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Hibernacula Site Selection of the Eastern Box Turtle, Terrapene c. carolina, in a West Virginia Population

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HIBERNACULA SITE SELECTION OF THE EASTERN BOX TURTLE, *TERRAPENE C. CAROLINA*, IN A WEST VIRGINIA POPULATION

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Approved by
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APPROVAL OF THESIS

We, the faculty supervising the work of Benjamin Owen Koester, affirm the thesis, *Hibernacula Site Selection of the Eastern Box Turtle, Terrapene c. Carolina, in a West Virginia Population,* meets the high academic standards for original scholarship and creative work established by the Biological Sciences and the College of Sciences. This work also conforms to the editorial standards of our discipline and Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

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ABSTRACT

Eastern Box Turtles (*Terrapene c. carolina*) are a terrestrially hibernating reptile found throughout the eastern United States. Despite their prevalence, little, outside of anecdotal observations, is known about their hibernacula selection. This study examines if they preferentially select hibernacula locations, and if so, what characteristics they select in a West Virginia population. Over the course of two years, radio-tagged turtles (n=12) were followed into hibernation in Wayne County, WV. Upon entering hibernation, ~36 data point were collected in a grid-like fashion around the hibernacula, with an additional point collected at the hibernacula. At each point, seven variables were recorded: soil temperature, soil compaction, soil moisture, soil pH, cover depth, cover moisture, and cover type. Results were analyzed using either categorical logistic regression for quantitative data or Ivlev's (E) and Vanderploeg and Scavia's (E*) electivity indices for categorical data. The conditional logistical regression showed a significant selection for both soil compaction (p=0.029) and cover depth (p=0.007). The two electivity indices showed a strong selection for mixed deciduous leaf litter as a cover type (E= 0.1264, E*= 0.4486). Thus, Eastern Box Turtles significantly select hibernacula sites with soft, friable soil, where they may dig easier, along with a thick cover of deciduous leaf litter, which provides increased insulation during the winter months. These results correspond with recorded anecdotal observations, thus suggesting this study may have validity throughout much of *Terrapene c. carolina's* range, and provide an avenue for further study of their winter ecology, which is necessary for their continuing protection.

BACKGROUND

Herpetofauna Winter Ecology

Winter ecology, the study of how living organisms acclimate to cold winter conditions, is a necessary area of study throughout the temperate and arctic regions of the world. In temperate regions, such as West Virginia, plant and animal species may be exposed to what could be considered winter conditions for up to six months a year. These organisms must adapt to changing conditions in order to meet the needs brought about by cold, food shortage, decreased solar radiation, and snow. Fortunately, animals throughout these regions have developed novel response to the reality of winter. While there are many variations in these responses, they can be generalized into three categories: migration, hibernation, or acclimatization (Halfpenny and Ozanne, 1989).

While many avian and a few mammalian species are capable of migration, the vast majority of terrestrial species are limited in the distance they can travel. For these species, hibernation or acclimatization are the only viable options. The choice becomes even more limited when dealing with ectothermic species. While endotherms have the capability of increasing bodily insulation to allow them to retain their warmth, ectotherms have no such capability, being regulated by external temperatures. Thus, hibernation (the significant lowering of metabolic rates in order to allow survival in unfavorable conditions on fat reserves alone) becomes the primary overwintering strategy in herpetological and other ectothermic species (Marchand, 2013).

Reptiles and amphibians make up a large, but often overlooked, portion of fauna in temperate region ecosystems. Unfortunately, winter studies relating to herpetofauna are scarce. This lack of winter studies of reptiles and amphibians may have many reasons, including the secretive nature of these species as well as a widespread belief that ectotherms respond to cold shifts exclusively by torpor and eventual hibernation (Adams *et al*., 1989). While this belief holds true in a wide variety of reptiles and amphibians, there are exceptions (Neill, 1948). Even species that hibernate often have as complicated behaviors during hibernation as seen during the rest of the year.

This scarcity of information leaves many questions pertaining to the winter ecology of herpetological species located in West Virginia, which provides ample opportunity for studies of winter behavior exhibited by these species.

Eastern Box Turtle Winter Ecology

Eastern Box Turtles (*Terrapene c. carolina*) are terrestrially hibernating reptiles who dig shallow hibernacula, often not even covering their carapace. They may be active late in the year and have been known to change hibernacula location during the winter. They will typically overwinter by burrowing into woodland soil beneath a cover of litter (Figure 1). Eastern Box Turtles are among the better studied reptiles in terms of winter survival. However, many studies conducted on them have primarily focused on internal physiological responses to cold rather than field studies looking at winter behavior and environmental impacts (Costanzo *et al*., 1993; Costanzo and Claussen, 1990; Gatten, 1987; Packard and Packard, 2001; Storey *et al*., 1993).

The reasons for this dearth of field studies seem to be, as stated above, the general assumptions about ectothermic overwintering strategies. However, field studies of Eastern Box Turtle's overwintering hibernation behaviors have been completed (Cahn, 1933; Carpenter, 1957; Claussen *et al*., 1991; Congden *et al*., 1989; Costanzo *et al*., 1995b; Dolbeer, 1970; Grobman, 1990; Neill, 1948; Reagan, 1974). The majority of these studies, stretching over the last century, are descriptive studies, describing how box turtles behave during hibernation.

Although Eastern Box Turtle hibernation has been fairly well described, few studies have attempted to delve deeper into mechanisms which influence hibernation behavior (Carpenter, 1957; Grobman, 1990; Reagan, 1974). Also, because this area of Terrapene ecology has received such little attention aside from these studies, influences such as geographic distribution and microhabitat variations, not to mention evolutionary adaptations, require additional field research to ascertain.

Figure 1: Eastern Box Turtle About to Emerge from Hibernation

As part of understanding Eastern Box Turtle winter ecology, the aforementioned studied physiological responses to cold must be briefly outlined. As befitting a terrestrially hibernating ectotherm, box turtles have been found to be one of the more cold and freeze adapted reptiles (Claussen and Kim, 1993; Claussen and Zani, 1991; Costanzo and Claussen, 1990; Costanzo and Lee, 1994; Costanzo *et al*, 1988, 1993, 1995a; Gatten 1974; Gatten, 1987; Risher and Claussen, 1987; Storey and Storey, 1992). They achieve this adaptation through a rather remarkable freeze tolerance and, to a lesser extent, a limited ability to supercool (Costanzo and Claussen, 1990).

Supercooling is a process by which the bodily formation of ice is generally retarded, and is synonymous with freeze avoidance. In normal circumstances, intra and intercellular water readily begins ice formation at 0°C. In animals that supercool, this freezing is blocked by antifreeze compounds (glucose, glycerol, uric acid) released throughout the body, allowing body temperatures to fall below 0°C without subsequent freezing of body fluids. This process, which works amazingly well in smaller ectotherms, is not particularly effective as a long-term strategy in Eastern Box Turtles due to their large body mass, resulting in a decreased ability to retard bodily freezing (Costanzo and Claussen, 1990). Also, because typical hibernacula are moist and cold, rapid cooling and ice formation will result even with excellent antifreeze production (Carpenter, 1957; Reagan, 1974). Due to this issue, supercooling has only been reported to be effective in *T. c. carolina* at -1.12°C ± 0.28°C between less than ten minutes to two hours before bodily ice formation begins (Costanzo and Claussen, 1990).

Since supercooling is such a limited strategy for box turtles, freeze tolerance presents a better alternative. Freeze tolerance is the process of allowing ice to form in a slow, controlled manner in the intercellular bodily fluids. When *T. c. carolina* approaches freezing temperatures, antifreeze compounds are released in internal organs and intercellular spaces (Costanzo and Claussen, 1990; Costanzo *et al*, 1993; Hutton and Goodnight, 1957). At the same time, organs and cells are dehydrated, with internal water being replaced with antifreeze compounds. Thus, organs and cells are much better protected, while freezable water is removed from where it can do the most damage. The intercellular water is then allowed to freeze in a controlled manner, while organs and intracellular spaces remain unfrozen due to a low concentration of water and a high concentration of antifreeze compounds. The results of this process are significant, with box turtles having been observed, in laboratory conditions, with between 33 to 58 percent of their bodily fluid frozen at temperatures as low as -3.1°C for periods up to three days with no negative after effects (Costanzo and Claussen, 1990). This ability greatly aids Eastern Box Turtles and allows them to hibernate terrestrially with such great success.

INTRODUCTION

Project Rationale

Despite the fact that winter conditions play a major role in the natural history and survivorship of Eastern Box Turtles and other herpetological species, studies concerning this topic are scarce and many areas of inquiry have yet to be studied. In West Virginia and other temperate states, almost no winter herpetological studies have been conducted in the last 30 years (Dr. Thomas Pauley, pers. comm.). This being the case, many areas of crucial natural history are unknown. Throughout the United States, amphibian and reptile species are decreasing in number (Houlahan *et al*, 2000; Lannoo, 2005). Due to herpetofauna's vital role in ecosystems and their invaluable capability of being bioindicators, it is necessary to preserve these species to the best of our abilities.

Consisting of up to half the year, winter conditions must be studied to determine the life history, seasonal activity patterns, habitat associations, and necessary requirements of these species which are so crucial to the environment.

Project Objectives

Mushinsky (1975) and Sugalski & Claussen (1997) reported significant preference for soil pH among a variety of terrestrial salamanders. If salamanders are influenced by soil pH values, there also is a possibility that Eastern Box Turtles are similarly influenced when selecting a soil site to reside in for several months (Dodd, 2001). Dolbeer (1970) reported a significant increase in Eastern Box Turtle movement during times of precipitation, suggesting moisture may play some role in hibernacula behavior. Carpenter (1957) and Costanzo *et al*. (1995a) reported that Eastern Box Turtles seem to prefer soft, friable, sandy soil texture for hibernacula locations. Dodd (2001) hypothesizes that the absence of quality sandy soil to hibernate in might be one of the leading restrictions on the Eastern Box Turtle's northern range. Congden *et al*. (1989) and Grobman (1990) reported significant effects of soil temperatures on entrance, movement, and emergence of box turtles from hibernation. Finally, Carpenter (1957) and Claussen *et al*. (1991) suggested that box turtles prefer leaf litter as a cover for hibernacula, while also preferring to be near natural cover such as trees, logs, or shrubs.

From these previous studies, it seems Eastern Box Turtles preferentially select a variety of characteristics when active and potentially before going into hibernation. These characteristics, however, have all been examined individually by a variety of researchers but have never been considered simultaneously. Thus, there is uncertainty for what specific characteristics Eastern Box Turtles might select, if they do. The literature is full of reports of Eastern Box Turtles relocating in the middle of winter (Carpenter, 1957; Claussen *et al*., 1991; Congden *et al*., 1989; Dolbeer, 1971) and I have personally observed a turtle digging through snow to relocate to a new hibernacula. Despite all these reports, there are no attempts to explain why they relocate in less than optimal conditions. Is it individual personality or are Eastern Box Turtles selecting for some combination of hibernacula characteristics? If the characteristics change at their current hibernacula, will they move to a new one? These are the questions I have attempted to shed light on during the course of this study.

Thus, my objectives in this study were to attempt to answer several interrelated questions, which are of vital importance to the survival and ecological necessities of *T. c. carolina*. My goals were to determine if Eastern Box Turtles preferentially select hibernacula locations, and if so, what site characteristics do they select. Then, if some combination of site characteristics influences hibernacula selection, do these characteristics influence other unexplained behaviors seen during hibernation in this species?

METHODS & MATERIALS

STUDY SITES

The study site was located in Beech Fork State Park (BFSP; 38˚18’19”N, 82˚20’21”W) adjacent to the campgrounds in Wayne County, West Virginia (Figures 2-5). The site was selected based on the high box turtle population density and diverse topography. Elevation in the study site ranged from 181m to 263m including a mountain ridge (Figures 2-5). Initial sampling and the adherence of radio transmitters were done in the lower elevation range (181-190m) of the study site (Figure 3).

Habitat was characterized by mesophytic eastern deciduous hardwood forest and fragmented by two frequently traveled trails, an intermittent stream bed, and open fields cleared and mowed for recreational use. The southern edge of the study area was bound by a perennial stream, with a well-used road and campgrounds on the southern side. Common over story vegetation at site comprised of mixed pine (*Pinus* sp.) and hardwood including maples (*Acer* sp.), American beech (*Fagus grandifolia*), hickory (*Carya* sp.), tulip poplar (*Liriodendron tulipifera*), white oak (*Quercus alba*), and sassafras (*Sassafras sp.*). Some dominant understory vegetation during the sampling period included autumn-olive (*Elaeagnus umbellata*), honeysuckle (*Lonicera* sp.), Japanese stiltgrass (*Microstegium vimineum*), multiflora rose (*Rosa multiflora*), and a variety of fern species.

Field work took place over the course of two field seasons covering the winter of 2011-2012 and the winter of 2012-2013.

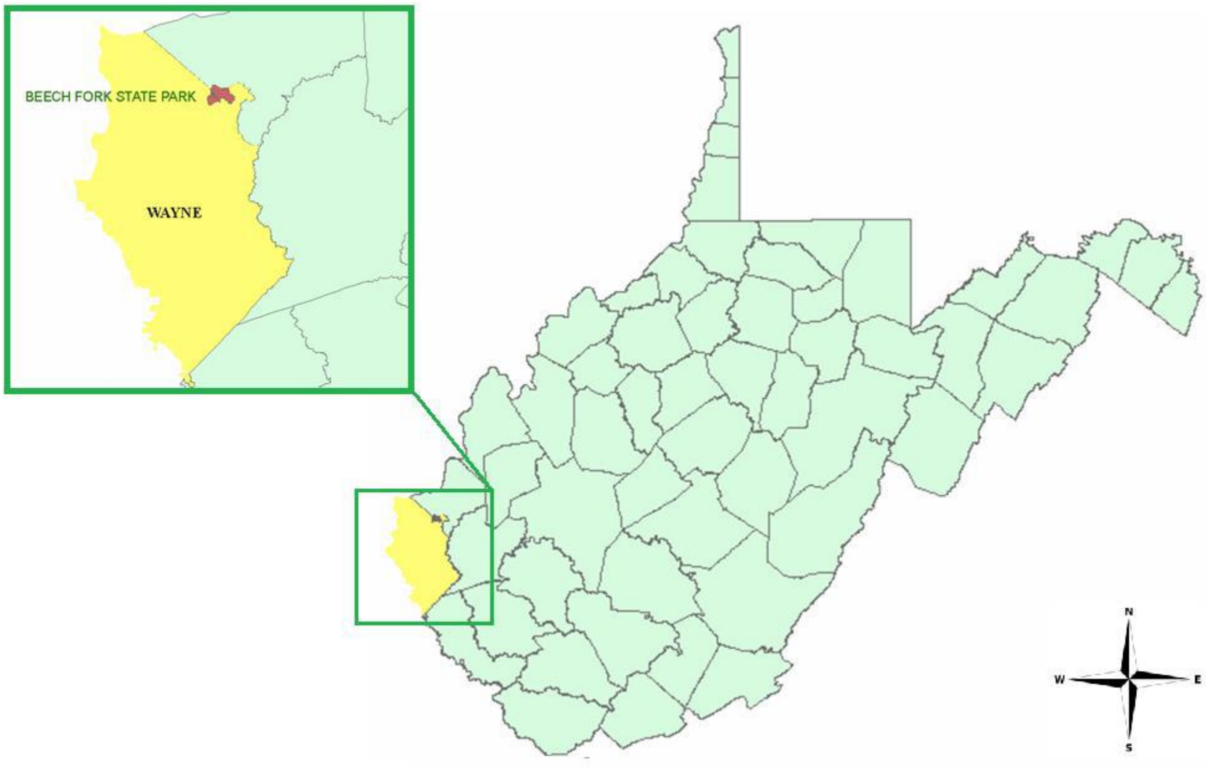


Figure 2: Map of West Virginia highlighting county in yellow and study area in pink. Map designed by Nathalie Aall. Used with permission.

Figure 3: Google Earth topography map of study site at Beech Fork State Park. Area outlined in red represents the area in which box turtles were initially tagged.

Figure 4: Top: ArcMap aerial imagery of the study area.
Figure 5: Bottom: USGS Winslow 7.5 minute topographic map, showing study area.

SURVEY METHODS

Radio Telemetry

This study employed radio telemetry, where a small radio transmitter is affixed to the carapace of the turtle allowing the location of the turtles to be pinpointed even when buried or under snow. Eastern Box Turtles used in this study were located onsite during early October and tagged in November 2010 with radio transmitters purchased from Wildlife Materials, Murphysboro, Illinois. The dark green transmitters weighed about 20g each which was less than six percent of the smallest turtle's body mass. The transmitter was affixed to the 3^{rd}-4^{th} pleural scute on the right side of each turtle's carapace using non-toxic one minute epoxy. This location was chosen so that the transmitter would not limit mobility, burrowing nor mating ability of the turtle (Figure 6). The turtles were tagged onsite and returned to where they were originally found.

After tagging the turtles, I tracked them using a Wildlife Materials TRX-2000 receiver and a three-prong directional Yagi antenna until they entered a hibernaculum in early November. The turtles were confirmed to be in hibernation once they failed to move for three days consecutively. While the turtles were in hibernation, I tracked them every third day until the weather warmed in late February. In order to minimize disturbance to the hibernating turtle, the cover over the hibernacula was not removed during these checks. The turtle was confirmed to be in the same location through a check with the receiver and by passing over the site with a metal detector, which detected the metal of the transmitter.

From the point where the weather warmed in mid-February, tracking occurred daily in order to determine when the turtles emerged from their hibernacula. After emergence, turtles were followed for one week to confirm that emergence was permanent and they were not going to reenter hibernation. The transmitters were then removed and the box turtles were released where they had been captured.

During the 2011-2012 field season, 15 Eastern Box Turtles were originally tagged. However, due to unknown circumstances ten transmitter signals disappeared within the first month. These turtles were never found and the radio signals were never detected during the following two years. Thus, five mature turtles, consisting of two males and three females were studied for the first year. In an attempt to make up for the loss of the turtles from the first year, seven additional mature turtles, consisting of four males and three females were tagged for the 2012-2013 field season.

Figure 6: Top: Radio tagged Eastern Box Turtle. Top right: Top view of turtle showing temperature recorder on opposite side. Right: Box Turtle in hibernation. You can see the transmitter emerging through the cleared litter.

Soil Mapping

Upon confirmation of each Eastern Box Turtle being in hibernation, a soil map was constructed around each hibernaculum. The soil map consisted of a 10,000 m^2 square (100m x 100m) with data points collected every 20m in a grid-like fashion, with the hibernacula in the very center of the map. This resulted in an ideal 36 data point collected per turtle, with an additional data point being taken at the hibernacula. The purpose of this grid mapping was to show what possible conditions are present on site, from which the turtle selects for a hibernacula. At any point where two or more turtles were close enough that the maps intersected, the maps were merged to form one large map. Also at any point where physical boundaries would naturally bound the movement of the Eastern Box Turtles, such as the large perennial stream on the southern side of the study area, the map was clipped at that boundary (Figures 7-12).

Figure 7: Location of 2011-2012 field seasons' turtles with collected soil maps highlighted. Top: ARCGIS aerial.

Figure 8: Bottom: USGS 7.5 minute topographic map, Winslow Quadrangle, WV. 1972.

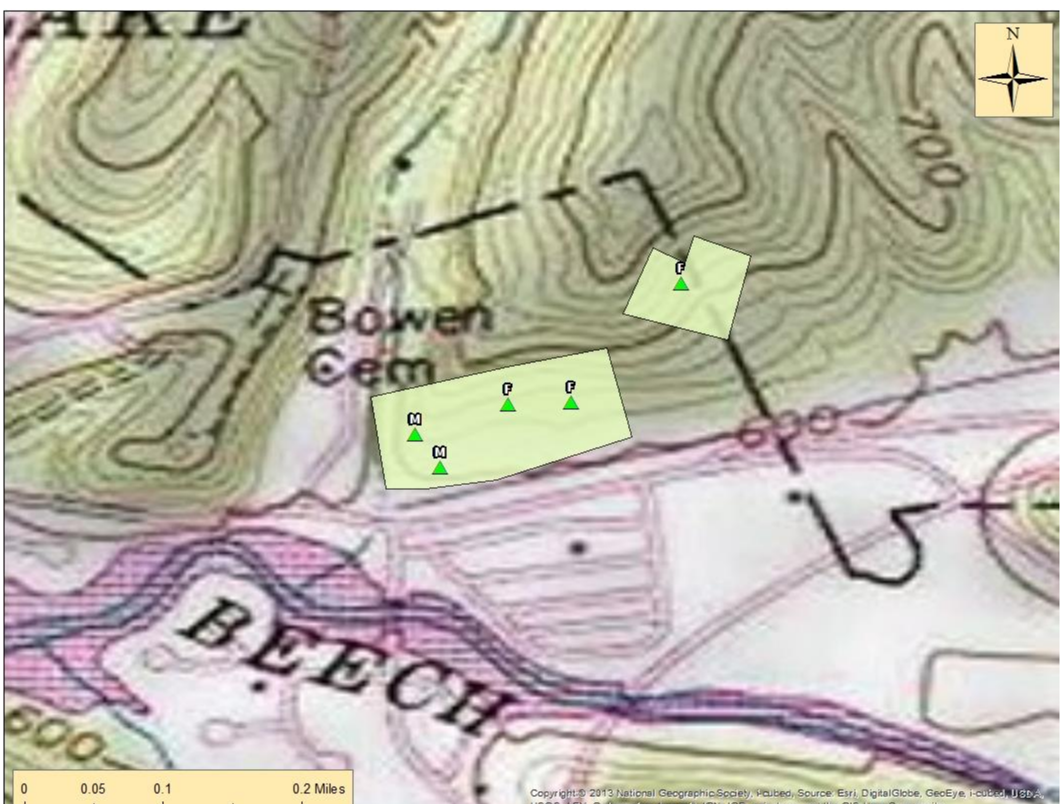


Figure 9: Location of 2012-2013 field seasons' turtles with collected soil maps highlighted. Top: ARCGIS aerial.

Figure 10: Bottom: USGS 7.5 minute topographic map, Winslow Quadrangle, WV. 1972.

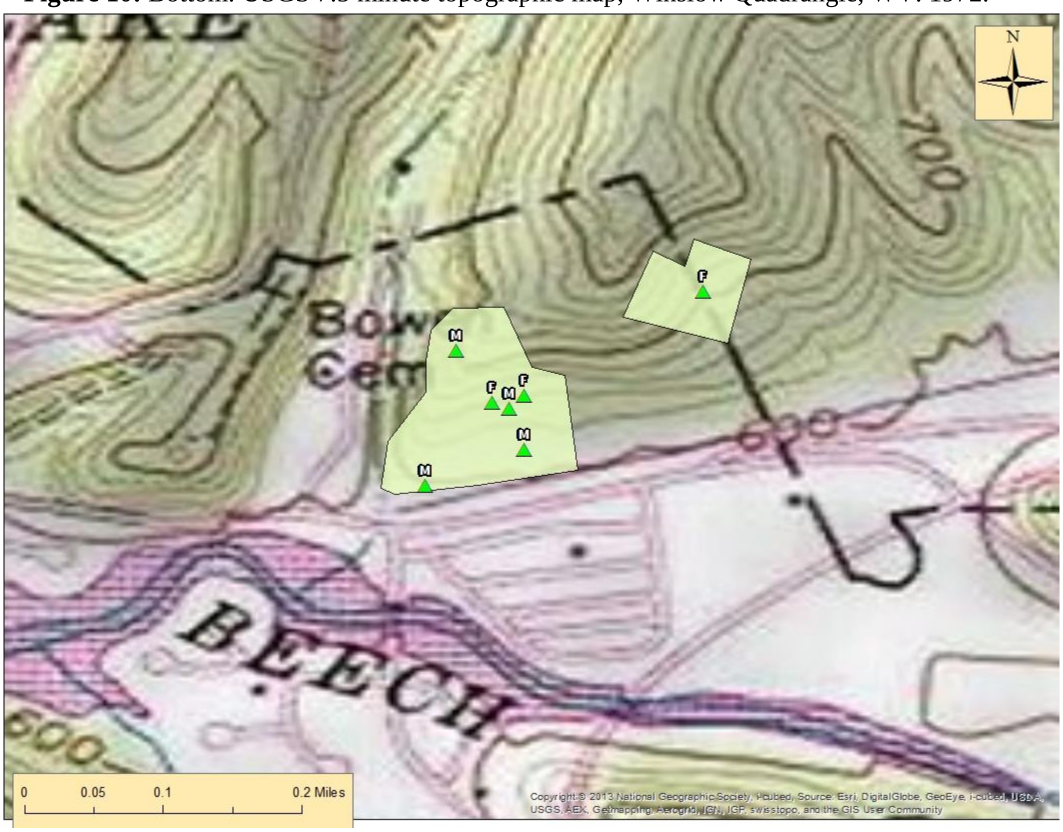
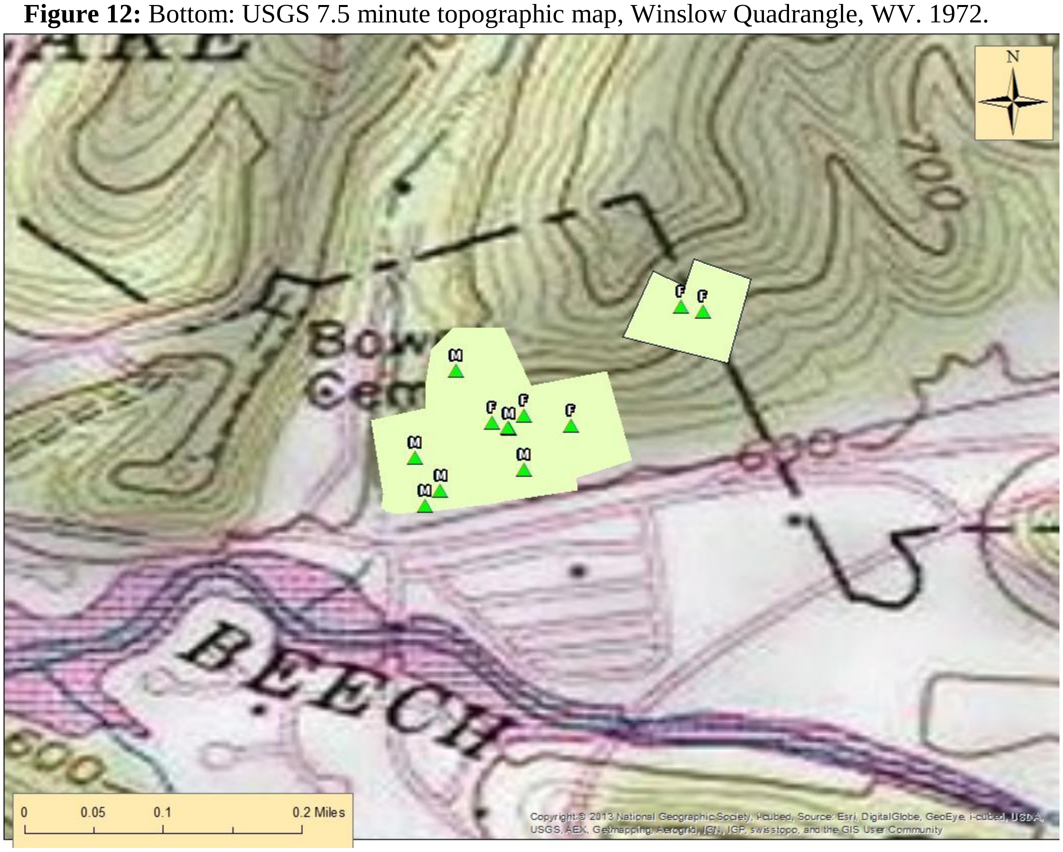


Figure 11: Combined location of both field seasons' turtles with collected soil maps highlighted. Top: ARCGIS aerial.
Figure 12: Bottom: USGS 7.5 minute topographic map, Winslow Quadrangle, WV. 1972.



DATA COLLECTION

At each point on the soil mapping, seven variables were collected, which can be broken down into either soil or cover characteristics. The soil characteristics collected were soil temperature, soil compaction, soil moisture, and soil pH. The cover characteristics collected were cover depth, cover moisture, and cover type.

Soil Characteristics

Soil temperature was recorded using a LaMotte pHPlus Direct pH Meter with thermometer at a depth of 15cm. Soil compaction was recorded in pounds per square inch (psi) using a Dickey-John Soil Compaction Tester penetrometer to a depth of 25cm. When compaction was tested, the highest psi shown during a slow controlled push from 0-25cm was recorded for that point. For each of these characteristics three sample points were taken within a square meter of the test point and the average was recorded. If an impenetrable substance such as a rock or root was encountered while using the penetrometer that sample point was discarded and another sample point was used for the average to avoid skewing the data. For the turtle hibernacula data point, the sample points were taken in a triangle around the turtle within 5cm of the actual animal.

Soil moisture was recorded by using a two inch bulb planter to collect a soil sample 8-10cm deep. A portion of this sample then was brought back to the lab, weighed, put into a drying oven at 110°C for 24 hours, and weighed again (Black, 1965). The soil moisture was then calculated by the difference between these two weights. Soil pH was recorded by using the remaining soil sample not used for soil moisture testing and making 1:10 part slurry of soil to distilled water (Wescott, 1978). This slurry was thoroughly mixed and recorded using a LaMotte pHPlus Direct pH Meter with pH probe. For the turtle hibernacula data point, the soil was collected directly abutting the turtle within 3cm of the actual animal.

Cover Characteristics

All cover characteristics recorded were taken by examining a square meter area centered on the data point. Thus, the recorded cover depth was the average cover depth over that square meter. The exception to this was for the hibernacula data point, where all characteristics recorded were taken either directly over or abutting the hibernacula.

Cover depth was recorded by measuring the depth of the surface litter or material from the soil surface to the highest point of that surface litter. This characteristic was recorded in the field with a standard ruler. Cover moisture was recorded by cutting a $100cm^2$ square (10cm x10cm) to the ground of the deepest section of litter for the examined square meter. This sample was then brought back to the lab, weighed, put into a drying oven at 110°C for 24 hours, and weighed again (Black, 1965). The cover moisture was then calculated by the difference between these two weights.

Cover type was recorded categorically by simply noting what surface litter or material types were present within the square meter. For simplicity of recording after observing the site, cover types were divided into four categories: mixed deciduous leaf litter, coniferous pine needle litter, dead herbaceous plant matter, and surface moss.

DATA ANALYSIS

In order to analyze the large data set resulting from the mapping, it was decided to use categorical logistic regression to examine the soil and cover characteristics collected. This test allows a multivariate examination of the data in a controlled manner. To use this test, each box turtle's hibernacula data was paired with five randomly selected data points from its soil map. In cases where multiple turtles shared a soil map, each data point could only be selected once for the statistical analysis. Thus, one categorical logistic regression was run for the soil characteristics consisting of compaction, pH, moisture, and temperature, and another was run on the cover characteristics consisting of depth and moisture. These tests were performed using the R statistical computing package, an open source program created by the R Development Core Team (Bloomfield, 2014; Hilbe, 2013; Husson *et al*, 2010; Stevens, 2009).

Due to the categorical nature of the cover type data collected not lending itself to traditional statistical analysis, it was decided to use an electivity index on the data to examine the Eastern Box Turtle's preference of one cover type to another. Electivity indices were designed to present foraging preferences but have been used in previous studies to examine habitat preferences as well (Jhala, 2009; Senko *et al*, 2010). Unlike the previous tests, all data points collected were used for the electivity indices. Using the paper by Lechowicz (1982), which examined many of the available electivity indices, it was decided to use two indices to determine and compare the results: Ivlev's Electivity index (E) and Vanderploeg and Scavia's Relativized Electivity index (E*). These indices are easily understood with Ivlev's and Vanderploeg and Scavia's indices resulting in a value where any number greater than zero indicates a greater selection for, and anything less than zero indicates a greater selection against.

RESULTS

Soil Characteristics Analysis

Collected soil characteristics from all turtles combined soil maps (n=60) are recorded in Table 1, while the combined turtle hibernacula soil data (n=12) are recorded in Table 2. This collected raw data showed that soil compaction across the site ranged from 60psi to 280psi, averaging 118psi. Meanwhile, the hibernacula soil compaction data ranged lower from 40psi to 120psi, averaging 75.8psi.

The other collected soil variables appeared more similar between the soil map and hibernacula data, with soil temperature across the site averaging 5.2°C, and ranging between 2.3°C and 8°C, while hibernacula soil temperature averaged 6.1°C, ranging between 3.6°C and 7.3°C. Site soil pH averaged 6.05, ranging between 4.68 and 7.91, while hibernacula soil pH was similar averaging 6.49, with a range between 5.51 and 7.76. Site soil moisture content averaged 29.53%, ranging between 17.24% and 50%, while hibernacula soil moisture content was very similar averaging 27.59%, with a range between 10.23% and 34.78%.

Characteristic	n	Min	Mean	Max	Median	Std. Deviation
Soil Compaction (psi)	60	60	118.833	280	110	35.595
Soil Temperature (°C)	60	2.3	5.213	8	5.1	1.092
Soil pH	60	4.68	6.0522	7.91	5.845	0.965
Soil Moisture Percent	60	17.239	29.528	50	29.594	6.285

Table 1: Soil characteristics (excluding hibernacula) from soil map data summary.

Characteristic	n	Min	Mean	Max	Median	Std. Deviation
Soil Compaction (psi)	12	40	75.833	120	75	23.259
Soil Temperature (°C)	12	3.6	6.075	7.3	6.3	1.044
Soil pH	12	5.51	6.494	7.76	6.12	0.941
Soil Moisture Percent	12	10.230	27.591	34.783	27.496	4.670

Table 2: Eastern Box Turtle hibernacula soil data summary.

The results of the conditional logistical regression on soil characteristics revealed only one statistically significant variable out of the dataset (Table 3). That variable was soil compaction with a p value of 0.0293. Soil pH was the next most significant with a p value of 0.1063. Meanwhile soil temperature (p=0.1914) and soil moisture (p=0.8739) showed no significant variation between the hibernacula and the randomized soil plot characteristics.

n=72, number of events=12

	coef	exp(coef)	se(coef)	z	Pr(>I z I)
Soil.Compaction	-0.08347	0.91992	0.03829	-2.18	0.0293 *
Soil.Temperature	0.92164	2.51342	0.70543	1.306	0.1914
Soil.pH	2.93146	18.75499	1.81534	1.615	0.1063
Soil.Moisture	1.88261	6.57064	11.86129	0.159	0.8739

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	exp(coef)	exp(-coef)	lower .95	upper .95
Soil.Compaction	0.91992	1.08705	8.534E-01	9.916E-01
Soil.Temperature	2.51342	0.39786	6.307E-01	1.002E+01
Soil.pH	18.75499	0.05332	5.344E-01	6.582E+02
Soil.Moisture	6.57064	0.15219	5.263E-10	8.203E+10

Rsquare	= 0.34 (max possible= 0.45)
Likelihood ratio test	= 29.95 on 4 df, p=5.005e-06
Wald test	= 5.89 on 4 df, p=0.2077
Score (logrank) test	= 24.51 on 4 df, p=6.3e-05

Table 3: Output of conditional logistic regression on soil characteristics.

Cover Characteristics Analysis

Collected cover characteristics from all turtles combined soil maps (n=60) are recorded in Table 4, while the combined turtle hibernacula soil data (n=12) are recorded in Table 5. This collected raw data showed that cover depth across the site ranged from 0.254cm to 10.16cm, averaging 1.80cm. Meanwhile, the hibernacula soil compaction data ranged similar from 0.635cm to 10.16cm, averaging much higher at 6.19cm.

The other collected cover variable, cover moisture content, appeared more similar between the soil map and hibernacula data, with moisture content across the site averaging 34.68%, and ranging between 0% and 64.71%, while hibernacula cover moisture content averaged 38.50%, ranging between 19.22% and 53.33%.

Characteristic	n	Min	Mean	Max	Median	Std. Deviation
Cover Depth (cm)	60	0.254	1.799	10.16	1.397	1.925
Cover Moisture Percent	60	0	34.680	64.706	34.100	13.341

Table 4: Cover characteristics (excluding hibernacula) data summary.

Characteristic	n	Min	Mean	Max	Median	Std. Deviation
Cover Depth (cm)	12	0.635	6.191	10.16	5.08	2.911
Cover Moisture Percent	12	19.224	38.495	53.333	38.531	10.151

Table 5: Eastern Box Turtle hibernacula cover data summary.

The results of the conditional logistical regression on cover characteristics also revealed one statistically significant variable out of the dataset (see Table 6). This variable was cover depth with a p value of 0.00725. Cover Moisture was shown to not be statistically significant with a p value of 0.10034.

n=72, number of events=12					
	coef	exp(coef)	se(coef)	z	Pr(>I z I)
Cover.Depth	1.843E+00	6.315E+00	6.686E-01	2.685	0.00725 **
Cover.Moisture	1.270E+01	3.282E+05	7.729E+00	1.643	0.10034
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

	exp(coef)	exp(-coef)	lower .95	upper .95
Cover.Depth	6.315E+00	1.584E-01	1.64497	2.424E+01
Cover.Moisture	3.282E+05	3.047E-06	0.08645	1.246E+12

Rsquare	= 0.295 (max possible= 0.45)	
Likelihood ratio test	= 25.13 on 2 df,	p=3.499e-06
Wald test	= 7.85 on 2 df,	p=0.01974
Score (logrank) test	= 26.5 on 2 df,	p=1.757e-06

Table 6: Output of conditional logistic regression on cover characteristics.

Cover Type Analysis

Ivlev's Electivity (E) index and Vanderploeg and Scavia's Relativized Electivity (E*) index both showed comparable results (Table 7, Figures 13 & 14). Both indices revealed a complete avoidance of surface moss as a cover type at hibernaculum (E= -1, E*= -1), and also showed a lesser avoidance of dead herbaceous plant matter (E= -0.5000, E*= -0.1910) and coniferous pine needle litter (E= -0.4922, E*= -0.1811) at the hibernaculum. Mixed deciduous leaf litter, on the other hand showed a strong selection for in both indices, especially in Vanderploeg and Scavia's Relativized Electivity index (E= 0.1264, E*= 0.4486).

	Cover Type Combined	Cover Type Combined	Cover Type Combined	Cover Type Combined
Type	Leaf Litter	Plant Matter	Pine Needles	Moss
number in sample	12	12	12	12
total selected for	12	2	1	0
abundance of choice (ri)	1	0.166666667	0.083333333	0
total number available	196	196	196	196
number available type	152	98	48	22
abundance in ecosystem(Pi)	0.775510204	0.5	0.244897959	0.112244898
Ivlev's Electivity Index				
score(Ei)	0.1264	-0.5000	-0.4922	-1.0000
Vanderploeg and Scavia's Relativized Electivity				
score(E*)	0.448647539	-0.191041135	-0.18108851	-1.0000

Table 7: Electivity indices calculations.

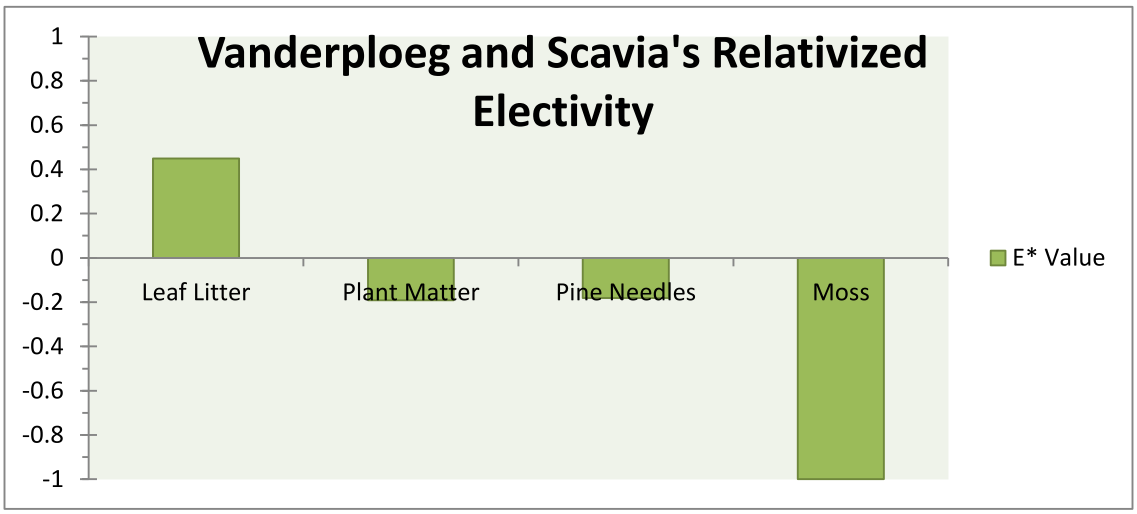


Figure 13: Graph of Vanderploeg and Scavia's Relativized Electivity index results.

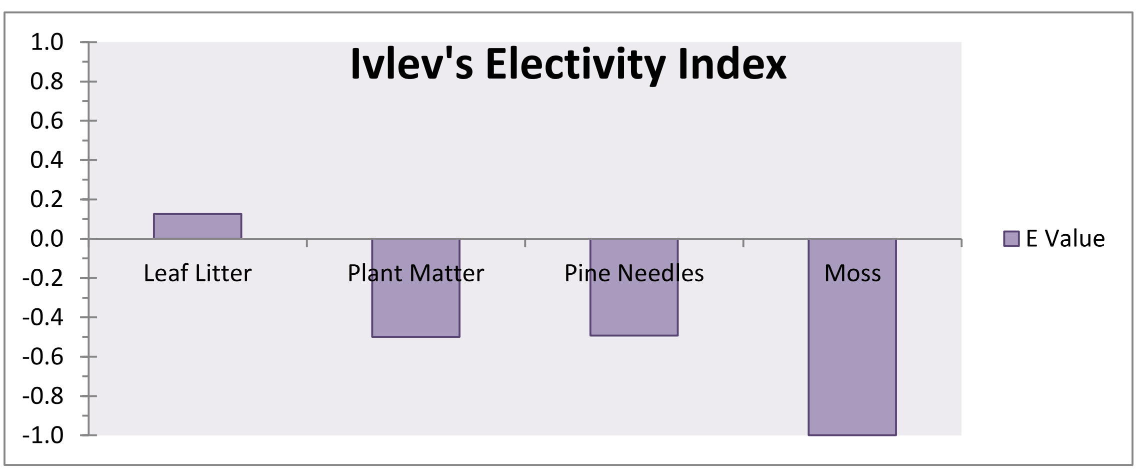


Figure 14: Graph of Ivlev's Electivity index results.

DISCUSSION

Interpretation of Results

The results of this study seem to indicate that Eastern Box Turtles preferentially select hibernacula location characteristics in comparison to the surrounding habitat. They preferentially select areas of soft, easily compactible soils, with a thick covering of mixed deciduous leaf litter. From an ecological and behavioral perspective this make sense, because the soft friable soil would allow box turtles to easily dig into the ground to create a desired form during periods of torpor and also create the final hibernacula with a minimum of energy expenditure.

The thick layer of mixed deciduous leaf litter also makes sense, because a thicker layer of litter would create a better insulating barrier between the rapidly changing air temperature and the more controlled thermal environment of the hibernacula, which would lend itself to the Eastern Box Turtle's physiological responses as outlined in the Background section. The slower decline in soil temperature compared to air temperature would allow the turtles to have the slow body temperature decline necessary for limited supercooling and eventual periods of freeze tolerance necessary for their survival. While the thick insulating layer of leaves would not keep the hibernacula warm, it would protect *T. c. carolina* from unseasonably warm or cool days during the period of hibernation which could adversely impact the survival of the animal. The other observed cover types (moss, plant matter, pine needles) do not form a thick and compact surface layer as the leaf litter, which is probably the reason these cover types show a negative selection in the data analysis.

The final results of this study are also supported by the field notes on *T. c. carolina* in the literature. This study revealed a preference for soft friable soils with a thick layer of deciduous leaf litter. This compares well with Carpenter's (1957) and Costanzo *et al*.'s (1995b) reports of Eastern Box Turtles seemingly preferring soft, friable soil for hibernacula locations, and with

Dodd's (2001) hypothesis that the lack of suitable soil may limit the box turtle's distribution. My study also agrees favorably with Carpenter's (1957) and Claussen *et al*.'s (1991) suggestion that box turtles prefer leaf litter as a cover for hibernacula.

Thus, my results are reinforced with these observational findings from across *T. c. carolina's* distribution range, which may suggest that these findings be similar across the entire range of this species. Of course, additional studies would be required to confirm this suggestion. Also unfortunately, the desired behaviors that I wished to explore during this study (such as box turtles changing hibernacula during the winter) were never experienced during the two winters on site. So it is still unknown if a change in hibernacula characteristics may influence this and other behaviors.

Issues with this Study

While the majority of this study ran smoothly, several large issues presented themselves, including one that severely curtailed my ability to collect additional data. The primary issue experienced was equipment failure, where early in the first winter of the study two-thirds of the tracked radio transmitters disappeared simultaneously. This disastrous situation both limited the data that could be collected that year, as well as how much data could be collected the following year as well. It is still unknown what happened to any of these transmitters.

The second primary issue with this study has already been alluded to. This was the lack of specific behaviors which I wished to observe during the winter. This lack of observations did not allow me to study them, which, in turn, did not allow me to answer questions I had. Even this was beyond my control; it is an unfortunate but integral part of field studies, where animal behavior limits what you are allowed to study.

Implications for Conservation

The study of winter ecology in herpetofaunal species is necessary in order to better understand and protect these valuable animals. Many reptile and amphibian population numbers are drastically dropping, in most cases, due to man's intervention (Houlahan et al., 2000; Lannoo, 2005). Throughout the United States many measures have been taken to help preserve these ecologically necessary species. However, any measure taken using present knowledge, with little regard to winter, is only a half-measure. Since many species spend such a significant portion of their lives in torpor, or some other overwintering state, the needs they express during the cold months of the year are surely just as important as their summer needs. Also, at no time in an animal's life is it as much a passive bioindicator as it is during hibernation.

Thus, the knowledge gained from this study will hopefully be used for furthering studies in this field and will hopefully be used to better preserve herpetological species in the future. This need for information may be especially true with the ongoing threat of global warming, which may cause enough significant alterations to winter conditions to prevent reptiles and amphibians from having their needs met during this already dangerous season (Cahn, 1933; Metcalf, 1979; Neill, 1948). Thus, the more information biologists know about the winter ecology of these species, the better they can safeguard all species of herpetofauna, and hopefully, preserve them for future generations.

Future Work

In the future I would like to continue examining the winter behavior of Eastern Box Turtles. While this study does reveal some interesting results, it is only applicable for one population of turtles in one part of West Virginia. Continuing work is needed to see if the results discovered in this study hold true for other populations outside of the study area.

I would also like to focus work on other interesting and little understood areas of Eastern Box Turtle winter ecology, such as what is the stimulus for hibernation, and, most interestingly, what is the stimulus that brings these turtles out of hibernation and makes them regain that “spark of life.” This stimulus out of hibernation is probably one of the most interesting physiological and behavioral aspects of these turtles that has yet to be understood.

Finally, going back to this project’s work, behaviors noted in the introduction and that have been personally seen still do not make sense for these animals. For instance, why do they move during the winter from one hibernaculum to another? This behavior was not noted during this study, so it was impossible to examine if something in the hibernacula changed or if it is a behavioral response with nothing to do with the hibernacula. Regardless, future work is needed to understand the rich and often overlooked winter ecology of this terrestrial turtle species.

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APPENDIX A

New Application (X) Renewal ( ) (Original IACUC No.__________) IACUC Project No. 467

APPLICATION FOR THE CARE AND USE OF
LABORATORY ANIMALS AT MARSHALL UNIVERSITY

Name: Dr. Thomas K. Pauley
Title: Professor

Department: Biological Sciences
Campus Phone No.: (304) 696-2376

Co-Investigator: Benjamin Koester
Title: Graduate Student Department: Biology

Co-Investigator email:koester1@marshall.edu Co-Investigator phone: (309) 825-3965

Title of Project/Course: Hibernacula site selection, hatchling overwintering behavior, and emergence from hibernation in the Eastern Box Turtle, Terrapene c. carolina (Linnaeus, 1758) in West Virginia.

Type of Activity: Research (X) Teaching ( )

Date of Project Period (limited to 3 years): January 31, 2011– June 1, 2013

Funding Source: Internal X External_____ Agency__________

1. OVERVIEW AND RATIONALE OF PROTOCOL: The following information is required to assist the committee with evaluating the appropriateness of the animal model and procedures to be used. All questions must be answered. **Abstracts from grant application forms are not acceptable.** Use additional pages if necessary. **In the following space, provide a paragraph, telling in lay terms what you plan to do in this project. Be concise and respond in language understandable to a non-scientist.**

This work is for Benjamin Koester's graduate thesis research. I will be conducting the fieldwork, and will write the report on the findings of this research. Dr. Pauley is my advisor and will supervise this work.

Eastern Box Turtle populations throughout the Eastern United States have been rapidly declining in recent years due to human interaction. Road building, deforestation, and climate change all play a role in this decline. At no point during the year is the effect of climate change and human interaction so likely to cause problems as during the winter. Variable temperatures, pollution, and changes in the land can all have drastic consequences on turtle mortality rates. Unfortunately, Eastern Box Turtle's overwintering requirements are unknown, and thus they cannot be protected from these human causes. In order to begin determining these requirements the objectives of this study are to determine hibernation site preference in overwintering populations of Eastern Box Turtles in relation to soil parameters, determine environmental parameters that trigger emergence from hibernation in the spring, and document hatchling Eastern Box Turtle's overwintering behavior at Beech Fork State Park, WV.

A. State the:

1) objective(s)

a) Determine hibernation site preference in overwintering populations of Eastern Box Turtles in relation to soil parameters.

b) Determine environmental parameters that trigger box turtle emergence from hibernation.

c) Document Overwintering behavior of Eastern Box Turtle hatchlings.

1

2) primary aim(s)

a) Determine if Eastern Box Turtles choose hibernation locations

b) Determine if environmental conditions determine emergence from hibernation

c) Determine how first-year hatchlings respond to winter conditions

3) significance or scientific merit of this protocol.

This study will provide information on the winter behavior and requirements of a decreasing reptile species. This information will assist developing conservation strategies for this species and other terrestrial turtle species. It will also determine if human impact on the environment is likely to threaten terrestrially hibernating turtles.

B. State:

1) the rationale for using animals in this project

To determine hibernation site preference and emergence requirements requires the use of live animals in their natural settings. To determine hatchling strategies also requires the use of live animals in a natural setting. Radio-tagging these animals are necessary to ensure I follow one population throughout the experiment.

2) the justification of the animal model chosen

Eastern Box Turtles is the most common terrestrially-hibernating turtle in the Eastern United States, and the only terrestrially-hibernating turtle in West Virginia. Since studying terrestrial hibernation and its possible implications based on hibernation site is the goal of this study and it is cost prohibitive to study outside the state, the Eastern Box Turtle presents an ideal study specimen.

3) the justification for the number of animals required. Address the number of animals used for control versus the number of experimental groups, if applicable.

100 Eastern Box Turtles will be used for the study. Since smaller sample sizes might not reveal actual preferences for hibernation sites, emergence, and behavior, this number of specimens is necessary. Previous experience with this model has shown distinct individual behaviors in the turtles. Thus, to eliminate individuals biasing the project, a large sample size is necessary. Also, since electronic devices are being used to track these animals, this large sample size allows for losses due to electronics failures.

C. Describe how animals are to be used in this protocol, noting the general experimental design and all animal procedures to be conducted. In short, everything that is done with an animal as part of this protocol must be described.

I will use hand sanitizer before and after contact with each box turtle throughout the study. Twenty turtles will be initially restrained for a half hour to be radio tagged, where a small radio transmitter and temperature sensitive ibutton will be glued to the rear shell of their carapace with all-purpose non-toxic epoxy (Mech, 1983) After the non-toxic epoxy has cured, the animal will be placed in its exact capture location. These animals will be checked several times a week with minimal handling, preferably none. All site characteristics will be taken directly adjacent to the turtles to prevent disturbance and temperature will be taken with a laser thermometer on the turtles shell. These same twenty turtles will be followed for two winters and then will have the radio transmitter removed. Removal will take place by securing the turtles, applying small amounts of acetone to the non-toxic epoxy, and gently peeling the epoxy off the turtle with a knife (Mech, 1983) This method will not harm the animal or leave any permanent mark on their shells. After removal, the Eastern Box Turtles will be returned to their collecting location.

Additional turtles will be handled during the study through the use of site surveys around the tagged turtle's hibernation sites. These turtles will not be bodily marked but will be handled to determine sex through visual checks of their plastrons (bottom shell) (Dodd, 2001).

Female tagged turtles will be radio tracked during the summer until they nest. Nests will be protected by wooden-framed screen enclosures to prevent predation. After hatching, hatchlings will be collected, radio tagged with light-weight transmitter using all-purpose non-toxic epoxy (Mech, 1983), and returned to the site they were collected from. After following these hatchlings into hibernation, they will have their radio transmitters removed by the same method described above and will be returned to their collection site.

2.

Animal Common Name	Total No. of Animals To Be Used for the Life of Project*	Discomfort, Distress, Pain Level**	Appropriate Pain Relieving Drugs Will Be Used (Yes/No)	Will Survival Surgery Be Conducted (Yes/NO)
Eastern Box Turtle	100	B	No	No

*Total number of animals used will be monitored and investigator will be notified when the total number is being reached. When the total number is reached, no additional animals will be ordered unless justified by the investigator and approved by the IACUC

**See Appendix 1

3. Federal law requires a written statement for A. and B. below:

A. Provide a narrative describing the methods, and list the sources, that have been utilized to determine that suitable alternatives, both for painful procedures and the animal model requested, are not available. **Include in this narrative a list of all literature reviewed and all reference sources utilized (i.e. MEDLINE, AGRICOLA with date of the search, key words and dates searched; relevant journals, personal communication, meeting/seminar attended, etc.).**

The animal model requested is necessary due to it being the only terrestrially hibernating turtle in this region of the country (Dodd, 2001). In order to study the natural history of the Eastern Box Turtle in its natural environment, it is necessary to use Eastern Box Turtles. Also, since baseline data for this study is not known, it is impossible to run theoretical studies using a "lower" animal or cell line to determine my results.

Dodd, K. 2001. North American Box Turtles. Norman: University of Oklahoma Press.

Mech, David. 1983. *Handbook of animal radio tracking.* Minneapolis: University of Minnesota. 42-44.

B. Provide written assurance that the activities do not unnecessarily duplicate previous experiments. **Include in this narrative a list of all literature reviewed and all reference sources utilized (i.e. MEDLINE, AGRICOLA with date of the search, key words and dates searched; relevant journals, personal communication, meeting/seminar attended, etc.).**

Marshall University's, Liberty University's, JSTOR's, Google Scholar's, and the Society for the Study of Amphibians and Reptiles' databases were searched on a monthly basis between October 2009 –November 2010 using key words: Eastern Box Turtle, turtle hibernation, turtle hibernacula, Reptile Hibernation, Herpetology hibernation, Hibernation site selection, turtle brumation, box turtle site selection, and reptile brumation. After this extensive literature search, it was concluded that the proposed research has never before been attempted and thus does not unnecessarily duplicate a previous experiment.

The following list of references was reviewed and their bibliographies were searched:

Adams, N, Claussen, D, & Skillings, J. 1989. Effects of temperature on voluntary locomotion of the Eastern Box Turtle, *Terrapene carolina* carolina. Copeia, 1989(4), 905-915.

Cahn, A. 1933. Hibernation of the Box Turtle. Copeia, 1933(1), 13-14.

Carpenter, C. 1957. Hibernation, hibernacula and associated behavior of the Three-toed Box Turtle (*Terrapene carolina triunguis*). Copeia, 1957(4), 278-282.

Claussen, D, Daniel, P, Jiang, S, & Adams, N. 1991. Hibernation in the Eastern Box Turtle, *Terrapene c. carolina.* Journal of Herpetology, 25(3), 334-341.

Congdon, J, Gatten, R, &Morreale, S. 1989. Overwintering activity of box turtles (*Terrapene carolina*) in South Carolina. Journal of Herpetology, 23(2), 179-181.

Costanzo, J, Iverson, J, Wright, M, & Lee, R. 1995. Cold hardiness and overwintering strategies of hatchlings in an assemblage of northern turtles. Ecology, 76(6), 1772-1785.

Dodd, K. 2001. North American Box Turtles. Norman: University of Oklahoma Press.

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Grobman, A. 1990. The effect of soil temperatures on emergence from hibernation of *Terrapene carolina* and *T. ornata.* The American Midland Naturalist, 124, 366-371.

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Neill, W. T. 1948. Hibernation of amphibians and reptiles in Richmond County, Georgia. Herpetologica 4:107-114.

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Strickel, L. F. 1950. Populations and home range relationships of the box turtle, Terrapene c. carolina (Linnaeus). Ecological Monographs, 20:351-378.

4. EUTHANASIA: Techniques for euthanasia shall follow current guidelines established by the latest Report of the AVMA Panel on Euthanasia. Other methods must be specifically reviewed and approved by the IACUC.

SPECIES	METHOD	DOSE (mg/kg body wt)	ROUTE
NA	NA	NA	NA

5.

NAMES OF INVESTIGATORS, TECHNICIANS, AND OTHERS HANDLING ANIMALS AT THE TIME OF APPLICATION (This list will be updated annually)	EXPERIENCE WITH THIS ANIMAL MODEL (Yrs)	MU TELEPHONE	EMERGENCY TELEPHONE
Dr. Thomas Pauley	40+	(304) 696-2376	
Benjamin Koester	2		

6. Specific location where animal research/teaching will be conducted: ____Beech Fork State Park, Wayne County, WV

7. If animals are taken from the ARF for research/teaching, will they be returned to the ARF?
____Yes ___X_No

8. OUTSIDE STUDY AREAS: Will animals be held in study areas outside of animal facility for more than 12 hours? Yes__ No__ If yes, list building and room number. _
Not applicable, animals left in their natural environment.

9. Outline any special requirements for caging, lighting, environmental control, diet, etc.
Animals will not be taken from habitat. They will be placed in their exact capture location a few minutes after they are caught.

ASSURANCE FOR THE HUMANE CARE AND USE OF ANIMALS
FOR TEACHING AND RESEARCH

The information included in this IACUC application is accurate to the best of my knowledge. All personnel listed recognize their responsibility in complying with university policies governing the care and use of animals.

All the experiments, described in this application, involving live animals will be performed under my supervision or that of another qualified scientist. Technicians involved have been trained in proper procedures in animal handling, administration of anesthetics, analgesics, and euthanasia as described.

The following signatures signify assurance that the individual(s) will comply with the protocol described herein. **Any changes in the above protocol must receive approval of the IACUC prior to implementation.**

Nov 16, 2010		11/17/10	
Principal Investigator (signature)	Date	Department Chairperson or Authorized Individual (signature)	Date

Date Original Application Received

Date Original Application Reviewed

Recommendations of IACUC

			Jan 24, 2011
Date Revised Application Received		Designated Reviewer (signature)	Date

Jan 24, 2011
Final Approval Date

Jan 24, 2011
Chairperson IACUC   Date

MARSHALL UNIVERSITY
www.marshall.edu

Joan C. Edwards School of Medicine
Animal Resource Facility

Dear Sir/Madam:

The following application and protocol to use laboratory animals at Marshall University was reviewed and received final approval by the Institutional Animal Care and Use Committee (IACUC) on January 24, 2011

Title of application: "Hibernacula site selection, hatchling overwintering behavior, and emergence from hibernation in the Eastern Box Turtle, Terrapene c. Carolina (Linnaeus, 1758) in West Virginia"

IACUC Project No.: 467

Name of Principal Investigator: Thomas Pauley

Co-Investigator: Benjamin Koester

As a condition of approval, the Institutional Animal Care and Use Committee required the following modifications to the above-referenced application:

None

Mon Valentovic

Monica A. Valentovic, Ph.D.
Chairperson, IACUC

1542 Spring Valley Drive • Huntington, West Virginia 25704-2901 9230
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APPENDIX B

Division of Natural Resources
Wildlife Resources Section
Operations Center
P.O. Box 67
Elkins, West Virginia 26241-3235
Telephone (304) 637-0245
Fax (304) 637-0250

Joe Manchin III
Governor

Frank Jezioro
Director

NUMBER 2010.252

SCIENTIFIC COLLECTING PERMIT

Under Authority Conferred by Chapter 20, Article 2, Section 50, Code of West Virginia, As Amended

Benjamin O. Koester
6288 Beech Drive, Apt. 8
Huntington, WV 25705

Marshall University

is hereby permitted to collect specimens according to the attached application and the Special Provisions on the reverse side of this permit.

This permit is not transferable and expires on December 31, 2010.

A complete list of all specimens collected will be kept and reported to the Director of the Division of Natural Resources of West Virginia no later than 45 days after the expiration date of this permit.

PERMIT PROVISIONS

I understand that (1) The privileges granted under this permit are not transferable, and to allow anyone other than myself to use my permit is unlawful and will be considered cause for revocation of said permit; (2) A Federal Scientific Collection Permit issued by the U.S. Department of Interior must be obtained before any migratory birds, or their nests or eggs, are collected or held in captivity; (3) The Federal Permit does not extend the privileges of the permittee beyond those granted by the Division of Natural Resources; (4) Permission must be obtained from either the owner or the custodian of any fenced or posted land before entering same for the purpose of collecting scientific specimens; (5) It is unlawful to carry a revolver or pistol contrary to Article VII, Chapter 61, Code of West Virginia; (6) It is unlawful to collect specimens with a gun on a Sunday; (7) It is unlawful to sell, offer for sale, barter, or offer to barter any wild animals, wild birds, fish or frogs collected; (8) When traps or nets or other devices are used UNATTENDED while exercising the privileges of this permit, said traps, nets, or devices must have attached thereto a tag bearing the name, address and number of the Scientific Collecting Permit; (9) It is unlawful to take or attempt to take any wild animals, wild birds, fish or frogs under said permit except for scientific and propagation purposes; (10) A hunting or fishing license must be obtained before specimens may be taken for sport; (11) Only those species or classes of wild birds, wild animals, fish or frogs listed below, and in the numbers stated, may be lawfully taken under said permit; and (12) I am required by law to carry my Scientific Collecting Permit, on my person while exercising the privileges granted thereunder, and to exhibit the permit to anyone requesting to see the same.

Must be signed before valid.

Benjamin Koester
Signature of permittee

Chief, Wildlife Resources, WVDNR

Date of issue 11-4-10

DIVISION OF NATURAL RESOURCES
Wildlife Resources Section
Operations Center
P.O. Box 67
Elkins, West Virginia 26241-3235
Telephone (304) 637-0245
Fax (304) 637-0250

Earl Ray Tomblin
Governor

Frank Jezioro
Director

NUMBER 2011.081

SCIENTIFIC COLLECTING PERMIT

Under Authority Conferred by Chapter 20, Article 2, Section 50, Code of West Virginia, As Amended

Benjamin O. Koester
6288 Beech Drive, Apt. 8
Huntington, WV 25705

Marshall University

is hereby permitted to collect specimens according to the attached application and the Special Provisions on the reverse side of this permit.

This permit is not transferable and expires on December 31, 2011.

A complete list of all specimens collected will be kept and reported to the Director of the Division of Natural Resources of West Virginia no later than 45 days after the expiration date of this permit.

PERMIT PROVISIONS

I understand that (1) The privileges granted under this permit are not transferable, and to allow anyone other than myself to use my permit is unlawful and will be considered cause for revocation of said permit; (2) A Federal Scientific Collection Permit issued by the U.S. Department of Interior must be obtained before any migratory birds, or their nests or eggs, are collected or held in captivity; (3) The Federal Permit does not extend the privileges of the permittee beyond those granted by the Division of Natural Resources; (4) Permission must be obtained from either the owner or the custodian of any fenced or posted land before entering same for the purpose of collecting scientific specimens; (5) It is unlawful to carry a revolver or pistol contrary to Article VII, Chapter 61, Code of West Virginia; (6) It is unlawful to collect specimens with a gun on a Sunday; (7) It is unlawful to sell, offer for sale, barter, or offer to barter any wild animals, wild birds, fish or frogs collected; (8) When traps or nets or other devices are used UNATTENDED while exercising the privileges of this permit, said traps, nets, or devices must have attached thereto a tag bearing the name, address and number of the Scientific Collecting Permit; (9) It is unlawful to take or attempt to take any wild animals, wild birds, fish or frogs under said permit except for scientific and propagation purposes; (10) A hunting or fishing license must be obtained before specimens may be taken for sport; (11) Only those species or classes of wild birds, wild animals, fish or frogs listed below, and in the numbers stated, may be lawfully taken under said permit; and (12) I am required by law to carry my Scientific Collecting Permit, on my person while exercising the privileges granted thereunder, and to exhibit the permit to anyone requesting to see the same.

Must be signed before valid.

Signature of permittee

Chief, Wildlife Resources, WVDNR

Date of issue 3-9-2011

DIVISION OF NATURAL RESOURCES
Wildlife Resources Section
Operations Center
P.O. Box 67
Elkins, West Virginia 26241-3235
Telephone (304) 637-0245
Fax (304) 637-0250

Earl Ray Tomblin
Governor

Frank Jezioro
Director

NUMBER 2012.088

SCIENTIFIC COLLECTING PERMIT

Under Authority Conferred by Chapter 20, Article 2, Section 50, Code of West Virginia, As Amended

Benjamin O. Koester
5037 West Pea Ridge
Huntington, WV 25705

Marshall University

is hereby permitted to collect specimens according to the attached application and the Special Provisions on the reverse side of this permit.

This permit is not transferable and expires on December 31, 2012.

A complete list of all specimens collected will be kept and reported to the Director of the Division of Natural Resources of West Virginia no later than 45 days after the expiration date of this permit.

PERMIT PROVISIONS

I understand that (1) The privileges granted under this permit are not transferable, and to allow anyone other than myself to use my permit is unlawful and will be considered cause for revocation of said permit; (2) A Federal Scientific Collection Permit issued by the U.S. Department of Interior must be obtained before any migratory birds, or their nests or eggs, are collected or held in captivity; (3) The Federal Permit does not extend the privileges of the permittee beyond those granted by the Division of Natural Resources; (4) Permission must be obtained from either the owner or the custodian of any fenced or posted land before entering same for the purpose of collecting scientific specimens; (5) It is unlawful to carry a revolver or pistol contrary to Article VII, Chapter 61, Code of West Virginia; (6) It is unlawful to collect specimens with a gun on a Sunday; (7) It is unlawful to sell, offer for sale, barter, or offer to barter any wild animals, wild birds, fish or frogs collected; (8) When traps or nets or other devices are used UNATTENDED while exercising the privileges of this permit, said traps, nets, or devices must have attached thereto a tag bearing the name, address and number of the Scientific Collecting Permit; (9) It is unlawful to take or attempt to take any wild animals, wild birds, fish or frogs under said permit except for scientific and propagation purposes; (10) A hunting or fishing license must be obtained before specimens may be taken for sport; (11) Only those species or classes of wild birds, wild animals, fish or frogs listed below, and in the numbers stated, may be lawfully taken under said permit; and (12) I am required by law to carry my Scientific Collecting Permit, on my person while exercising the privileges granted thereunder, and to exhibit the permit to anyone requesting to see the same.

Must be signed before valid.

Benjamin Koester
Signature of permittee

Chief, Wildlife Resources, WVDNR

Date of issue 2-15-2012

CURRICULUM VITAE

Curriculum Vitae

Benjamin O. Koester
Environmental Specialist
Environmental Services Division, GAI Consultants
343A Central Avenue,
South Charleston, WV 25303
Phone: 309-825-3965
Email: b.koester@gaiconsultants.com

Current Position

2012-Pres. **Environmental Specialist**. GAI Consultants
2010-2012. **M.S. Candidate** in Biological Sciences: Herpetology, Department of Biological Sciences, Marshall University
Advisor: Dr. Thomas Pauley
2010-2012. **Graduate Teaching Assistant**, Department of Biological Sciences, Marshall University
2011-2012 **GIS Digitizer**, West Virginia Division of Natural Resources

Education

2010-Pres. M.S., Biological Science: Herpetology, Marshall University, Huntington, WV
Expected Graduation Date: December 2014
Course Work Completed: May 2012
GPA: 4.0
2006-2010 B.S., Biology: Environmental Science, Liberty University, Lynchburg, VA
Degree Conferred: July 2010
Minor: History
2010 Graduate Record Examination (GRE)
600 Verbal; 620 Quantitative: 1220 Combined

Professional Experience

2012-Pres. Environmental Specialist at GAI Consultants
Responsibilities: Wetland and Stream Delineation, Writing Reports and Nationwide permits
2010-2012 Graduate Teaching Assistant at Marshall University
Courses Taught: Human Anatomy, Comparative Vertebrate Anatomy, Herpetology

Research Experience

Undergraduate Experience
2009
Population Density Study of Migrating Saw-Whet Owls
Advisor: Dr. Gene Sattler Ph.D. (Liberty University)
Behavioral Differences between Resident and Translocated Eastern Box Turtles
Advisor: Dr. Norman Reichenbach Ph.D. (Liberty University)
Home Range Preferences between Resident and Translocated Eastern Box Turtles
Advisor: Dr. Norman Reichenbach Ph.D. (Liberty University)
Mark-Recapture study of Red Spotted Newts
Advisor: Dr. Norman Reichenbach Ph.D. (Liberty University)
Impact Assessment of Waste-Water Treatment plant on Stream ecosystems
Advisor: Dr. Norman Reichenbach Ph.D. (Liberty University)

2009-2010

Allopatric and Sympatric Relationships between Peaks of Otter Salamanders and Red-backed Salamanders
Advisor: Dr. Norman Reichenbach Ph.D. (Liberty University) and Dr. Timothy Brophy Ph.D. (Liberty University)

Effect of Environmental Factors on Eastern Box Turtle Hibernation
Advisor: Dr. Norman Reichenbach Ph.D. (Liberty University)

Effect of Waste-water treatment plant effluent on avoidance behavior in central Virginia fish
Advisor: Dr. Norman Reichenbach Ph.D. (Liberty University)

Differing Thermal Minimum's effect on diversity of fish in low and high altitude streams
Advisor: Dr. Norman Reichenbach Ph.D. (Liberty University)

Graduate Experience

Master's Thesis

Hibernacula Site Selection of Eastern Box Turtles in West Virginia
Advisor: Dr. Thomas Pauley Ph.D. (Marshall University)

Grant and Funding

2010

Unfunded

National Science Foundation Graduate Research Fellowship
Virginia Herpetological Society Research Grant

Skills & Qualifications

- Fluent in SPSS, SAS, & Excel
- Telemetry
- Environmental Impact Assessment
- Mist-netting
- Bird Banding
- Mark-recapture studies
- Toe-clip and Injected elastomer methods of marking animals
- Knowledgeable with GPS technology
- Using Weather Stations and I-buttons
- Electro-shocking
- Museum curation
- Preparing and preserving specimens
- Collecting Marine Invertebrates
- Seining for fish and newts
- Removal Studies
- Proven ability to work on team research projects and alone
- Skilled at Bird and Reptile Identification
- Other lab and field experiences corresponding to classes taken
- GIS
- Wetland Delineation

Professional Memberships

- Association of Field Ornithologists
- Virginia Herpetological Society

- Society for the Study of Amphibians and Reptiles
- Chelonian Foundation

Interests

- Winter Ecology
- Reptile and Amphibian Ecology and Behavior
- Physiological and behavioral adaptations of ectothermic and endothermic animals to cold weather
- Hibernation Physiology

Public Outreach & Appeareances

2010-2012

I have coordinated and supervised graduate students going into elementary schools, providing underprivileged children and underfunded schools with free herpetology shows and biology talks in order to widen the horizons for these children.

Publications and Presentations

Publications

Koester, B.O. 2011. Eastern Box Turtle (*Terrapene carolina carolina*). **Catesbeiana** 31(1): 43-44

Presentations

Koester B. O., Pauley T.K. 2012. Hibernacula site selection in the Eastern Box Turtle, Terrapene c. carolina (Linnaeus, 1758) in West Virginia. Association of Southeastern Biologists in Athens, Georgia

Koester, B. O. and Reichenbach, N. 2011. Winter environmental conditions and their influence on Eastern Box Turtle (*Terrapene carolina carolina*) overwintering behavior in Central Virginia. Association of Southeastern Biologists in Huntsville, Alabama

Peters D. E., Kroschel W. A., Koester B. O., Pauley T.K. 2011. Morphometric differentiation between aquatic adult and terrestrial eft stages in West Virginia populations of Eastern Red Spotted Newts (*Notophthalmus v. viridescens*). Association of Southeastern Biologists in Huntsville, Alabama

References

Thomas Pauley, Ph.D.
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Marshall University
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Professor
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Liberty University
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300 Summers Street, Suite 1100
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