. From a management prospective, a small change in any parameter that allows for large positive changes in growth rate may be more cost effective than having to implement large scale changes to any one specific class.

Perhaps the most difficult parameter to estimate is first year survival. Rates of egg survival without predation can reach 47% in the Blackwater Creek study, but survival beyond year 1 is probably no higher than that estimate regardless of condition. Because of this uncertainty, changes in egg survival will be implemented in our model. Additionally, management strategies may increase survival for some, most, or all of the stage classes. How each of these stages affect population growth rate may suggest which management plan would be most effective. These can take two directions: managing for
headstart programs and managing for adults. For headstarts, this could involve rearing eggs through year 1 or rearing through year 4. Judging from our previous study, long term headstarting could effectively reduce the duration of the juvenile and subadult stages to the point where they could be combined. These 4 year headstart individuals were already in the subadult stage class and exhibited mating behaviors. Management efforts to increase the survival of adults may or may not influence the survival of subadults. Therefore we will examine these “best case” possibilities by: 1) increasing egg survival through a headstart program to 50%; 2) increasing egg survival and raising turtles through the juvenile stage (estimated success rate of juveniles = 95%); 3) increasing survival of adults to 95%; and lastly 4) increasing subadult and adult survival alone to 95%. 5) increase egg survival to 17% (20 eggs raised from the population), juvenile survival to 95% and only three stage classes (most similar to the scenario presented in Chapter 1).
2.3 Results

The Lynchburg population at Blackwater Creek was projected for 50 years using the 88 adult females found in the study as the starting population. Based on our stage based population matrix (Table 8), the growth rate of the population ($\lambda$) reached the stable stage distribution at $R = 0.956$ indicating a slight population decline. The stage distribution in the wild is estimated to be composed of mostly eggs, followed by adults, juveniles and subadults (Table 9). Reproductive value indicates that the adult stage, followed by the subadult stage are the most important (Table 9). After 50 years, the original population levels were drastically reduced to 3 adults. When applied to our sensitivity analysis, changes in fecundity have minimal impact on $\lambda$ (Fig 6). And while survival to transition stages is more important, the strongest impact is clearly on survival of individuals in the same stage. Slight changes to adult survival have the greatest effect on population growth, as a 5.5% increase in adult survival could allow the population to rebound (Fig 7). However, through effective management strategies, large changes to survival may be possible to all stages. Without predation, egg survival is 47% in the Blackwater Creek study. If we simulate egg and hatchling survival at 47% our $\lambda$ increases to 1.033. If juvenile survival is increased by 21%, the population will begin to recover. The greatest increases in $\lambda$ are when management strategies incorporate large increases in egg survival (Table 10; Fig 8). When these management strategies are implemented, the stage most sensitive to change remains adult stage class survival (Table 11). The
management scenario with the highest increase in the population growth rate was the 5
year headstart program, followed closely by the 1 year program.

Table 8: Stage based population matrix for the eastern box turtle.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>0.105</th>
<th>0</th>
<th>0.170</th>
<th>1.123</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.699</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>.105</td>
<td>0.699</td>
<td>0.178</td>
<td>0.744</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.133</td>
<td>0.880</td>
<td></td>
</tr>
</tbody>
</table>

Table 9: Stable stage distribution and reproductive values of the eastern box turtle based on estimates from Table 12.

<table>
<thead>
<tr>
<th>Stage Class</th>
<th>Stable stage distribution (dominant eigenvector)</th>
<th>Reproductive value (left eigenvector)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs/hatchlings</td>
<td>0.383</td>
<td>1.000</td>
</tr>
<tr>
<td>Juvenile</td>
<td>0.168</td>
<td>8.756</td>
</tr>
<tr>
<td>Sub adult</td>
<td>0.148</td>
<td>12.131</td>
</tr>
<tr>
<td>Adult</td>
<td>0.300</td>
<td>17.112</td>
</tr>
</tbody>
</table>
Figure 6: Sensitivities to changes in growth rate ($\lambda$) by stage and parameter. Growth and fecundity show no real change, while adult survival is the most sensitive to change.
Population growth rates are shown by stage class based on various degrees of survival. All classes are dependent on the adult survival (e.g., growth rate cannot go below 0.88 because that is the adult survival). Any point above 1 shows a growing population. Adult survival shows the greatest percentage increase (or widest range) in $\lambda$ because of sensitivity to small changes in survival (See Figure 6).
Table 10: Changes to $\lambda$ based on management scenarios for the eastern box turtle displaying positive responses from all groups, particularly headstart options.

<table>
<thead>
<tr>
<th>Management Scenario</th>
<th>Old $\lambda$</th>
<th>New $\lambda$</th>
<th>Change in $\lambda$</th>
</tr>
</thead>
<tbody>
<tr>
<td>First year survival = 50%</td>
<td>0.946</td>
<td>1.035</td>
<td>+ 0.089</td>
</tr>
<tr>
<td>First year = 50% and Juvenile survival = 95%</td>
<td>0.946</td>
<td>1.058</td>
<td>+ 0.133</td>
</tr>
<tr>
<td>Adult survival increased by 10%</td>
<td>0.946</td>
<td>1.004</td>
<td>+ 0.058</td>
</tr>
<tr>
<td>Adult, subadult survival increased 10%</td>
<td>0.946</td>
<td>1.012</td>
<td>+ 0.066</td>
</tr>
<tr>
<td>Three stage classes, First year = 17%; Juvenile = 95%</td>
<td>0.946</td>
<td>1.033</td>
<td>+ 0.087</td>
</tr>
</tbody>
</table>
Table 11: Changes in elasticities based on influence of possible management strategies. Bold numbers indicate the stage most sensitive to change.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Fecundity</th>
<th>Growth</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Old</td>
<td>New</td>
<td>Old</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>First Year (1 year headstart program with first year survival at 50%)</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Juveniles</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Subadults</td>
<td>0.015</td>
<td>0.049</td>
<td>0.041</td>
</tr>
<tr>
<td>Adults</td>
<td>0.041</td>
<td>0.053</td>
<td>0</td>
</tr>
<tr>
<td>5 year headstart program with first year survival at 50% and juveniles at 95%</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Juveniles</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Subadults</td>
<td>0.015</td>
<td>0.046</td>
<td>0.041</td>
</tr>
<tr>
<td>Adults</td>
<td>0.041</td>
<td>0.048</td>
<td>0</td>
</tr>
<tr>
<td>Adult survival at 95%</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Juveniles</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Subadults</td>
<td>0.015</td>
<td>0.008</td>
<td>0.041</td>
</tr>
<tr>
<td>Adults</td>
<td>0.041</td>
<td>0.033</td>
<td>0</td>
</tr>
<tr>
<td>Subadult and Adult survival at 95%</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Juveniles</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Subadults</td>
<td>0.015</td>
<td>0.010</td>
<td>0.041</td>
</tr>
<tr>
<td>Adults</td>
<td>0.041</td>
<td>0.036</td>
<td>0</td>
</tr>
<tr>
<td>Three stage classes, First year = 17%; Juvenile = 95%</td>
<td>0</td>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td>Juveniles</td>
<td>0</td>
<td>0.014</td>
<td>0.056</td>
</tr>
<tr>
<td>Adults</td>
<td>0.041</td>
<td>0.079</td>
<td>0</td>
</tr>
</tbody>
</table>
Chapter 2.4 Discussion

Box turtles have long periods of maturity with high survival of juveniles and adults to outweigh low survival rates of eggs and hatchlings. Because egg production is very low in the eastern box turtle, the egg/hatchling stage has more value than it does in marine chelonids. For the Blackwater Creek study, less than a 100% increase in first year survival can rebound the population. This may sound daunting, but when housed in predator free pens, egg survival is 47%, over a 400% increase in survival. The concern with this approach is sustainability (Heppell 1996). As populations increase a larger number of individuals need to be headstarted to continue to increase population growth. The most effective use of headstarting appears to be restarting ghost populations, though success can still take decades (Nelson et al., 2007). Each of our headstart models out performed our management strategies for adults (Table 10). Regardless, all of our simulated management scenarios indicated that survival of the adult stage had the highest sensitivity to change in $\lambda$ (Table 10). The least amount of effort needs to be applied to the adult class to increase lambda above 1 (Fig 6). Conversely, a reduction in adult survival would also have the largest decrease in $\lambda$. It would seem that management should be placed predominantly on the adult stage class. The inherent danger in presuming that methodology can be found in percentages. The adult age class can conceivably be increased by just under 14%, raising overall survival to 100%. For the egg/hatchling stage, survival can, in theory, be increased to over 900%. Practically, the highest possible
increases to egg survival are unknown and most likely are contingent on the population in question. As with all management efforts, determining success requires long term monitoring. The Blackwater Creek data may be not extensive enough to truly understand the dynamics of the population. Over time, the amount of gravid females could change and any one of the survival indices could increase or decrease substantially. However, there are turtle populations similar to this one across the turtle’s range (Dodd 2001).

It is important to note that while the adult stage has the strongest effect on $\lambda$, it can, contextually, be the most difficult stage to manage (Hall et al., 1998). Survival of RRT turtles varies depending on the relocation effort, but it is significantly lower than resident adults. Success of these projects is often determined early when long term monitoring was required to determine true success. Those that are successful require vast amounts of continuous habitat (Cook 2004). Currently, there are no management strategies for eastern box turtles akin to using turtle excluder devices for marine turtles and RRTs are often the most feasible, though not the most desirable, management strategies (Dodd 2001). Headstart programs for the eastern box turtle may be practical for several reasons: 1) egg mortality has more weight because of the low reproductive output, 2) the turtles are relatively easy to raise in captivity, 3) captive rearing can mimic real world habitat, 4) site fidelity of RRT juveniles may be higher than RRT adults, 6) a wider selection of sites can be used as juvenile RRT turtles require less available space, and 7) raising a turtle on a high protein diet can reduce the length of the juvenile stage and sexual maturity could be reached at an earlier date. The duration of the conservation effort will be dependent on the survival of hatchlings after being hatched. The longer a
turtle is raised in captivity, the higher the cost, but potential benefits may outweigh cost. Headstarting may be used as a supplementary tool as well. The goal of management should be to establish baseline recovery populations and allow them to become self-sufficient. Since there are scenarios for all stage classes to positively influence the population growth rate, long term management should focus on adult survivorship.
2.5 Conclusion

Juvenile data are difficult to interpret and rarely definitive. Hatchling data are nearly impossible to find due to their extremely small size. That said, our model has extrapolated the best possible scenarios for both the Blackwater Creek population and other similar populations of box turtle. Headstart programs to rebound local populations can conceivably be beneficial, but long term population pressures will still exist. The instant the program is halted, the underlying issues in the population will resurface. The same could be said for any management class however, so the issue becomes one of cost. Creating a cheap alternative that could increase adult survival with minimal effort is the best scenario, but not always realistic. Rebounding local populations of eastern box turtles seem viable as relatively low numbers of individuals need to be headstarted compared to other similar species. Until more consistent data on box turtle morphology throughout their range are presented, the model will have to undergo modifications based on situational dynamics of the study population. Therefore, determining how effective a headstart program could be across the range of *T. carolina* is unknown.
Literature Cited


Appendix A: Site Maps with Turtle Movements

Figure 1-A: Site map of the VCU Rice Center.
Figure 2-A: No Pen turtle movement for the duration of the active season in 2008. Each color represents one individual turtle.
Figure 3-A: No Pen turtle movement for the duration of the active season in 2009. Each color represents one individual turtle.
Figure 4-A: Pen turtle movement for the duration of the active season in 2009. Each color represents one individual turtle.
Figure 5-A: Habitat types at the Rice Center based on a 30 X 30 m habitat analysis.
Figure 6-A: Delineated habitat use by the No Pen turtles in 2008 where the majority of the data points are found in the pine forest.

Figure 6-A: Delineated habitat use by the No Pen turtles in 2009 where the majority of the data points are found in the pine forest. Activity areas are reduced from 2008.
Figure 7-A: Delineated habitat use by the Pen turtles in 2009 where the majority of the data points are found in the mixed forest.
Appendix B: Study Images

Figure 8-A: Construction of the pen where ten turtles were housed for the first year of the study.

Figure 9-A: Taking swabs from a native turtle for PCR analysis.
Figure 10-A: Diagram of the venipuncture technique used to extract blood from the eastern box turtle.

Figure 11-A: Taking measurements for body condition.

Figure 12-A: Turtle 212 of the no pen group engaging in mounting behavior most often associated with mating.
Figure 13-A: Turtle 217 of the no pen group with an apparent eye infection.
Vita

Nicolas Barret Frederick was born on November 19, 1983 in Lynchburg, Virginia. He graduated from E.C. Glass High School, Lynchburg, Virginia in 2002. He received his Bachelor of Science in Biology from Virginia Polytechnic and State University, Blacksburg, Virginia in 2006, where he conducted undergraduate research with Dr. Franklin Carvajal and Dr. Robert Hoffman. He then worked with the Duke Medical Center’s Cancer and Leukemia Group B, for the following year.