Investigation of nest predation as a cause of turtle population declines on the Sequoyah National Wildlife Refuge, Oklahoma

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By

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Science

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May 23, 2019

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Acknowledgments

This research was funded in part by the federally recognized tribe, the Cherokee Nation of Oklahoma. Boats for the nest predation and turtle population studies, and housing were provided by the Sequoyah National Wildlife Refuge. I would like to thank my major advisor, Dr. Jeffrey Lodge, for his guidance planning my academic and thesis schedules. I would also like to thank my committee members, Dr. Susan Smith Pagano and Dr. Karl Korfmacher for their input and support through this process. Most importantly, I am thankful to Rochester Institute of Technology and the Environmental Science program who allowed me to complete my thesis research with my father, Dr. Paul Shipman, to whom I owe a debt of gratitude and love. It was very special for me to partake in turtle research that my father has been working on for the past twenty years and more! Special thanks to Darrin Unruh, manager of Sequoyah National Wildlife Refuge, for his hospitality and patience with the turtle crew and nest crew. I also thank the members of the turtle crew and nest crew, citizens of Cherokee Nation: K. Kindle, C. Davis, A. McDonald, S. Potter, and D. Faddis, for their assistance in the field. Wado (thank you). Lastly, I thank my mother, Lori Shipman, fiancé, David Messner, along with family and friends for their unwavering support and love.
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Chapter 1

Investigation of nest predation as a cause of turtle population declines on the Sequoyah National Wildlife Refuge, Oklahoma

Abstract

Investigators of a turtle population study spanning 19 years reported major declines in capture rates of red-eared sliders (*Trachemys scripta*; 4.05 to 0.44) and Ouachita map turtles (*Graptemys ouachitensis*; 0.18 to 0.01) populations within Sequoyah National Wildlife Refuge in Vian, Oklahoma. Although capture rates of alligator snapping turtles (*Macrochelys temminckii*) collected in 2016 were comparable to those found in a 1997-2000 study (0.33 compared to 0.35), there was concern that the cause(s) of observed declines in smaller, shorter-lived turtle species could also impact alligator snapping turtle populations, and may not yet be apparent due to differences in life histories, such as longevity and age of sexual maturity.

Artificially constructed nests and trail cameras helped determine that nest predation, a common cause of turtle mortality is extremely high along six tributaries within the refuge (100%). Raccoons (*Procyon lotor*) were the most common nest predators, contributing to 71% of all nest predation. Alligator snapping turtle captures were higher in tributaries with higher nest predation suggesting that alligator snapping turtles may be contributing further to turtle mortalities. To alleviate the high rates of nest predation, the refuge should take action to manage common turtle nest predators, as well as investigate alligator snapping turtles as an additional turtle predator.
Introduction

1.1 Statement of the Problem

Research sponsored by the Cherokee Nation of Oklahoma on Sequoyah National Wildlife Refuge in Eastern Oklahoma found major population declines in the red-eared slider (*Trachemys scripta*), and the Ouachita map turtle (*Graptemys ouachitensis*) over a 19-year period (Richey et al., 2016). It was observed that red-eared sliders captured per net night (net night = one turtle trap set overnight for one night) was 0.44 in 2016 compared to 4.05 in 1997-2000 (Riedle et al., 2008), and Ouachita map turtle captured per night was 0.01 compared to 0.18 (Richey et al., 2016). These noted declines correspond to similar results found in unpublished surveys on the refuge in 2014 (Darrin Unruh, Sequoyah National Wildlife Refuge Manager, personal communication, 2016). While recent capture rates of the alligator snapping turtle (*Macrochelys temminckii*) were comparable to those found in the 1997-2000 study (0.35 to 0.33), the cause(s) of this decline in smaller, shorter-lived turtle species could also impact alligator snapping turtle populations, but may not yet be apparent due to differences in life histories (Richey et al. 2016).

The alligator snapping turtle is a large, freshwater turtle species with a long lifespan of approximately 80-100 years in the wild, has a low relative reproductive rate reaching sexual maturity at about 12-13 years of age (Pritchard, 1989). In contrast, the smaller red-eared slider reaches sexual maturity at 3-5 years of age in males, though the size of males is more important when determining sexual maturity at plastron lengths between 90-110mm (Gibbons & Greene, 1990). Female red-eared sliders reach maturity around 8 years old (Rhen et al., 1999), and Ouachita map turtles at 2-3 years of age in
males and 6-7 years in females (Ernst & Lovich, 2009). These smaller turtle species are expected to survive 20-25 years in the wild. Due to the gaps in age of sexual maturity between species and shorter longevities, the cause(s) affecting red-eared sliders and Ouachita map turtles could possibly be delayed in the alligator snapping turtle populations at the Sequoyah National Wildlife Refuge. Nesting for all three species takes place between the months of April through July (Carr, 1969; Behler, 1979). Hatchling emergence occurs anywhere between 60-100 days after nest construction (Carr, 1969; Ernst & Barbour, 1972.; Behler, 1979).

1.2 Nest Predation

A noticeable increase in potential turtle nest predators: feral hogs (*Sus scrofa*), raccoons (*Procyon lotor*), and nine-banded armadillos, (*Dasypus novemcinctus*) was observed in recent years (Darrin Unruh, Sequoyah National Wildlife Refuge manager, personal communication, 2016). However, population counts have not been recorded. The increased presence of these invasive species on the refuge may have a role in the observed turtle declines. Surveyors in the most recent turtle population study and preliminary artificial nest study also found evidence of nest predation (Fig. 1). Nest predation is recognized as one of the major causes of turtle mortality in both freshwater and marine turtles (Dawson et al., 2016; Holcomb & Carr, 2013).
Artificially constructed nests are commonly used to investigate turtle nest predation (Marchand et al., 2002; Burke et al., 2005; Hamilton & Freedman, 2002; Holcomb & Carr 2013; Dawson et al., 2016) without disturbing natural nests. This method was adopted from ornithological studies focusing on bird nest predation. Methods for artificially constructed nests in ornithology have improved over time, especially with the considerations of Major and Kendall (1996) who criticized past methods including the potential impact that human scent or disturbance could have on these studies with the recommendation that scientists take extra care in reducing human scent. Artificial nests are meant to be constructed as similarly as possible to natural nests. In comparison to artificial nests, natural turtle nests can be difficult to locate (Hamilton et al., 2002; Holcomb & Carr, 2013), especially for locations on the refuge that are only accessible by boat.

Figure 1. Evidence of nest predation on a group of turtles nests (unidentified species). Predation is determined by the spread of eggshells around the nests leading inland. Photo taken in June of 2017.
1.3 Preliminary Study

I investigated the possibility of nest predation as a cause of turtle population declines, through a preliminary study conducted in June of 2017. The study took place along Big Vian Creek, one of the refuge’s smaller tributaries of the Arkansas River. Following similar methods to the study conducted by Holcomb and Carr (2013), I found a 90% predation rate on ten artificially constructed nests. The nests were monitored using trail cameras and eight out of nine nests were observed to be depredated within the first 24 hours after construction. This rate is detrimental to turtle populations when considering that turtle hatchling incubation periods generally last two to three months. Alligator snapping turtles have a mean laboratory incubation period of 87 days (Holcomb & Carr, 2011). Nests were reset with eggs each morning after predation occurred for a total of three days for observation. The predation rates mentioned above were taken from the first observed predation on each nest. The sole purpose of resetting the nests was to observe nest predators. Raccoons were the most prevalent predators responsible for predation at 73%. The second most predominant predator was the nine-banded armadillo at 11%. All other potential predators were unidentifiable (16%). These results from this preliminary study were also comparable to the results of Holcomb and Carr’s study (2013). A continuation of this nest predation study was conducted to determine if nest predation is the major cause of turtle mortality and thus the cause of turtle population declines on the refuge. I hypothesized that creeks with higher nest predation would have lower turtle captures. Raccoons were expected to be the predominant nest predator due to prior observations.
Materials and Methods

2.1 Site Description

Sequoyah National Wildlife refuge, located in eastern Oklahoma, was established in 1970 as part of the construction of the Robert S. Kerr Reservoir built by the U.S. Army Corps of Engineers. The refuge covers 20,800 acres and within its boundaries contains part of the Arkansas River, a navigation waterway system, with adjacent lakes, streams and wetlands, as well as a lock and dam system.

The population surveys and artificial nest studies took place along six accessible tributaries within the refuge: Dirty Creek, Hezekiah Creek, Big Vian Creek, Little Vian Creek, Negro Creek, and Canadian River (Fig. 2).

Figure 2. Sequoyah National Wildlife Refuge boundary and the six study sites (Riedle et al. 2008).

2.2 Turtle Nest Predation Study
During the months of May, June, and July of 2018, I conducted the turtle nest predation study and population survey in the selected streams and rivers. To be consistent with previous population surveys, methods were the same as those used by previous investigators (Riedle et al., 2008) for the duration of three to four days per site. Ten specifically designed turtle hoop nets from Memphis Net and Twine Co. were set upstream of structures (i.e. submerged logs) and baited with fish suspended on a hook within the net. The nets were 2.1m in length with four 1.05m hoops that are covered with 2.5cm mesh. The nets sat overnight and were checked the following morning; this is referred to as a trap night. Capture rates were determined by the average of each species captured per trap night per site. Turtles captured in each hoop net were counted, identified, and recorded. Captured alligator snapping turtles were further studied by taking body measurements, weight, and were marked (unless it was a recapture). Alligator snapping turtles were marked by drilling harmless holes in the marginal scutes following a numbering system established by previous principal investigators (Riedle, et al., 2008).

Artificial nests were constructed by digging a hole approximately 20-22cm deep and 15-20cm wide using a shovel (Holcomb & Carr, 2013; Fig. 3). Five to six chicken eggs were placed in the hole and covered with the displaced soil to create a mound that was about 10-12cm high. Gloves and rubber boots were worn while constructing nests to reduce human scent. A trail camera (Bushnell Trophy Cam) was secured to a tree trunk near the constructed nest site about 1-2 feet high off the ground. Images from the trail cameras were used to determine predator species, time to first predation, as well as other species visits. The nests were left overnight (one nest night), checked, and reset every day.
for three to four days. The eggs used for this study were both store bought and rejected eggs from a local chicken farm. Ten nests were constructed in each tributary in areas that were easily accessible from the river, and ideally, areas with sandy soils in a low vegetated area (Congdon & Gibbons, 1990), not located near any natural nesting sites. Artificial nest sites were constructed along the same tributaries as the population surveys.

![Figure 3. Example of an artificially constructed nest.](image)

The following environmental measures that may impact nest predation rates collected were: percent overhead canopy cover, slope of bank, soil type (clay, silt, sand, soil), percent ground vegetation coverage in a 5 meter radius from the nest, and temporal data: minimum and maximum daily temperature, precipitation, and moon phase. Temporal data and moon phases were collected from Mesonet (Brock et al., 1995; McPherson et al., 2007), an Oklahoma climatological survey. The data were collected from the Webber Falls monitoring station, northwest of the wildlife refuge (5-17 miles from each tributary). Collecting nest site characteristics is important to understanding and compiling complete natural histories for freshwater turtles (Geller, 2012). Riverine turtles


have the highest proportion of threatened species when compared to other vertebrates, including most North American turtle species that are threatened with extinction in this century (Barko & Briggler, 2006). A complete natural history and understanding of nesting habitats and behaviors helps one to better understand the land turtles need to carry out this process for population recruitment. Similarly, it is important to understand how habitat features affect nest predation since predators can affect turtle population dynamics (Dawson et al., 2014; Strickland et al., 2010).

2.3 Analysis

Predator species, first time to predation, predation rates, and the overall count of species visits and predator richness for each tributary were determined by the collected trail camera images. Nest site visits were counted by identifying the species in an image, and visits by the same species were considered unique if there was an hour long gap between visits. Predation that was visibly apparent at a nest check but had no camera evidence due to activity not detected by the camera or blown out images, were labeled ‘unknown’.

The number of red-eared slider, Ouachita map turtle, and alligator snapping turtle captures were compared to tributaries with the highest and the lowest predation using a t-test. ANOVA (Zar, 1984) was used to determine significance in trends for predator visits, predator species richness, and predation. A canonical correspondence analysis (CCA), a multivariate analysis technique that shows the relationships among sample sites, species, and environmental variables (ter Braak & Prentice, 1988), was used to find a relationship between species visits (predator and non-predator) versus temporal and physical nest site characteristics for depredated nest sites only. Any species that were recorded with two or fewer visits were not included in the CCA. All analyses were computed using PAST, a
Results

3.1 Nest Predators and Predation

Out of 218 nest nights, the total nightly predation for the six tributaries was 84%. Total first nightly predation was 73% out of 60 nest nights. Big Vian and Dirty Creek both had 100% predation on the first nest night, with 100% and 95% average nightly predation rates respectively. Little Vian, Negro Creek, Hezekiah Creek, and Canadian River averaged 80%, 78%, 75%, and 73% per night. All artificial nests were depredated within 1-4 nights in all tributaries, resulting in an overall nest predation rate of 100%. One nest each on Hezekiah Creek and Canadian River remained undisturbed for three nest nights but were depredated by the fourth night. There was significant difference in nightly nest predation among tributaries (ANOVA p-value < 0.05, F-value = 2.289, df = 5; Fig. 4). Big Vian and Dirty Creek had significantly greater nightly predation than Hezekiah Creek and the Canadian River (Mann-Whitney Pairwise p-value < 0.05).
Figure 4. Nightly nest predation for each tributary. Big Vian had 100% nest predation overall for each night. Hezekiah had the lowest with 72.5% nest predation for each night. Nightly nest predation on the Canadian River and Hezekiah Creek were significantly different from nightly nest predation on Big Vian and Dirty Creek. The error bars denote the standard deviation.

The average time to first predation overall was 11.38 hours from nest construction with the average nest night time between set and checks at 23.39 hours. Nineteen total species (potential nest predators and non-predator species) were observed through the trail camera images with eleven of those species identified as nest predators. The eleven predator species included: raccoon (*Procyon lotor*), nine-banded armadillo (*Dasypus novemcinctus*), Virginia opossum (*Didelphis virginiana*), feral hog (*Sus scrofa*), bobcat (*Lynx rufus*), small rodent (unknown species.), turkey vulture (*Cathartes aura*), crow
(Corvus spp.), coyote (Canis latrans), river otter (Lontra canadensis), and egret (Ardea alba).

The number of predator visits among tributaries were significantly different (ANOVA p-value = 0.001, F-Value = 3.914, df = 5). Predator visits along the Canadian River were significantly different from the rest of the tributaries (Mann-Whitney Pairwise p-value < 0.05; Fig. 5). This is also true for predator species richness (Mann-Whitney Pairwise p-value < 0.05; Fig. 6).

**Figure 5.** Mean nightly predator visits for all six tributaries. Canadian River predator visits were significantly lower compared with four other tributaries (not significantly different from Hezekiah Creek which was significantly different from Dirty Creek) with raccoons and feral hogs noted as the only predators. In comparison to raccoons, nine-banded armadillos, opossums, and turkey vultures observed on Dirty Creek, and raccoons, nine-banded armadillos, opossums, coyote, and crows on Negro Creek. The error bars denote the standard deviation. Sample sizes are found in Appendix 1.
Figure 6. Mean predator species richness for each tributary. Canadian River had the lowest count for species richness and was significantly different from the five other tributaries. The error bars denote the standard deviation.

Raccoons were the most common nest predator with 371 unique visits overall, contributing to 68% of all nest predation and 71% of first nest night predation (Table 1). Nine-banded armadillo was the second most common nest predator with 34 unique visits overall, 8% of all nest predation and 9% of first night predation. Opossum had 54 unique visits and feral hog had 3 unique visits. Both species account for 2% and 1% of nest predation respectively. Other predators such as mouse (Spp.), turkey vulture, coyote, river otter, and egret were observed as predators that visited an artificial nest after a nest was depredated by another species (Appendix 1).
**Table 1.** Summary of first nest night predation and total nest predation by species (%).

<table>
<thead>
<tr>
<th></th>
<th>Raccoon</th>
<th>Nine-Banded Armadillo</th>
<th>Opossum</th>
<th>Feral Hog</th>
<th>Crow</th>
<th>Bobcat</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>First nest night predation (%)</td>
<td>71</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>n=44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predation (%) of all depredated nest nights</td>
<td>68</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>n=184</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2 Turtle Captures

A total of 367 red-eared sliders (Table 2) were captured for a capture rate of 1.59 individuals per trap night (n=230). Capture rates for Ouachita map turtles and alligator snapping turtles was 0.09 and 0.13 respectively (Table 3).
Table 2. 2018 summary of turtle captures per species. Common species names are found in appendix table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Trachemys scripta</th>
<th>Graptemys ouachitensis</th>
<th>Macrochelys temminckii</th>
<th>Sternotherus odoratus</th>
<th>Chelydra serpentina</th>
<th>Apalone spinifera</th>
<th>Graptemys kohnii</th>
<th>Kinosternon subrubrum</th>
<th>Pseudemys concinna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big Vian</td>
<td>20</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dirty Creek</td>
<td>78</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Little Vian</td>
<td>46</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hezekiah Creek</td>
<td>100</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Negro Creek</td>
<td>81</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Canadian River</td>
<td>42</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total Captures</strong></td>
<td><strong>367</strong></td>
<td><strong>21</strong></td>
<td><strong>31</strong></td>
<td><strong>9</strong></td>
<td><strong>9</strong></td>
<td><strong>13</strong></td>
<td><strong>2</strong></td>
<td><strong>2</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>

To determine if turtle capture rates were related to nest predation rates, two-sample t-tests were used to compare capture rates between the two tributaries with the highest predation (Big Vian and Dirty Creek) and lowest predation (Hezekiah and Canadian River). Red-eared slider capture rates are not significantly different between tributaries with the lowest and highest predation (t-test = 0.71, p-value > 0.05, low predation df = 6, high predation df = 7; Fig. 7).
However, Ouachita map turtle (t-test = 2.00, p-value < 0.05, low predation df = 6, high predation df = 7; Fig. 8) and alligator snapping turtles were significant (t-test = 4.56, p-value < 0.05, low predation df = 6, high predation df = 7; Fig. 9).

**Figure 7.** Mean capture rates of red-eared sliders between tributaries with high and low predation rates. Although the data was insignificant there is a general trend that there are fewer red-eared slider captures in tributaries with higher nest predation rates. The error bars denote the standard deviation.
Figure 8. Ouachita map turtle mean capture rates between tributaries high and low predation rates. Capture rates are significantly lower in tributaries with higher predation rates than in tributaries with lower predation rates. The equal variance assumption has been met between the two groups. The error bars denote the standard deviation.
Figure 9. The mean alligator snapping turtle capture rates between tributaries with high and low predation rates. Unlike captures of red-eared sliders and Ouachita map turtles, there were higher capture rates of alligator snapping turtles in tributaries with higher predation rates. The error bars denote the standard deviation.

Table 3. Summary of turtle capture rates through the years.

<table>
<thead>
<tr>
<th>Year</th>
<th>Red-eared Slider</th>
<th>Ouachita Map Turtle</th>
<th>Alligator Snapping Turtle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997-2000</td>
<td>4.05</td>
<td>0.18</td>
<td>0.35</td>
</tr>
<tr>
<td>2016</td>
<td>0.44</td>
<td>0.01</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>2018</strong></td>
<td><strong>1.60</strong></td>
<td><strong>0.09</strong></td>
<td><strong>0.13</strong></td>
</tr>
</tbody>
</table>
3.3 Nest site Temporal and Physical Characteristics

Physical nest site characteristics: tributaries (location), canopy coverage (%), ground cover (%), slope of bank, and soil type, are significantly related to depredated nest sites and nest predator species (Canonical Correspondence Analysis p-value = 0.015, N = 148 Fig. 10). The first three axes explained 71.63% of the explainable variation in the data. Raccoons, small rodents, nine-banded armadillos, white-tailed deer, and gray squirrels were central to physical nest site characteristics as shown in Figure 9. Big Vian and the Canadian River are associated with steeper slopes, and soils with more organic matter. Little Vian had the most canopy coverage by percent, and clayey soils. Dirty Creek was more associated with sandier soils and is most different from Hezekiah Creek.

Temporal data which included: minimum, maximum, and average daily temperature, precipitation, and moon phase, were not significantly related to depredated nest sites or nest predator species (Canonical Correspondence Analysis p-value=0.56).
Figure 10. Canonical correspondence analysis of physical nest site characteristics, predators and non-predators. The data included the characteristics of all depredated nests (blue) with identified predators (red) and non-predators.

Discussion

4.1 Nest Predation

The overall predation on six tributaries within Sequoyah National Wildlife Refuge, based off of first nest night predation alone (73%), was high which is a cause for concern for turtle populations. All artificial nests were depredated by the end of each study week and only two nests survived a total of three nest nights before predation was observed. Raccoons and nine-banded armadillos accounted for 76% nest predation overall. With
371 unique raccoon visits between 218 nest nights, raccoons dominated first night predation at 71%. Nine-banded armadillo is the second most common nest predator contributing to 9% of first night predation. It is also likely that most of the unknown nest predators, due to faulty trail cameras or unclear images that accounted for 18% of nest predation, were raccoons and nine-banded armadillos. Overall predation contributed by opossum and feral hog were low at 2% and 1%. Neither were observed as first night predators. Crows and bobcats contributed 2% and 1% of overall nest predation as well.

There was no surprise that raccoons were the dominant nest predator because it is not a controlled species within the refuge, it is a generalist with respect to habitat, and is an opportunistic feeder (Heske & Ahlers, 2016; Fig. 11). It is also a well-known turtle nest predator (Frazer, Gibbons, & Greene, 1990; Wilbur & Morin, 1994). I made the same observations with respect to raccoons as the dominant nest predator made during my preliminary study in 2017. Other artificial nest studies also found raccoons to be the dominant turtle nest predator. Holcomb and Carr (2013) studied nest predation of alligator snapping turtles in Black Bayou National Wildlife Refuge, Louisiana. Ninety artificial alligator snapping turtle nests were constructed using chicken eggs, all of which were depredated. The study concluded that raccoon and the nine-banded armadillo were the two most common predators. Burke et al. (2005) concluded that raccoons preyed upon artificially constructed diamond-backed terrapin (*Malaclemys terrapin*) nests similarly to natural nest sites and most nests were depredated the first night after construction. Hamilton et al. (2002) observed an 89% predation rate on artificially constructed red-eared slider nests in Florida where raccoon and opossum tracks were observed in all habitat types. Geller (2015) reported 97% first night predation on empty
artificial nests that lacked non-egg or turtle olfactory cues in comparison to 95% predation on natural nests. All nest types were believed to have been depredated by raccoons.

**Figure 11.** One of the many images collected showing a raccoon depredating an artificial turtle nest (Canadian River, 2018).

4.2 Turtle Captures

Overall, the capture rates for the three species of interest were still less than the original study capture rates in 1997-2000, and differed from the 2016 capture rates (Table 3). Red-eared slider captures were not as low as reported in the 2016 population survey but were still lower than the 1997-2000 capture rates (4.05, 2287 individuals over 565
trap nights; Riedle et al., 2008) with a capture rate of 1.60 (367 individuals over 230 trap nights, 2018). Ouachita map turtle captures in 2018 were half as much as the 1997-2000 capture rate of 0.18 (103 individuals over 565 trap nights; Riedle et al., 2008) with 0.09 (21 individuals over 230 trap nights, 2018). Alligator snapping turtle populations have declined since 2016 (0.33) with a capture rate of 0.13 (30 individuals over 230 trap nights). In the 1997-2000 study there were 197 alligator snapping turtle captures over 565 nest nights (Riedle et al., 2008). Ouachita map turtle and alligator snapping turtle capture rates within tributaries with high predation (Big Vian and Dirty Creek) and low predation (Hezekiah and Canadian River) are significantly different. When comparing data, there was an apparent trend that there were less captures of Ouachita map turtles while there are more captures of alligator snapping turtles in tributaries with high predation. Although captures of red-eared sliders between high and low predation was insignificant, there were nominally less captures in tributaries with higher predation. These trends are important to note because this suggests that alligator snapping turtles may potentially be contributing to turtle population declines in addition to high rates of nest predation.

4.3 Evidence of Alligator Snapping Turtles as Turtle Predators

Alligator snapping turtles eat other turtles including their own kind and are also likely to eat turtle nest predators, including raccoons. Elsey (2006) collected stomach and intestinal contents of 109 alligator snapping turtles in Louisiana and Arkansas. The study found that between five tributaries, 30% of the stomach and intestinal content samples collected were turtles (species type was not specified). Raccoons, armadillos, and opossums made up 1% each of the total collected contents. Almost 2% of the contents
were feral hog and 7% percent of the contents were unidentified mammals. This observation may explain higher capture rates of alligator snapping turtles within streams with higher predation rates. Two particular instances of potential alligator snapping turtle predation of other turtles was found within Big Vian (Fig. 12). A melanistic red-eared slider was found with an injury to its hind left leg and shell, and a stinkpot (Sternotherus odoratus) was missing its tail and part of its hind shell. A similar incident was observed on Onion Creek in Kansas in 1991 by Shipman, Edds, and Blex (1994). Two common snapping turtles (Chelydra serpentina) were found with injuries to the lower left shell and limb within a reasonable distance from an alligator snapping turtle.

Additionally, evidence of turtle nest predation was found in all tributaries though alligator snapping turtle nesting sites within the refuge did not appear to be disturbed nor depredated, unlike other natural nests along each tributary (Fig. 13 & 14). That being said, there is certainly more to be discovered concerning alligator snapping turtle community interactions, nest site selection, as well as its effect on other turtle populations.
Figure 12. Instances of potential alligator snapping turtle predation. Both the red-eared slider (left) and stinkpot have injuries to their carapaces. The red-eared slider is missing part of its left hind limb, and the stinkpot is missing its tail.

Figure 13. Five tributaries within the refuge boundary had intact alligator snapping turtle nests. Dirty Creek had two sites for a total of six nesting sites that were marked.
Figure 14. Alligator snapping turtle nest mounds were located on five tributaries within the refuge. The nesting mounds were often found in groups (rookery).

4.4 Tributary Characteristics

There are differing habitats between Big Vian and Dirty Creek compared with Hezekiah and Canadian River that may contribute to the differences in nest predator activity. Big Vian and Dirty Creek are adjacent farm fields that could contribute to higher predation since there is more habitat disturbances (i.e. forest edge, and forest-farmland edges) and available food sources for opportunistic feeders such as the raccoon (Heske & Ahlers, 2016) and nine-banded armadillo. The canonical correspondence analysis supports this as nine-banded armadillos, opossums, and especially raccoons, were shown central to the physical nest site characteristics. The canonical correspondence analysis also showed that Dirty Creek and Hezekiah differ from one another as either creek was
located on opposite ends of the graph (axis 2). Hezekiah Creek and the Canadian River are more isolated from anthropogenic disturbances such as farmland and major roadways. The Canadian River is thermally different from the five other tributaries because this portion of the river is a cold water release from the Eufaula Dam upstream. Several nests were also constructed on a large island within the river. The theory of island biogeography may come into play, where smaller islands such as the one within Canadian River, will not support the same number of species (in this case, nest predators) as a larger island or mainland habitat (MacArthur & Wilson, 1967). This was supported by the predator species richness ANOVA. Thus, both thermal and island isolation attributes may explain lower predation on the Canadian River.

4.4 Conclusion

Even though the species of interest are currently not on the endangered species list, there are known threats to habitats and recruitment due to habitat disturbance, habitat destruction, and nest predation (Baker et al., 2013; Bolton, 2007; Dieter et al., 2014; Oddie et al., 2015; Sterrett et al., 2011; Wirsing et al., 2012). All map turtles (*Graptemys spp.*), common snapping turtles, spiny and smooth softshell turtles, and alligator snapping turtles are listed by the Fish and Wildlife Service (2005 and 2016) on Appendix III of the Convention of International Trade in Endangered Species of Wild Fauna and Flora. This ruling protects these species (or attempts to) from the additional threat to survival through harvest and trade, though this ruling does not protect the environments in which they reside outside of the refuge boundary. Alligator snapping turtle populations on the refuge were once thought to be the most robust in the state of Oklahoma (Riedle et al., 2008). However, analyses conducted by Folt et al. (2016) found that populations of alligator
snapping turtles in Oklahoma are at imminent risk of extinction in less than 15 years likely caused by habitat disturbances and lasting impacts from historical harvests.

Although my research did not include monitoring of natural nests, the aftermath of nest predation on natural nests was observed on all six tributaries. Due to the high rates of predation observed throughout Sequoyah National Wildlife Refuge on artificial nests and evidence of natural nest predation, I recommend that the refuge take action to reduce turtle nest predation, especially predation caused by raccoons. I also recommend that the refuge investigate alligator snapping turtles as potential turtle predators and the steep declines in alligator snapping turtle populations. Research is already planned for the summer of 2019 to confirm the lower alligator snapping turtle capture rates.

The refuge currently has an eradication plan in place for feral hogs to protect the farmland that provides food for migrating birds. However, the refuge currently does not control raccoon populations. Eradication methods have limited success and can be costly, assuming management resources are limited (Engeman et al., 2016). Eradication methods have the added complication of needing to dispose of deceased predators and risk affecting the ecosystem when removing an established population (Lashley et al., 2018). Eradication does not guarantee that a population will not return to an area which also creates the issue of knowing when eradication programs should begin again when a population does return (Engeman et al., 2016). Because of these issues presented with eradication programs, I propose a different method utilizing behavioral conservation management: Batesian mimicry nest modeling. This method also utilizes artificially constructed nests but with the addition of chicken eggs injected with castor oil and dish soap, or a tobacco mixture A successful execution of the method will be determined
when nest predators learn to avoid turtle nests after encountering the model nests and falling ill due to the noxious chicken eggs. With further study and experimentation, this could be an alternate method and less-intrusive method when compared to eradication programs, and other predator management methods as discussed in the next chapter.

**Literature Cited**


Chapter 2

Preliminary study on Batesian mimicry modeling: A novel predator management method

Abstract

In response to high turtle nest predation on the Sequoyah National Wildlife Refuge, I investigated an alternate predator management method utilizing artificially constructed nests. Nests were constructed with both plain and treated eggs which served as the Batesian mimicry learning model for nest predators. The two egg treatments consisted of a tobacco mixture, and a mixture of castor oil and dish soap to deter nest predators. Overall, both treatments reduced nest predation on artificial turtle nests by 5%. This is a novel method and with further experimentation, it could potentially be recommended to the refuge as a predator management method.

Introduction

Eating noxious species can be energetically taxing to foragers (Halpin et al., 2017) such as raccoon and nine-banded armadillos. Turtle eggs are a seasonal food source for predators because the eggs are only available during the spring and summer months. Predators have to determine if it is worth the time and energy to forage for turtle eggs. Batesian mimicry is the portrayal of a harmless species imitating characteristics of an unpalatable species (Honma, Takakura, Nishida, 2008, Gamberale-Stille et al., 2011). The idea for this learning model is that when predators attempt to eat tainted chicken eggs (unpalatable model), they will become ill. After repeated exposure to the unpalatable model, predators will eventually learn to avoid natural nests (palatable mimic) because of the similar appearance to the noxious, artificially constructed nest
eggs (Fig. 14) as well as learn that it is not worth their time to forage for turtle eggs (Honma, Takakura, Nishida, 2008).

**Figure 14.** Batesian mimicry artificial turtle nest model concept.

As mentioned in the previous chapter, the refuge currently does not have a conservation management method in place to address the decline in turtle populations and high nest predation. If this method was successful, this learning model would be recommended for the refuge’s use in order to improve turtle populations, as opposed to resorting to other more invasive methods such as predator eradication.

This learning model addressed a knowledge gap in behavioral conservation management. Learning behavior in conservation management practices is fairly new,
with studies surfacing within the past 10-15 years. Published articles have failed to provide applicable advice that do not include implementation and emphasis on behavioral methods in conservation management (Berger-Tal et al., 2015). Additionally, if turtle nest predation is reduced by 5-10% using the Batesian mimicry model, this could potentially double the recruitment for heavily depredated turtle populations. In other words, if the number of surviving nests are doubled, that would be a 100% increase in surviving nests. I predicted that artificial nests containing eggs treated with unpalatable chemicals would experience lower predation rates than artificial nests with untreated eggs. Additionally, nest predators would learn to avoid the nests after poor experiences with the treated nests and

**Materials and Methods**

Artificial nests for the Batesian mimicry learning model study were constructed similarly as described in the previous chapter, though the chicken eggs underwent treatment using a non-toxic dose of an unpalatable chemical. The two treatments included a tobacco water mixture and a mixture of castor oil and dish soap. In addition to its bitterness, tobacco leaves also contains the neurotoxin, nicotine that may cause vomiting. Castor oil is a laxative and may cause diarrhea in high doses. Castor oil is also unofficially listed as a repellent for small mammals. The addition of dish soap was to add bitterness to the mixture. This study took place within the refuge’s Sandtown Bottom auto tour route on both open and restricted access roads. Ten transects with eight constructed nests each were placed 10 strides apart (5-10m). Each transect site was constructed at random within the refuge auto tour loop (Fig. 15) and did not take place
near the tributaries used for the turtle nest predation study. The beginning and end of transects were marked using pink tape attached to a tree branch or tall grass.

**Figure 15.** Ten transects were dispersed throughout the refuge auto tour loop along both public and restricted access roads.

Unlike the nest predation study, these nests were observed and reset once per week. Nests that were not depredated on the first night were checked daily until predation was observed. For 2-3 weeks the transect nests were set without treatment to train predators to return to the nests. The first treatment consisted of 1 part table salt, 1 part mustard powder 2.5 part tobacco leaves soaked in one gallon of distilled water. The
second treatment comprised of one part castor oil and one part dish soap. Five to ten milliliters of egg white in each treatment egg was removed using syringes and replaced with 5-10mL of the treatment mixture. The hole created by injection was covered using paraffin wax. All control eggs were also painted with paraffin wax for consistency when the treated eggs were deployed. After the training period, the treatment eggs were applied to four nests each (every other nest at each transect) at five transects for three weeks. The remaining nests were constructed with control eggs. The nests were checked the following day or until the nest was depredated. No trail cameras were used for this experiment because predation activity was based solely on nest disturbance or predation indicators. A t-test of nightly, first night predation was run to determine the effectiveness of both treatments combined versus the predation of control nests for the first nest night using PAST (Hammer et al., 2001). The tobacco mixture was used for four study weeks (80 nest nights each for controls and treated nests), while the castor oil and dish soap was used for one study week (20 nest nights each for the controls and treated nests).

**Results**

Much like the nest predation study on the six refuge tributaries (Chapter 1), all nests were eventually depredated (Fig. 16), but treatments delayed nest predation on both controlled and treated nests (Fig. 17 & 18).
Figure 16. A depredated control nest (foreground).
Figure 17. Mean of first night predation on control nests and treated nests observed after nest construction or nest reset (total of 80 nest nights each).
Figure 18. Mean of first night predation (predation observed after the first night of construction/reset) between control nests, and nests treated with castor oil and dish soap (20 nest nights each).

The first three weeks of the tobacco treatment did not show any change (100% first night predation). Overall predation of the nests treated with the tobacco mixture was 91%, while control nest predation was 98%. After one application of castor oil and dish soap, treated nests had 60% predation while control nests had 80% predation. While there was no significant variance in control nests between weeks three and four of the tobacco treatment, there were two control nests that were not depredated on the first night during the fourth week of the treatment (t-test = 1.41, p-value > 0.5, N=40; Fig. 19). Predation of the control nests prior to the addition of the castor oil treatment was significantly different from the predation of control nests during the treatment week (t-test = 2.18, p-value < 0.05, N = 20; Fig. 20). Tobacco and castor oil treatment predation counts
combined showed a 5% difference in first night predation on treated and control nests (t-test = 2.09, p-value < 0.05, N = 101; Fig. 21). There was also evidence of vomit or scat (Fig. 22) on two transects, one treated with the tobacco mixture, the other with castor oil mixture on opposite ends of the study area (transects 2 and 8).

**Figure 19.** The means of first night predation between control nests during weeks 1-2 and 3-4 along transects of nest treated with tobacco. Predation between the treatment weeks were not significant.
**Figure 20.** Mean first night predation of control nests prior to treatment vs control nests after castor oil and dish soap treatment. Predation between the control week and treatment week was significantly different.
Figure 21. Mean first night predation of control nests and all treatment nests (tobacco and castor oil). The difference was significant. The error bars denote standard deviation.

Figure 22. Evidence of wildlife vomit/scat near nest sites that were treated with the tobacco mixture (left) and castor oil and dish soap mixture.
Discussion
The Batesian Mimicry model method is labor intensive but it is unlike other common predation management methods because this method allows for multiple replicates (Hamilton, 2002) and does not require disturbance of natural nests. Other nest predation management methods include: wire cages, screens (mesh), pepper powder, hot sauce, and substrate sweeping to name a few. However, all of these methods disturb natural nests and may risk the survivability of turtle eggs. Wire cages have been used to protect turtle nests, but the method has mixed reviews. Engeman et al. (2016) found that cages on marine turtle nests did not prevent predation by feral hogs, but reduced predation by raccoon. Mroziak et al. (2000) reported that raccoons actually used the cages to locate turtle nests. The same is true for turtle nests protected by metal or plastic mesh screens (Lamarre-DeJesus and Curtice, 2013). Habanero pepper powder sprinkled over natural nests was also reported with conflicting outcomes. For loggerhead sea turtle (Caretta caretta) nests in South Carolina, predation was reduced (Lamarre-DeJesus and Curtice, 2013), but predation by raccoons on diamondback terrapin (Malaclemys terrapin) nests increased (Burke et al., 2015). Another method that attempted to reduce nest predation included sweeping of nest mound substrate to remove any visual and olfactory cues of Ouachita map turtle nests (Geller, 2015). This method was also unsuccessful.

Further study is necessary to determine the effects of the Batesian mimicry model on natural turtle nests. Due to time and resource constraints, I was unable to conduct the study long enough to determine the effectiveness of the treatments (other than the 5% reduction in predation between both treatments) and to determine the proper time needed for the treatment to be deemed effective. To improve this experiment, I would take a
more rigorous approach to reset the nests on a daily basis. Since each treatment was only applied on a weekly basis it is likely that nests were visited by multiple different individual predators over time which may have interfered with the learning model. It is promising that there was evidence of either vomit or scat along two transects, which indicated that the treatments had some form of effect on a nest predator. As mentioned before, reducing nest predation by 5-10% has the potential to double turtle recruitment for the following year. With further research this method could potentially be used to manage the high nest predation along the six tributaries on Sequoyah National Wildlife refuge.
Literature Cited


### Appendices

#### Appendix 1. Species Key

**Predator Species**

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Acronym</th>
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