Head-start Juvenile and Adult Resident Eastern Box Turtles (Terrapene carolina carolina): Winter Ecology Including Microhabitat Selection, Temperature Tolerance and Philopatry

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Head-start Juvenile and Adult Resident Eastern Box Turtles (Terrapene carolina carolina): Winter Ecology Including Microhabitat Selection, Temperature Tolerance and Philopatry

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Abstract

The Eastern box turtle (*Terrapene carolina carolina*) is one of the most common reptiles in North America, but is soon to be threatened due to increased urbanization, disease, and the pet and food trades. In order to assist resource managers in choosing suitable sites for relocation studies, more information on the winter ecology of this species is necessary. We examined the temperature tolerance, microhabitat and degree of philopatry to hibernacula for juvenile and adult E. box turtles for two consecutive winters in Charles City Co, VA. The turtles were tracked via radio telemetry to record point locations during the active and inactive seasons. iButton data loggers were attached to each turtle to record temperature throughout winter. Vegetation data were collected and analyzed to determine if there are microhabitat differences between adult and juvenile turtles, and between occupied and random plots. Philopatry to wintering hibernacula was determined by measurements in the field and using ArcGIS. Adults had significantly higher minimum temperatures than juveniles during the first winter (P=0.027), but not during the second winter (P=0.327). Shrub and canopy cover were marginally higher for random plots than for occupied turtle hibernacula plots (P=0.066 and P=0.092, respectively); however there were no significant differences for any of the vegetation variables between adults and juveniles. Some of the turtles demonstrated site fidelity of their hibernacula. The temperature data from this study suggests that juveniles may have poorer hibernacula selection and therefore lower minimum temperatures compared with adults, but this was not seen in our second season suggesting that juvenile turtles may learn to choose better hibernacula or to dig deeper. The difference in findings for the two winters could be attributed to illness by several of the turtles the first winter, by increased maturity in the juveniles the second winter or by the significant difference in ambient temperature for the two winters (P=0.0001). Juveniles do not differ from adults in microhabitat selection, therefore resource managers may not need to plan differently for juveniles and adults in relocation studies. We also conclude that both age groups of *T. c. carolina* exhibit site fidelity of hibernacula.
Introduction

Turtles are long-lived reptiles whose evolutionary successes in tolerance of heat, cold, dehydration, and hypoxia permits them to thrive in diverse environments (Costanzo et al. 2008) such as aquatic or terrestrial ecosystems with seasonal variation in temperate regions or consistent climate in tropical regions. The Eastern box turtle (Terrapene carolina carolina) is one of the most common reptiles in North America ranging in the eastern United States west from Michigan to southern Maine and south to southern Florida (Congdon et al. 1989). They inhabit moist woodlands, pastures, and marshy meadows (Nazdrowicz et al. 2008) that are exposed to varying weather severity and duration throughout their range (Congdon et al. 1989). These turtles move throughout their home range to find food, water, nest sites, and wintering hibernacula (Gibbons et al. 1990; Iglay et al. 2007) and how they occupy these habitats may vary seasonally and by age (Dodd 2001). Depending on variation in the landscape and availability of resources, T. c. carolina home ranges can vary from 1.2 to 4.7 hectares (VFWIS, 1998-2010).

Numerous turtle and other reptile species are experiencing population declines due mainly to anthropogenic causes. The primary causes of declines are destruction or fragmentation of habitat, poaching for the pet and food trades, and the spread of disease such as Ranavirus and bacterial infections. The introduction of invasive species, environmental pollution and global climate change are among other factors contributing to the loss of many reptile species (Gibbons et al., 2000). Urbanization poses a great threat to T. c. carolina because increased habitat fragmentation and wetland loss leads to turtles being more likely to encounter hazards such as roads (Gibbons et al. 2000). Because T. c. carolina are a long-lived species with delayed sexual maturity and low
fecundity (clutch size of 1 to 7 eggs per year (Dodd 2001) they are unable to recover quickly from major losses within the adult population (Dodd and Dreslik 2008). This was demonstrated in a 50 year study in Maryland where population declines were seen after extensive flooding in 1972 with recovery not occurring until 1995 (Hall et al. 1999). To survive, this species depends on high juvenile survival and low adult mortality (Budischak et al. 2006). Furthermore, because of the turtle’s intimate connection with the ground, they are at a high risk of accumulating harmful polychlorinated biphenyls (PCB’s). These PCB’s can cause endocrine disruption, sex reversal, abnormal gonads and many other potentially lethal health problems (Gibbons et al., 2000). Currently *T. c. carolina* is considered a Tier III Species of Greatest Conservation Need in Virginia’s Wildlife Action Plan and unless conservation of this species is enforced, extirpation or extinction may be inevitable.

Animal relocation is one possible solution to recover populations that is increasingly being used in wildlife management plans (Dodd and Seigel 1991). Relocation is defined as the movement of a free-ranging animal away from an area where they are threatened to an area historically or currently occupied by that animal (Hester et al. 2008) (*i.e in this experiment we will be monitoring head-start juveniles and resident adults*. Head-start is the relocation of captive bred animals). Overall success rates of 44% have been reported for relocation of birds and mammals and only a 19% success rate for relocation of reptiles and amphibians (Dodd and Seigel 1991). Since 1991, the success of reptile relocation has doubled 41% as a result of increasing efforts (Germano and Bishop 2008). However, these statistics may not be an accurate measure of actual success of relocations when considering the long life expectancy of many reptile species.
and the differences in success between species. Only when long-term studies have been conducted that support successful relocations should the movement of animals be used as a definitive conservation strategy (Dodd and Seigel 1991).

Relocation of T. c. carolina adults proves to be a difficult task since they have strong philopatry or site fidelity. When displaced, this species will attempt to return to its original location. Studies show that resident adult T. c. carolina restrict their movements to established home ranges that contain food, water, shelter resources, and potential mates, whereas relocated turtles have irregular patterns, larger home ranges and move greater distances when taken from their original locations (Hester et al. 2008). Alterations in home range size can be lethal due to greater daily energy expenditures, decreased ability to find important resources, and increased chances of hazards causing relocated turtles to experience higher mortality rates or disappearance than resident turtles (Hester et al. 2008).

Due to the strong site fidelity of this species, it has been proposed that placing the turtles in a pen for an extended period of time, with the intention that the turtles will become imprinted on the release site, may improve the success rate of relocation (Tuberville et al. 2005). Penning of relocated adult Gopher tortoise (Gopherus polyphemus) for 1-year demonstrated a significant decrease in the dispersal pattern and an increase in site fidelity (Tuberville et al. 2005). As part of Nicolas Frederick's Masters Thesis (2009), 10 of 20 head-start juvenile T. c. carolina were placed in a 736m² pen from June 2008 until June 2009. Frederick (2009) concluded that T. c. carolina is a prime candidate for the penning treatment, as his data demonstrated reduced movement patterns in the pen-treated turtles compared with no pen-treated turtles, and 87.5% site
fidelity for both treatments in the study (2009). This study is a continuation of Frederick’s work and all juvenile turtles used in his research are the juvenile turtles in this study.

For relocation to have a positive impact on survivability, appropriate microhabitat must be considered when selecting a site. It is thought that the most important factors in this species microhabitat are surface temperature, relative humidity and understory plant cover (Rossell et al. 2006). Suitable microhabitat is important because *T. c. carolina* may hibernate up to half of the year depending on location throughout its range in North America (Congdon et al. 1989). Hibernation is a critical time for this species since wintering mortality is the leading cause of non-anthropogenic influenced turtle deaths (Claussen et al. 1991). It is important to understand the wintering ecology of this species so that head-start or relocation project designs can take into account suitable winter habitat during the site selection process.

*Temperature Effects*

Temperature plays a critical role in physiological processes in turtles by affecting the metabolic rate and cardiorespiratory function, and the amount of lactic acid accumulation (Warren and Jackson 2007). To escape low temperatures *T. c. carolina* overwinter by burrowing below the leaf litter into the soil at varying depths (Claussen et al. 1991). Typically they burrow in areas with direct sunlight or under shrubs (Bernstein 2005) and may occur on south or west-facing slopes, flat terrain or in water (Dodd 2001). The turtles have the ability to dig deeper during cold temperatures and emerge during warm spells. This suggests that they do not enter a state of dormancy like many
hibernating animals. In the southern portions of their range, prolonged warming periods may cause the turtles to leave their hibernacula, putting them at risk for being exposed to sudden drops in temperature (Dodd 2001). One study recorded a 68% mortality of a population of *T. c. carolina* when they emerged from hibernation during a warming period and were exposed to freezing temperatures (Schwartz and Schwartz, 1974).

However, freezing temperatures do not necessarily kill box turtles as seen in Costanzo et al. (1995) where Ornate box turtle (*Terrapene ornata ornata*) hatchlings demonstrated a tolerance to freezing, which has also been seen in overwintering adult *T. c. carolina*. *T. c. carolina* are one of the few vertebrates known to withstand harsh freezing conditions (Costanzo and Claussen 1990). In a laboratory setting, *T. c. carolina* were placed in freezers for 3-4 days at -3.6°C and survived with 44-58% of their body water frozen (Costanzo and Claussen 1990). The freezing point of turtle tissue is -0.4°C and freezing may enhance the survival of this species by inhibiting further body cooling from the release of heat of fusion (Claussen et al. 1991). This ability to supercool can be attributed to the organism’s anoxia tolerance and antioxidant defenses to ischemia occurring during freezing periods (Storey 2006). Soil type can also affect the freeze tolerance of turtles as seen when Blanding’s turtles (*Emydoidea blandingii*) were able to withstand temperatures of -4°C for seven days and continued to cool to -14°C, but when placed in moist soils were only able to cool to -1.3°C (Storey 2006). Sub-zero temperatures are common throughout the box turtle’s range and data suggests that body freezing is neither rare nor lethal to *T. c. carolina* (Claussen et al. 1991).

To provide protection from freezing temperatures and loss of moisture the turtles must find suitable hibernacula. This is why microhabitat selection for this species is
thought to be based on thermoregulation and minimization of evaporative water loss (Rossell Jr et al. 2006). Studies show that turtles prefer hibernacula exhibiting high humidity levels coupled with variable woody debris, thick mats of leaf litter, or brush often next to fallen logs, rocks or stumps (Dolbeer 1971; Lue and Chen, 1999; Rossell Jr et al. 2006). Thermoregulation is provided by the surrounding soils the turtles burrow into, as previous studies indicate that the body temperature of turtles is similar to that of the surrounding soils since they are ectotherms (Bernstein 2005). Thermal protection provided by leaf litter and soils also assists in safely thawing the turtles’ body after freezing events (Costanzo and Claussen 1990).

Burrowing depth varies greatly between turtles where some individuals burrow deeper to escape the cold others have very shallow burrows where the top of the carapace is visible (Dodd 2001). In the colder northern regions, T. c. carolina have been known to burrow up to 20cm below the soil surface with additional leaf litter coverage to find protection from freezing temperatures (Congdon et al. 1989). In a study in Ohio, T. c. carolina hibernacula depths were recorded at 14cm with an average of 8cm of leaf litter above the burrows, typically preventing the turtle’s body temperatures from dropping below 0°C (Clauseen et al. 1991). In the southern ranges, burrow depths tend to be shallow typically less than 4cm and often flush with the soil surface (Congdon et al. 1989), but can burrow deeper as the weather becomes colder (Claussen et al. 1991). Deeper leaf litter stabilizes both temperatures and soil moisture for the hibernacula, whereas open canopy areas may expose the turtles to higher ambient temperatures (Steen et al. 2007). This may explain why turtles hibernating in open areas may emerge sooner than turtles in greater depths of leaf litter. Regardless of how deep the turtles burrow,
their temperatures are moderated by the environment and buffered against the fluctuating ambient temperature.

Previous studies indicate that turtles emerge from hibernacula based on ambient and soil temperatures. Grobman (1990) found that emergence of Three-toed box turtle (*Terrapene carolina triunguis*) and *T. o. ornata* occurred after five consecutive days of subsurface temperatures of 7°C or higher, occasionally emerging prematurely resulting in fatality. After the initial emergence from their hibernacula, the turtles will remain in the vicinity of their hibernacula, until the first warm spring rains, so they can submerge if temperatures drop (Grobman 1990). On the contrary, after analyzing 48 hours of soil temperature data, Bernstein and Black's (2005) study of *T. o. ornata* did not support that emergence is related to soil temperatures alone, rather they found that a combination of temperature and precipitation were important. None of these studies considered the condition of individual turtles and how this may affect emergence and temperature effects. Turtles have been documented to emerge from hibernacula in the middle of winter (Dolbeer 1971), perhaps to change hibernacula or due to poor health. If a turtle is in poor body condition they will not be able to efficiently thermoregulate which could cause early emergence. The inability to thermoregulate could result in lower temperatures experienced during the winter months and lead to winter mortality. Turtles in poor health also may not have enough fat stores resulting in delayed submergence into overwintering sites. Emergence during winter has been described in an anecdotal observation of Canebrake rattlesnake (*Crotalus horridus horridus*) moving above ground during the overwintering period in years that it was unable to find sufficient food prior to submergence (personal communication by John D. Kleopfer, 2011).
Vegetation and microhabitat

Surrounding vegetation plays a major role in the success of turtles. In addition to providing protection from predation and the elements, complex understory vegetation provides these omnivores with a mixture of food sources such as blackberry (*Rubus sp.*), elderberry (*Sambucus Canadensis*), low-bush blueberry (*Vaccinium vacillans*), viburnum (*Viburnum sp.*), muscadine grape (*Vitis rotundifolia*), wild strawberry (*Fragaria virgiana*) and various fungi (Mitchell 1994). Turtles are found in both deciduous and pine dominated forests with open canopies and dense understory (Mitchell 1994). In the eastern part of their range they occupy mesic woodlands that include various deciduous and pine species, soils rich in moisture, and an abundance of vegetation (Dodd 2001).

Overstory tree species provide differing types of leaf litter that may play a role in microhabitat selection, but dense understory species may provide good cover and camouflage from predators and shelter from harsh weather conditions. One study suggests *T. c. carolina* selects areas with a high density of shrub, sapling and vine species to provide heavy understory coverage (Dolbeer, 1971). However, a study conducted in a mountain wetland in North Carolina found that *T. c. carolina* selected microhabitat with moderate canopy cover and less understory cover to potentially allow greater air circulation; thus aiding the turtles in dissipating heat during periods of high temperatures (Rossell et al. 2006). This study also concluded that woody debris and leaf litter may not be of importance when selecting microhabitat since they are not directly attributed to thermoregulation (Rossell et al. 2006), but they did agree that *T. c. carolina* occupy habitat with dense, tangled vines and large amounts of woody debris (Rossell et al., 2006).
Juveniles vs. Adults

Age of *T. c. carolina* is often estimated by counting annular rings; however, recently this method has been criticized by some researchers because older turtles typically have additional wear on their carapace (Dodd 2001) and the rings may be indicative of seasonal growth rather than years of age (St. Clair 1998). A better estimate of age is based on body size using carapace length rather than weight as there is no definitive weight class for indicating age (Yahner 1974). Assessing the size is also more relevant since *T. c. carolina* sexual maturity is based on size rather than actual age. Sexual maturity was determined to be reached at ~100mm for males and from 130-150mm for females in two close relatives of *T. c. carolina* in a study in Oklahoma (St. Clair 1998). The *T. c. carolina* is considered juvenile from hatching up to 110mm carapace length, sub-adult 110-119mm, and adults greater than 120mm (Dodd 2008). However, other studies define juveniles as carapace lengths up to 75mm, sub-adults 75-125mm and adults above 125mm (Budischak et al. 2006). For this study we use the term juvenile loosely as their size is not typical for their age since they were captive bred for the first three years of life putting their size close to twice that of the expected size at age four.

Adults and juveniles also have age-specific differences with regard to microhabitat selection, which is another key element to successful relocation and site selection. Adults have shown to use a greater variety of habitats in larger areas, whereas juveniles may choose smaller, more concealed areas with better protection (Dodd 2001). According to a study on the voluntary locomotion of *T. c. carolina*, movements in this species increase with increasing body mass (Adams et al. 1989) indicating that adults
may require a larger home range to suit their needs than juveniles. In a study in Florida, Florida box turtle (*Terrapene carolina bauri*) demonstrated stronger preferences for certain locations, whereas adults tend to be more flexible in their choice of habitat during the active season (Jennings 2007). If these differences exist in the active season, it is important to determine whether there are also differences between juveniles and adults with regards to winter habitat selection.

*Philopatry*

Philopatry instinctually leads displaced aquatic and terrestrial turtles back to their originating location and is the main reason for the low success rate of relocation. Philopatry has been documented for specific behaviors in the active and non-active season. Turtle species have been known to move within their home range to densely forested areas for overwintering and areas of less vegetation during the summer in consecutive years (Lue and Chen 1999; Dodd 2001). Overwintering sites have been chosen near previously used sites that proved successful in past years (Dodd 2011). A three year study on *T. o. ornata* revealed that females selected nest sites within one meter of previously occupied sites, and one male turtle selected winter hibernacula in adjacent burrows for all three years (Bernstein et al. 2007).

During another study, *T. c. carolina* demonstrated site fidelity of its hibernacula when it overwintered in the exact location for three winters in a row (Claussen et al. 1991). In a 40 year study by Stickel (1989), *T. c. carolina* demonstrated consistency of site selection over time, where year after year the hibernacula were only a short distance apart (≤15m). This study also concluded that travel to overwintering sites from summer
sites is not random, but a definitive aspect of the turtles’ home range (Stickel 1989). In addition, a study on Yellow margined box turtle (*Cuora flavomarginata*) revealed two turtles returning to the same overwintering site within 10m (Lue and Chen 1999).

Philopatry by Common musk turtle (*Sternotherus odoratus*) was found in a Virginia lake to be exhibited by both males and females when displaced from their original location (Andres et al. 2006). More recently a thermal environment study of *T. c. carolina* in Maryland confirmed that this species returns to almost the same site to overwinter each year (Savva et al. 2010). Turtle species exhibition of returning to the same habitats and sub-habitats seasonally within their home ranges suggests that turtles are capable of orientation during travel (Lue and Chen 1999). Philopatry of habitats and sub-habitats for turtle species could lead to reduced survivability for relocated turtles or turtle populations faced with habitat fragmentation or destruction (Bernstein et al. 2007). However, turtles that have been raised in captivity and pen-treated their first year in the wild have not been assessed with regard to their fidelity to hibernacula.

**Objectives and Hypothesis**

The main objective of this study is to investigate the wintering ecology of *T. c. carolina* with hopes of providing recommendations for site selection in turtle relocation studies. Increasing knowledge of the winter biology and habitat needs of turtles could assist resource managers in developing conservation strategies, as well as allow them to predict the effects of climate change (Costanzo et al. 2008).

Specifically, we will *i)* determine if overwintering temperatures differ between the two age-classes of turtles (head-start juveniles and resident adults), *ii)* determine if
overwintering has an effect on body condition, iii) determine if there are any differences in hibernacula microhabitat between the adults and juveniles and between occupied and random sites, and iv) determine if this species exhibits site fidelity for its overwintering site and if site fidelity is the same for adults and juveniles. We hypothesize that more adults will find suitable hibernacula than juveniles, specifically shown by adults having higher minimum temperatures. We do not expect any of the adults to have temperature readings below 0°C, whereas we expect juvenile turtles to spend time in below freezing temperatures due to lack of experience from having spent fewer winters in the wild than the adult residents. We expect there to be a difference in microhabitat selection of the hibernacula sites between the juveniles and adults. Adults may have different summer foraging and overwintering preferences, whereas juveniles who have not fully developed home ranges will stay within the same habitat year round. We expect that both juveniles and adults will actively select their hibernacula such that microhabitat features at occupied sites would be different from randomly selected locations. Lastly, we also hypothesize that both juveniles and adults that find successful hibernacula will return the following year within a 15m distance based on site fidelity. This distance was chosen based on the variation of distances (1m to 25m) used in previous studies.

Methods and Materials

This study took place at the VCU Walter and Inger Rice Center, which is situated on the James River in Charles City County, Virginia. The VCU Rice Center is a 138 ha research property. Our study site is located in the eastern portion of the Rice Center property and onto the adjoining property owned by Berkley Plantation totaling 80.76 ha.
and is comprised mainly of mixed deciduous and pine forest, including a small percentage of both tidal and non-tidal wetlands (Figure 1). As part of an on-going head-start program through the Virginia Department of Game and Inland Fisheries, in 2009, 13 adult *T. c. carolina* were found on multiple occasions on the Rice center property to add to the 15 head-start juvenile box turtles in the initial sample for this study. We tracked these 28 turtles during the winter of 2009 – 2010; however, due to transmitter failure of one adult and one juvenile, the death of one adult and two juveniles, and the chance finding of one previously lost juvenile from transmitter failure during the summer of 2009, the sample size for the winter 2010 – 2011 was 24 (11 adults and 13 juveniles).

The turtles’ marginal scutes were notched using Cagle’s (1939) system to assign a unique identifier in the order they were found. Adults were given identities in the 100 series and juveniles in the 200 series. In September, each adult and juvenile turtle was fitted with 3.6g R1680 transmitters (*Advanced Telemetry Systems, Isanti MN*) and in October each turtle had an iButton temperature data logger (*Thermocron iButtons, Dallas Semiconductors, Maxim Integrated Products, Inc., Sunnyvale, CA*) attached via epoxy putty. Both transmitter and data loggers with epoxy putty did not exceed 7% of the total body weight for each individual turtle.

**Radio Telemetry**

All adult and juvenile turtles were radio tracked using an ATS R-410 Receiver and Yagi 3-prong antennae, two to four times per week from the time of spring emergence to when they entered their hibernacula the following winter. During the winter months, the turtles were tracked once per week. The turtles were tracked to their
hibernacula and the exact location was found using a White’s DFX Spectrum E series metal detector and marked with flags. Every occasion the turtles were tracked, the location was recorded using a Garmin 60CSx GPS Unit.

Health Assessment and Morphometric Data

To monitor the health of the population of turtles and to determine the effects of hibernation on body condition, we collected morphometric data, blood samples and observational data. Observational data included examination of the external body for any signs of abnormality, swelling, injury, deterioration of scales or body parts, and abnormal moving or behavior. Analysis of blood smears was conducted for the quality of the cells and evidence of infection. Blood was collected from each turtle every six months using a 21G1 needle for adults and a 27G1/2 needle for juveniles. The needle was inserted into the supravertebral vein and placed on three to four blood slides. The smears were stained with Diff Quick and covered with a clear cover glass. Each slide was then examined for the presence of parasites, the morphology of the erythrocytes and other cells, and any evidence of bacterial-related disease in the blood. For turtles exhibiting conjunctivitis, both the eye and cloaca were swabbed, cultured for bacteria, and analyzed. Morphometric data were also collected for all adult and juvenile turtles using 40 cm Haglof calipers to measure carapace length, carapace width, plastron length, plastron width and shell height. Each turtle’s mass was recorded every six months using an Ohaus CS2000 scale. Weights were recorded several times after emergence from hibernacula to calculate an average emergence weight. Weights were recorded again in the fall during transmitter replacement and iButton attachment prior to overwintering. A
mass versus carapace length regression analysis was performed and the residuals were calculated to serve as an index of body condition post hibernation for both juveniles and adults.

*Temperature data*

Using OneWireViewer 0.3.14.7 (*Maxim Integrated Products Software, Inc.*, Sunnyvale, CA), 40 iButton temperature data loggers were programmed to begin data collection 1 November at midnight and record the temperature every three hours until 1 May. In October 2009, a total of 28 of the 40 iButtons were coated twice in Plasti Dip and allowed to dry before attaching to the third marginal scute of the carapace of each turtle to improve water resistance. The remaining buttons were placed in ziplock plastic bags and nailed to the nearest tree at 12 randomly selected turtle hibernacula to record ambient air temperature. In October 2010, a total of 34 iButtons were coated twice in Plasti Dip; 24 of which were attached to turtles and the remaining 10 attached to trees at randomly selected hibernacula. Average ambient temperature from the tree-mounted iButtons was used in analysis of turtle versus ambient temperatures. A previous study indicates the temperature data loggers are a good measure of core body temperature of a burrowed turtle as they found no significant difference in data logger temperatures and body temperatures measured by drilling a hole in the plastron to collect deep body temperature (Congdon et al. 1989). We compared head-start juvenile and resident adult temperatures (mean, maximum, minimum, standard deviation, and mean difference to ambient temperature) and date of emergence using a Tukey’s HSD t-test. Adult 123 was
omitted from the winter 2009 – 2010 analysis as he emerged very early with an aural abscess and we believed this turtle was not representative of the adult population.

Vegetation Analysis

A thorough vegetation analysis was performed during June 2010 using the One-Tenth Acre Circular Plot Method (James and Shugart 1970) at each of the winter 2009 – 2010 turtle hibernacula, as well as 10 randomly chosen locations in the summer of 2010. Randomly selected plots were generated by creating a minimum convex polygon around the perimeter of the known turtle locations and using Hawth’s tool in ArcMap 9.3 to randomly select 10 locations (Figure 2). The 10 random points were then found using the Garmin 60CSx GPS Unit and flagged. Within each circular plot we identified all tree and herb species and estimated the DBH of woody stems less than 3 inches by counting the number of stems in each of the following categories: A (3-6in.), B (6-9in.), C (9-15in.), D (15-21in.), E (21-27in.), F(27-33in.) and G(33-40in.). The amount of downfall was calculated by measuring length and width of fallen trees above 3in DBH with a survey tape measure. Canopy cover was measured using ocular tube readings at the center of the plot, and understory growth was estimated visually by determining how much of the plot was covered in herbaceous species. Without disturbing the hibernating turtles, leaf litter depth and soil depth to the crown of the carapace were measured to the nearest millimeter. We compared vegetation data between hibernacula and random plots as well as between juvenile and adult hibernacula using Tukey’s HSD t-tests. After observing several turtles hibernating under *Ilex opaca* (American holly), a two-tailed t-test was performed to determine if there was a relationship between *Ilex opaca* and total burrow depth, and minimum temperature.
Philopatry and GIS

Using the location data, the distance between the 2009 - 2010 and 2010 – 2011 winters were calculated both by measuring on site with a survey tape measure and using ArcGIS 9.3 to determine if individuals returned the second winter within 15m of the first year’s hibernacula. In ArcGIS 9.3 a 15m buffer was placed around each 2009 – 2010 winter and checked for an attribute intersect from the 2010 – 2011 winter hibernacula point locations. We also calculated home range sizes by creating minimum convex polygons around each individual using 2009 – 2011 summer and winter point locations. Any individual that we did not have point location for both years was omitted from the home range analysis. We compared adult and juvenile distances to hibernacula and home range sizes using Tukey’s HSD t-tests. A distance versus minimum temperature regression analysis was also performed.

Results

Pilot Study

We conducted a pilot study beginning in October 2008, where we collected data on the first winter for ten head-start turtles that were not pen-treated or provided hibernacula as well as ten pen-treated that were given suitable hibernacula. The juvenile turtles with no penning were radio tracked to their hibernacula and monitored once a week until their emergence the following spring. Microhabitat selection was varied between the turtles from areas of dense canopy cover or down-fall with little leaf litter coverage to open areas under thick leaf litter coverage.
We collected iButton data logger information for 14 of the 20 juvenile turtles (7 not penned & 7 penned). We found no significant difference for mean, maximum, minimum or standard deviation between the non-penned and penned turtles (Table 1 and Figure 3). The lowest recorded temperature for the non-penned turtles was -2.5°C and -2°C for the penned turtles (Figure 4).

**Health Assessment and Morphometric Data**

Two juvenile turtles presented with apparent health concerns. Juvenile turtle 217 suffered from conjunctivitis caused by an unknown type of infection. The cloaca swab from this turtle revealed the presence of *Bacillus sp.*, but was not confirmed if this was the causal agent. Turtle 217 suffered for 11 months with the eye infection until he was found deceased on 2 June 2010. The necropsy was inconclusive due to excessive deterioration of the body. Juvenile turtle 206 and adult turtle 123 presented with an aural abscess post winter emergence in 2010. The cloaca swab revealed the presence of *Alcaligenes faecalis*, bacteria commonly found in the soil and water, typically in areas of human activity (Otte et al., 1996). The pharyngeal swab indicated the presence of both *Bacillus sp.* and *Micrococcus sp.*; however, none of these species could be designated as the aural abscess causing agent. Juvenile turtle 205 presented with conjunctivitis in November 2009 and again in December 2010; this turtle was found deceased outside of wintering hibernacula on January 22, 2011. The necropsy for this turtle attributed the death to winter kill, or freezing. Only one turtle emerged with a health concern in the spring 2011. Adult turtle 150 emerged with conjunctivitis. No parasites, viruses or
bacterial-related infections were detected in the analyzed blood smears for any of the remaining adult and juvenile turtles.

We found seven turtles (201, 217, 215, 216, 136, 150, and 151) to be in poor body condition post hibernation after the winter 2009 – 2010 based on our size adjusted mass body condition index, with juvenile turtle 217 determined to be in the worst condition (Figure 4). For the winter 2010 – 2011 we found three turtles to be in slightly poor condition (213, 136, and 150) post hibernation, but much less so than the poor condition turtles in 2009 – 2010 winter (Figure 5).

Temperature Data

Adults had a significantly higher mean minimum temperature (N=13, $\bar{x} =0.96^\circ$C, SE=0.31) than juveniles (N=14, $\bar{x} =0.14^\circ$C, SE=0.28; t=-1.96, P=0.027) during the 2009 – 2010 winter period (Figure 6), however no significant differences were found for the remaining response variables (Table 2). During the 2010 – 2011 winter period we found no significant differences in the response variables between the adult and juvenile turtles for winter 2010 – 2011 (Table 3). A minimum temperature of below 0°C was reached by five juveniles and one adult during the winter 2009 – 2010 (Figure 5) and by three juveniles and two adults during the winter 2010 – 2011 (Figure 6).

Adults were found to have a significantly later Julian date of emergence (N=13, $\bar{x} =96.77$, SE=2.25) than juveniles (N=14, $\bar{x} =87.79$, SE=2.17; t=-2.88, P=0.0043) for the winter 2009-2010 (Table 2). Adult turtles, with the exception of two outliers, emerged from hibernacula between 3 April and 6 April 2010; whereas juveniles emerged between 18 March and 8 April 2010 (Figure 9). No significant difference was found for the Julian
date of emergence in the winter 2010 – 2011 (Table 3). A juvenile and an adult emerged on 19 March, nearly a month prior to the bulk of the individual’s emergence between 16 April and 24 April 2011 (Figure 10). The emergence dates for the second winter were very sporadic with no apparent pattern.

Vegetation Analysis

The data for herbaceous cover and percent downfall was not normally distributed and thus transformed prior to analysis. When comparing vegetation in unoccupied random plots and occupied turtle hibernacula, we found no significant difference for the ratio of pine to deciduous trees, percent herbaceous cover, or downfall cover (Table 3). Unoccupied random plots (N=10, $\bar{x}$ =65.5, SE=8.1) had a slightly higher percentage of shrub cover than occupied turtle hibernacula (N=28, $\bar{x}$ =50.93, SE=4.93, t=-1.53, P=0.06) (Table 3). The unoccupied random plots (N=10, $\bar{x}$ =79.0, SE=7.66) also had a slightly higher percentage of canopy cover than the occupied turtle hibernacula (N=28, $\bar{x}$ =66.85, SE=4.66, t=-1.35, P=0.09) (Table 3).

When comparing vegetation in adult and juvenile hibernacula we found no significant difference for the ratio of pine to deciduous trees, percent herbaceous cover, shrub cover, or canopy cover (Table 4). Adult turtles (N=13, $\bar{x}$ =2.79, SE=0.65) had slightly more downfall around their hibernacula than juveniles (N=15, $\bar{x}$ =1.59, SE, 0.56, t=-1.38, P=0.08) (Table 4). Adult turtles (N=13=, $\bar{x}$ =69.17, SE=9.59) burrowed significantly deeper than juvenile turtles (N=15, $\bar{x}$ =46.36, SE=8.88, t=-1.74, P=0.053) in winter 2009 – 2010, however there was no significant difference in burrow depths in winter 2010 - 2011 (Table 4).
Several of the turtles (9 of 28 total in 2009 - 2010 winter and 6 of 25 in 2010 - 2011 winter) chose hibernacula under *Ilex opaca*. Statistical analysis revealed no correlation between *Ilex opaca* and burrow depth for either year (Table 5). However, a marginally significant relationship was found between *Ilex opaca* and minimum temperature for the first winter (N=9, \( \bar{x} = -0.11 \), SE=0.37, t=-1.83, P=0.07), but no relationship was found the second winter (N=6, \( \bar{x} = 0.61 \), SE=0.82, t=0.27, P=0.78) (Table 5).

**Philopatry and GIS**

In 2010 – 2011, three adult turtles and three juvenile turtles hibernated within 15m of their 2009 – 2010 hibernacula according to field calculations. ArcGIS determined three adults and four juveniles hibernated within 15m (Figure 11). All other turtles (75% of total) had hibernacula that were > 15m in consecutive years. No relationship was found between adult and juvenile distance to hibernacula (adult N=11, \( \bar{x} = 54.25 \), SE=18.85; juvenile N=13, \( \bar{x} = 69.83 \), SE=19.08, t=0.58, P=0.28). Minimum temperature did not have an effect on the distance to hibernacula (N=23, \( R^2=0.000456 \), P=0.92). Adults had significantly larger home ranges than juveniles (adult N=11, \( \bar{x} = 5.12 \), SE=0.83; juvenile N=13, \( \bar{x} = 2.94 \), SE=0.76, t=-1.83, P=0.04).

**Discussion**

**Temperature effects**

Our temperature data from the 2009 – 2010 winter supported the hypothesis that more adults than juveniles would find suitable hibernacula because adults had
significantly higher minimum temperatures than juveniles (P=0.0027). However, our hypothesis was not supported for the second winter as no significant differences were found between adults and juveniles. We suspect that the second winter showed no difference for three potential reasons: maturation and learning of juveniles, several turtles were in poor health condition prior to and after emergence, and the ambient temperature for the second winter was significantly higher than ambient temperature the first winter (P=0.0001). Winter 2009 – 2010 was the first winter without man-made hibernacula for the eight pen-treated and only the second winter for the seven non-pen-treated juveniles. More of the penned turtles experience temperatures at or below 0°C (5 of 8) than did the non-penned turtles (3 of 6) during the first winter. The same trend was seen the second year when the only three juveniles to experience temperatures below 0°C were all pen-treated turtles on their second year without man-made hibernacula. The reduced number of pen and non-pen treated turtles experiencing below freezing temperatures in their second year suggests that they may indeed be gaining experience and learning what constitutes a suitable hibernacula. Aside from maturity, the no-pen-treated juveniles have a significantly higher body mass (P=0.0428) than the pen-treated turtles which could be an indicator that smaller size turtles may not have the mass necessary to buffer against freezing temperatures.

Poor health could also be the reason for the turtles to not successfully find suitable hibernacula and experience freezing temperatures. Among the juveniles to fall below 0°C during the first winter were the three juveniles that presented with either an eye infection or aural abscess (two of which died over the course of the study). The only adult that experienced below freezing temperatures also presented with an aural abscess.
However, during the second winter, none of the turtles with freezing temperatures emerged exhibiting signs of sickness or poor body condition to date, with the exception of the one juvenile (205) that died in January 2011 due to not finding a hibernaculum. Similar results to our second winter were found in Maryland when hibernacula temperatures varied by only 1% between *T. c. carolina* juveniles, sub-adults and adults (Savva et al. 2010). In a post hoc analysis we compared the calculated residuals for body condition in both years with the minimum temperature, standard deviation and the burrow depth to determine if body condition had an effect on these variables. We found that body condition did not have an effect on the temperature variables or burrow depth (Table 6). This most likely is due to the few numbers of individuals in poor body condition within our sample size.

The significant difference in ambient temperature for the two winters could be the best explanation for no differences being found the second winter. With milder temperatures than the first winter, fewer turtles reached low minimum temperatures as seen during the first winter. In order to determine if there is a difference between the two age-groups, a long term study should be conducted to account for variation in ambient temperature and to determine if our results were due simply to several sick turtles in our sample.

After analyzing the results for temperature, an additional analysis determined that as burrow depth increased, minimum temperature significantly increased (*P*=0.0012). The first winter adults burrowed significantly deeper than juveniles (*P*=0.046) but not the second winter (*P*=0.486). The turtles burrowing at significantly greater depths the second winter could explain why no differences were found between the two groups and
why the second winter had a significantly higher mean minimum temperature than the first winter (P=0.0267).

The Julian date of emergence for winter 2009–2010 revealed juveniles emerging significantly earlier than adults. During the spring of 2010, two juveniles emerged on 18 March followed by two more juveniles on 20 March. Of these four early emerging turtles, three of them were turtles presenting with an eye infection or aural abscess further pointing to poor health being the causal agent of early emergence and non-ideal overwintering experience as three of the four reached minimum temperatures below °C. One juvenile did not emerge until 1 May; however, this turtle was not included in any of the temperature data analysis for winter 2009–2010 due to loss of the iButton data. All but two adults emerged within three days of each other suggesting a specific variable may be influencing emergence. However, emergence for winter 2010–2011 revealed no significant differences in the Julian date of emergence for adults and juveniles. Adult 151 was removed from the temperature analysis due to failure of the iButton, in turn not allowing us to determine its emergence date. Both a juvenile and an adult emerged nearly one month before the majority of the other turtles on 19 March 2011. Juveniles and adults emerged sporadically from 4 April to 3 May. This change in results from the first winter suggests that the factors leading to emergence from hibernacula are likely varied and complex. While age may play a role in this, there are likely several interacting factors such as temperature, precipitation, and the health of an individual.

Grobman’s (1990) study of *T. t. carolina* and *T. o. ornata* resulted in emergence after five consecutive days of 7°C temperatures. However, during the first winter of our study the majority of the turtles emerged after five or more consecutive days of
temperatures above 10˚C. This is similar to what was seen in South Carolina where *T. c. carolina* emerged when ambient temperature was closer to 12˚C (Congdon et al. 1989). This trend was not apparent the second winter studied as temperatures were well above 10˚C for 1-2 weeks before the emergence of most turtles. A warm spell in the middle of March led to the early emergence of two turtles, both of which had developed an aural abscess after the first winter; however both of these turtles cleared their infections prior to winter 2010 – 2011. During the warm spell, temperatures were close to 10˚C for the six days prior to their emergence on 19 March. After this warm spell temperatures dropped below 10˚C until early April. It is assumed the fluctuation in temperatures was a cue for the turtles to emerge later in April. It is also possible that significant precipitation is needed before the turtles will emerge as other studies have demonstrated rainfall increasing the activity level in turtles and being a driver in spring emergence since precipitation is a key element in box turtle ecology (Dolbeer 1970; Grobman 1990). Further study on precipitation effects and humidity levels could prove to have more of an influence on emergence than temperature.

In the middle of the winter hibernation period for both winters, three turtles (2 adults and 1 juvenile) moved to new hibernacula. Activity during the winter is not uncommon according to Dolbeer (1970) who reported several instances of winter movements. Short distance movements were also seen in *C. flavomarginata* in Northern Taiwan when the turtles moved from one overwintering site to another (Lue and Chen 1999). This could be due to poor health, a hibernation site lacking in moisture, or not enough protection from the elements.
One of the adult turtles that moved during the first winter was found in hibernacula submerged under 9.3 cm of water. A juvenile during the pilot study winter 2008 – 2009 was also consistently submerged under several centimeters of water for the duration of the hibernation period. The survival of these turtles can be explained by oxygen uptake through the integument (Jackson et al. 2004) coupled with lowered metabolism. Jackson et al. (2004) demonstrated that in a submerged Chrysemys picta bellii (Western painted turtle) cloacal exchange did not significantly affect oxygen uptake; however, oxygen uptake was greatly reduced when integument diffusion was prohibited. Jackson et al. (2004) also showed the turtles developing lactic acidosis at a slower rate when paralyzed, which could be the cause of a turtle in our study having decreased metabolism. Highly vascularized skin in the hind limb of the Elseya latisternum (Serrated snapping turtle) also supports integument uptake of oxygen (King and Heatwole 1994). It is not likely that cloacal respirations are the attributing factor as in aquatic turtles, since the cloacal bladder and cloacal bursae that have respiratory functions are vestigial or absent in most terrestrial species including box turtles (Dodd 2001; Peterson and Greenshields 2001).

Vegetation and microhabitat

Our vegetation results do not strongly support our hypothesis that the turtles will select microhabitat for hibernation rather than randomly burrowing; however, we did find a couple of potentially important factors that may have been more significant had we had a larger sample size. Unoccupied random plots had a marginally higher percentage of shrub and canopy cover than occupied turtle hibernacula plots. This suggests that our
turtles may be selecting areas with less overstory to allow for additional sunlight to the forest floor where they are hibernating. Additional sunlight could assist in thermoregulation, especially for turtles burrowed at shallow depths. Turtles using hibernacula with less overstory is consistent with Mitchell’s (1994) description of *T. c. carolina* preferring areas with open canopies and dense understory. The fact that occupied sites tended to be areas with less shrub and canopy cover suggests that this species selects microhabitat that is suitable for hibernation. Our ability to find differences between hibernacula and random plots may have been affected by our small sample size; only ten random plots were used and eight of these were in the pine forest.

Our vegetation results also fail to support our hypothesis that a difference would be found in microhabitat selection between adults and juveniles. These results suggest that habitat selection may be instinctual and very little learning takes place such that juveniles have the same preferences for microhabitat as adults. The adults did have a marginally higher percentage of downfall around their hibernacula than juveniles (*P*=0.08). Adults have a larger body mass than juveniles which could give them the ability to dig below large downed trees. Our results are consistent with Savva et al. (2010), who also found no pattern in microhabitat selection for overwintering sites between juveniles, sub-adults, or males and females.

After noticing the apparent preference for hibernacula under the evergreen *Ilex opaca*, we were curious to see if hibernacula under this species might differ in burrow depth and minimum temperature from hibernacula not found under this species. We found no significant influence from *Ilex opaca* on burrow depth; however, individuals with hibernacula under *Ilex* had slightly lower minimum temperatures (*P*=0.07). It is
likely that snow cover acts as an insulator for turtles buried into the soil, and there was little to no snow cover under this evergreen as it acts as a shield from snow. It is also possible that the shade provided by this evergreen causes a decrease in the temperature.

**Philopatry**

We failed to support our hypothesis that turtles finding successful hibernacula would return to that site the following year within 15m. Even though the distance to hibernacula was not influenced by minimum temperature or by age, we did find six out of 24 (3 adults and 3 juveniles) hibernating within 15m of the previous winter’s hibernacula site. A juvenile turtle, 214, returned within 15m of its hibernacula for three straight winters (12.8 and 11.7m respectively). The intersecting buffer created in ArcGIS revealed seven turtles (3 adults and 4 juveniles), including two juveniles that were not physically measured in the field to be within 15m. The difference in physical and GIS calculations is most likely due to inaccuracies in the GPS coordinates recorded. We experienced loss of satellite signal on occasion throughout this study due to the dense canopy cover. Regardless of the discrepancies between the physical calculations and the ArcGIS calculations, we found that some turtles will demonstrate site fidelity of their hibernacula by returning in close proximity to their previous overwintering site fidelity.

The demonstration of site fidelity for overwintering sites is consistent with other studies that have found box turtles returning to the same location year after year (Claussen et al. 1991; Lue and Chen 1999; Bernstein et al. 2007; Savva et al. 2010). Of the six turtles returning to the previous year’s hibernacula, two (adult 123 and juvenile 210) had minimum temperatures below 0°C indicating that the first winter site was potentially not suitable; however both turtles returned within 6.5m of the first winter site
but only one (juvenile 210) recorded temperatures below 0°C for the second winter. Because the turtles were not in the exact location both winters, it is still possible to find suitable hibernacula within the same vicinity of non-suited hibernacula and to alter the depth at which they burrow. Adult 123 had a burrow depth almost 100 mm deeper than the previous winter, whereas juvenile 210 burrowed to a shallower depth most likely being the reason for experiencing a second winter with below freezing temperatures.

The adults in our study had significantly larger home ranges than juveniles (P=0.04). A larger home range exposes the turtles to a greater amount of resources to increase fat stores. Larger home ranges also expand the number of possible overwintering sites, whereas smaller home ranges may be limited. In a post hoc discussion of philopatry, we analyzed the effect of home range size and age on the distance to hibernacula for both winters. We found that 60% of the variation of the distance between hibernacula could be explained by age and home range size (Table 7 and Figure 12). The larger the home range size the greater the distance to previous hibernacula (P=0.0001). When age was included in the analysis with home range size we found that juveniles had greater distances between hibernacula (P=0.0097), even though they had significantly smaller home ranges than adults. Individuals with small home ranges have a greater chance of hibernating in a close proximity to previous hibernacula. Individuals with large home ranges that hibernate in close proximity to previous hibernacula can more definitively be deemed site fidelity. When studying site fidelity of hibernacula, the home range size must be taken into consideration before concluding that this species exhibits site fidelity.
Conclusions

Although our hypotheses were not consistently supported by the data collected for the two winters, our conclusions can still assist resource managers in developing comprehensive management plans for *T. c. carolina*. From this study we can conclude that juveniles and adults do not vary significantly in their microhabitat selection of overwintering sites and can hence be treated with the same management strategy when selecting relocation sites. We can conclude that since all but two of the head-start juveniles were successful throughout the duration of this study, that these captive-bred individuals still have the instinctual capability to find the necessary resources for survival. This species has the ability to withstand below freezing temperatures up to a certain threshold as demonstrated by only losing one individual to winter mortality from extensive freezing temperatures, whereas other turtles survived up to -2.5°C temperatures. Long-term data could show definitive trends in emergence dates and if early emergence is often due to poor body condition. Additional studies could determine the cue for emergence, whether it be temperature or precipitation driven. This species may exhibit site fidelity of its overwintering site, however it is not in direct relation to the success of the site and researches should take into account the home range size prior to analyzing the site fidelity.

Further information is needed to determine if the turtles are selecting their hibernacula or if they randomly burrow when temperatures decline. GPS transmitters could assist in tracking their movements during the fall to better analyze the movement patterns just prior to hibernation. Finally, resource managers should consider the
differences is home range sizes when selecting relocation study sites to accommodate for the juveniles growing range as it reaches maturity.

**Vita**

In August 2008, Amberly Moon received her Bachelor of Science, Major in Biology and Minor in Chemistry from Virginia Commonwealth University. During her undergraduate degree she completed an independent study under graduate Nicholas Frederick on his research with the juvenile Eastern box turtles used in this study. While maintaining employment at the Department of Conservation and Recreation’s Division of Natural Heritage as an environmental review assistant, she entered the Master’s program at Virginia Commonwealth University in January 2009.
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Table 1. Mean values of response variables for pilot study in winter 2008-2009 temperature data analysis including mean, maximum, minimum and standard deviation for 14 juvenile E. box turtles. Pen turtles were given man-made hibernacula, whereas no pen treatment turtles found their own hibernacula.

<table>
<thead>
<tr>
<th></th>
<th>No Pen (SE)</th>
<th>Pen (SE)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.96 (0.23)</td>
<td>6.37 (0.23)</td>
<td>1.26</td>
<td>0.115</td>
</tr>
<tr>
<td>Max</td>
<td>14.92 (2.11)</td>
<td>19.00 (2.11)</td>
<td>1.36</td>
<td>0.099</td>
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<tr>
<td>Minimum</td>
<td>0.21 (0.65)</td>
<td>0.86 (0.65)</td>
<td>0.7</td>
<td>0.248</td>
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<tr>
<td>Std. Deviation</td>
<td>2.67 (0.21)</td>
<td>2.61 (0.21)</td>
<td>-0.2</td>
<td>0.576</td>
</tr>
</tbody>
</table>

Table 2. Mean values of response variables for both winter’s temperature data for 13 adult and 14 juvenile E. box turtles at the VCU Rice Center, Charles City Co, VA USA.

<table>
<thead>
<tr>
<th></th>
<th>Adult (SE)</th>
<th>Juv. (SE)</th>
<th>t</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>2009 -</td>
<td>Mean</td>
<td>4.97 (0.16)</td>
<td>4.92 (0.15)</td>
<td>-0.23</td>
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<tr>
<td>2010 Winter</td>
<td>Max</td>
<td>14.23 (2.51)</td>
<td>17.11 (6.90)</td>
<td>1.42</td>
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<tr>
<td>Winter Minimum</td>
<td>0.96 (0.31)</td>
<td>0.14 (0.28)</td>
<td>-1.96</td>
<td>0.027*</td>
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<tr>
<td>Std. Deviation</td>
<td>2.68 (0.17)</td>
<td>2.96 (0.16)</td>
<td>1.2</td>
<td>0.880</td>
</tr>
<tr>
<td>Mean diff. to ambient</td>
<td>2.14 (0.16)</td>
<td>2.09 (0.15)</td>
<td>-0.22</td>
<td>0.414</td>
</tr>
<tr>
<td>Julian Emergence Date</td>
<td>97 (2.25)</td>
<td>88 (2.17)</td>
<td>-2.88</td>
<td>0.004*</td>
</tr>
<tr>
<td>2010 -</td>
<td>Mean</td>
<td>5.75 (0.35)</td>
<td>6.01 (0.3)</td>
<td>0.55</td>
</tr>
<tr>
<td>2011 Winter</td>
<td>Max</td>
<td>16.65 (1.42)</td>
<td>15.7 (1.2)</td>
<td>-0.5</td>
</tr>
<tr>
<td>Winter Minimum</td>
<td>1.1 (1.13)</td>
<td>0.43 (0.96)</td>
<td>-0.45</td>
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<tr>
<td>Std. Deviation</td>
<td>2.77 (0.23)</td>
<td>2.80 (0.19)</td>
<td>0.09</td>
<td>0.536</td>
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<td>Mean diff. to ambient</td>
<td>1.43 (0.42)</td>
<td>1.59 (0.36)</td>
<td>0.3</td>
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<td>Julian Emergence Date</td>
<td>466.7 (3.81)</td>
<td>469.62 (3.35)</td>
<td>0.57</td>
<td>0.714</td>
</tr>
</tbody>
</table>
**Table 3.** Vegetation analysis response variables between occupied adult and juvenile hibernacula and random unoccupied potential hibernacula including the ratio of pine to deciduous, transformed herbaceous and downfall cover (%) and shrub and canopy cover (%) at the VCU Rice Center, Charles City Co, VA USA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Occup. (SE)</th>
<th>Unocc. (SE)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio Pine/Deciduous</td>
<td>0.12 (0.02)</td>
<td>0.12 (0.03)</td>
<td>-0.28</td>
<td>0.389</td>
</tr>
<tr>
<td>Herbaceous Cover</td>
<td>4.21 (0.51)</td>
<td>4.10 (0.83)</td>
<td>0.12</td>
<td>0.545</td>
</tr>
<tr>
<td>Shrub Cover</td>
<td>50.93 (4.93)</td>
<td>65.5 (8.1)</td>
<td>-1.54</td>
<td>0.066</td>
</tr>
<tr>
<td>Canopy Cover</td>
<td>66.85 (4.66)</td>
<td>79.0 (7.66)</td>
<td>-1.35</td>
<td>0.092</td>
</tr>
<tr>
<td>Downfall</td>
<td>2.10 (0.39)</td>
<td>1.66 (0.63)</td>
<td>0.59</td>
<td>0.719</td>
</tr>
</tbody>
</table>

**Table 4.** Vegetation analysis of adult and juvenile hibernacula including the ratio of pine to deciduous trees, transformed herbaceous cover and downfall (%), shrub and canopy cover (%) and the total soil and leaf litter burrow depth (mm) for winter 2009-2010 and 2010-2011 at the VCU Rice Center, Charles City Co, VA USA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adult (SE)</th>
<th>Juv. (SE)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio Pine/Deciduous</td>
<td>0.12 (0.03)</td>
<td>0.11 (0.03)</td>
<td>-0.12</td>
<td>0.454</td>
</tr>
<tr>
<td>Herbaceous Cover</td>
<td>4.61 (0.76)</td>
<td>3.89 (0.68)</td>
<td>-0.69</td>
<td>0.249</td>
</tr>
<tr>
<td>Shrub Cover</td>
<td>50.00 (7.64)</td>
<td>51.67 (6.84)</td>
<td>0.16</td>
<td>0.564</td>
</tr>
<tr>
<td>Canopy Cover</td>
<td>72.08 (6.91)</td>
<td>62.67 (6.18)</td>
<td>-1.02</td>
<td>0.159</td>
</tr>
<tr>
<td>Downfall</td>
<td>2.79 (0.65)</td>
<td>1.60 (0.56)</td>
<td>-1.39</td>
<td>0.088</td>
</tr>
<tr>
<td>Burrow Depth 09-10</td>
<td>69.17 (9.59)</td>
<td>46.36 (8.88)</td>
<td>-1.74</td>
<td>0.046*</td>
</tr>
<tr>
<td>Burrow Depth 10-11</td>
<td>101.05 (19.34)</td>
<td>100.15 (16.96)</td>
<td>-0.03</td>
<td>0.486</td>
</tr>
</tbody>
</table>

**Table 5.** Presence/absence of *Ilex opaca* effect on burrow depth (mm) and minimum temperature (°C) for both adult and juvenile E. box turtles for both 2009 – 2010 (Y1) and 2010 – 2011 (Y2) winters studied.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ilex (SE)</th>
<th>No Ilex (SE)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burrow depth (mm) Y1</td>
<td>56.89 (11.44)</td>
<td>58.5 (8.09)</td>
<td>-0.12</td>
<td>0.909</td>
</tr>
<tr>
<td>Burrow depth (mm) Y2</td>
<td>98.5 (28.32)</td>
<td>95.79 (14.53)</td>
<td>0.09</td>
<td>0.766</td>
</tr>
<tr>
<td>Min Temp Y1</td>
<td>-0.11 (0.37)</td>
<td>0.72 (0.26)</td>
<td>-1.83</td>
<td>0.078</td>
</tr>
<tr>
<td>Min Temp Y2</td>
<td>0.61 (0.82)</td>
<td>1.1 (1.6)</td>
<td>0.27</td>
<td>0.784</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009-2010 Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>-0.004</td>
<td>0.008</td>
<td>-0.5</td>
<td>0.621</td>
</tr>
<tr>
<td>Std. deviation</td>
<td>-0.001</td>
<td>0.004</td>
<td>-0.18</td>
<td>0.857</td>
</tr>
<tr>
<td>Burrow depth</td>
<td>0.031</td>
<td>0.236</td>
<td>0.13</td>
<td>0.895</td>
</tr>
<tr>
<td>2010-2011 Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>-0.003</td>
<td>0.018</td>
<td>-0.15</td>
<td>0.882</td>
</tr>
<tr>
<td>Std. deviation</td>
<td>-0.003</td>
<td>0.006</td>
<td>-0.59</td>
<td>0.563</td>
</tr>
<tr>
<td>Burrow depth</td>
<td>-0.006</td>
<td>0.013</td>
<td>-0.46</td>
<td>0.647</td>
</tr>
</tbody>
</table>

Table 7. Effect of age (adult and juvenile E. box turtles) and home range size (ha) on the distance between hibernacula for the 2009 – 2010 and 2010 – 2011 winters.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>P</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-27.43</td>
<td>9.59</td>
<td>-2.86</td>
<td>0.0097*</td>
<td>0.59</td>
</tr>
<tr>
<td>Home range</td>
<td>17.58</td>
<td>3.29</td>
<td>5.33</td>
<td>0.0001*</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. VCU Walter and Inger Rice Center study property boundary with study site including adjacent Berkley Plantation property.
Figure 2. Aerial image of the vegetation analysis location plots conducted in the summer 2010. A minimum convex polygon was created around the adult (100 series-squares) and juvenile (200 series-circles) in ArcGIS and using Hawth’s Tool the random vegetation plots (triangles) were generated. Note that all but three random plots are within the pine forest.
Figure 3. Pilot study results for minimum recorded temperature ranges for both pen and no pen treated Eastern Box Turtles for the winter 2008 – 2009 at the VCU Rice Center, Charles City, VA USA.
Figure 4. Minimum iButton data logger recorded temperatures for penned (201-210) and non-penned (211-219) Eastern box turtles for winter 2008-2009 at VCU Rice Center, Charles City, VA USA. Turtle 209 and 219 reached a minimum temperature of 0°C.
Figure 5. Body condition index using fit of least squares of carapace length (mm) and weight (g) for juvenile and adult E. box turtles post hibernation winter 2009 - 2010. Seven turtles (201, 215, 216, 217, 136, 150, and 151) were identified as having slightly poor body condition. Adults range from >120mm and 300g. Juveniles range from < 90mm and >100g.
Figure 6. Body condition index using fit of least squares of carapace length (mm) and weight (g) for juvenile and adult E. box turtles post hibernation winter 2010 - 2011. Three turtles (213, 136, and 150) were identified as having poor body condition. Adults range from >120mm and 300g. Juveniles range from <90mm and >100g.
Figure 7. Adult (100 series) and juvenile (200 series) turtle minimum temperature (°C) during the winter 2009-2010 for the critical period of 20 December 2009 until 31 March 2010. Turtles 135, 151, 201, 204 and 215 reached minimum temperatures of 0°C. Adult 123 was removed for the analysis as it biologically was not representative of the adult population due to illness.

Figure 8. Adult (100 series) and juvenile (200 series) turtle minimum temperature (°C) during the winter 2010 – 2011 for the critical period of 20 December 2010 until 31 March, 2011. Juvenile 205 was statistically an outlier as it reached extraordinarily low temperatures and perished.
Figure 9. Adult and Juvenile minimum temperature ranges (°C) for the 2009 – 2010 and 2010 – 2011 winters. Adults had significantly higher mean minimum temperature than juveniles (P=0.027) in the 2009 – 2010 winter.
Figure 10. Julian date of emergence range for adult and juvenile Eastern box turtles during the months of March, April and May 2010 and 2011. Adults emerged significantly later than juveniles (P=0.004) in the spring of 2010.
Figure 11. Aerial image of 2009-2010 and 2010-2011 hibernacula locations for the adult (100 series-squares) and juvenile (200-circles) series at the VCU Rice Center. Red circles are placed around the 7 turtle’s hibernacula (123, 126, 135, 201, 207, 210 and 215) that were within 15m for both winter 2009 – 2010 and winter 2010 – 2011.
Figure 12. Multiple regression analysis of home range size and age (adult and juveniles) effect on the distance between hibernacula for 2009 – 2010 and 2010 – 2011 winters ($R^2=0.59$).