AN ABSTRACT OF THESIS OF

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Title:

Male response to female chemical signals in Painted Turtles (*Chrysemys picta*) and changes in populations of Painted Turtles and Red-eared Sliders (*Trachemys scripta)* in two Kansas ponds.

Abstract approved:____

Turtles and other sexually reproducing animals require communication between males and females to identify each other. Although chemical signals are widely used by reptiles to communicate beneficial information for both sender and receiver, chemical communication studies in turtles are rare. I recorded the amount of time a male Painted Turtle (*Chrysemys picta)* spent in three sections of a pool: a section with female a Painted Turtle, a section with a male Painted Turtle, and an empty (control) section. Each section contained an opaque, plastic box with drilled holes that allowed chemical signals to be exchanged but prohibited the turtles from seeing into or out of the box. I completed 27 trials and recorded the time male turtles spent in each section of the pool (male, female, or empty). ANOVA and a Tukey's test showed that male Painted Turtles spent significantly more time in the section of the pool containing the female Painted Turtle than in the control section of the pool. No statistically significant difference was found for the time male Painted Turtles spent in sections of the pool containing another male vs. a female, and no statistically significant difference was found between the time spent in sections of the pool containing another male vs. an empty pool section. The preference of

the female section over the empty sections shows communication through chemical signals in Painted Turtles. Developing a better understanding of turtle communication may help improve the success of captive breeding programs for these animals.

Turtles are relatively long-lived animals. Studying such organisms requires long-term research as any population changes may take many years to observe. Studies focused on changes in turtle populations over time, especially within ponds, are rare. Nall and Thomas (2009) set turtle traps in two Lyon County, KS, ponds in 2007, and I set traps in the same ponds again in 2015; Painted Turtles and Red-eared Sliders (*Tracheyms scripta*) are found in both of these ponds. Species, sex, and plastron length were recorded for each turtle captured. Painted Turtles were more abundant than Red-eared Sliders in both ponds during both years. None of the individual turtles caught in 2007 were captured in 2015. Chi-square tests revealed that the proportion of male, female, and juvenile Painted Turtles varied significantly from 2007 to 2015 for both Gladfelter Pond and Fence Pond. However, low capture numbers of Red-eared Sliders at either pond prohibited a chi-square test to determine changes in the population. While a chi-square test could not be completed with meaningful results for Red-eared Sliders, the percentage of the population made up by juvenile turtles decreased in both species. T-tests revealed that there were no significant changes in mean plastron lengths for Painted Turtles at either pond. Again, too few Red-eared Sliders were captured at either pond to complete a t-test.

Male response to female chemical signals in Painted Turtles (*Chrysemys picta)*

A Thesis

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PREFACE

This is a multi-chaptered thesis with four chapters. Chapter 1 gives a short introduction to the whole thesis. Chapters 2 and 3 will both be submitted to *Transactions of the Kansas Academy of Science* and are formatted in the style required for that journal. Chapter 4 provides a summary of the major conclusions of chapters 2 and 3.

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CHAPTER 1

INTRODUCTION

Reproduction in sexually reproducing animals requires communication, as individuals must be able to locate and identify potential mates (Bradbury and Vehrencamp 1998). Chemical communication (e.g., olfaction and gustation) has been used by animals longer than other sense (Bradbury and Vehrencamp 1998; Wyatt 2003) and remains essential. Chemical signals can efficiently share information, are often inexpensive to produce (Bradbury and Vehrencamp 1998), and can be transmitted when the sender and recipient are separated by relatively long distances (Mason 1992). Chemical signals are widely used by reptiles to communicate information that may benefit both the sender and receiver (Mason 1992); however, few studies have focused on chemical communication in turtles. The Painted Turtle (*Chrysemys picta)* is a species commonly found in Kansas ponds. I studied male Painted Turtles and their response to chemical signals exuded by conspecifics within a plastic pool. I tested whether male Painted Turtles spent more time in close proximity to male or female Painted Turtles or a control (empty) section of a pool. Within each of the three pool sections, a black, plastic box with drilled holes allowed for chemical signals to flow through, but inhibited the turtles from seeing in or out. I completed 27 trials and recorded the time male turtles spent in each section of the pool (male, female or empty). Turtle life history characteristics make them susceptible to population declines (Gibbons et al. 2000; Ficetola et al. 2004; Daigle and Jutras 2005; Cheung and Dudgeon 2006), and it is important to study how turtles use communication to find mates and successfully reproduce. Developing a better understanding of how turtles communicate may help improve the success of captive breeding programs for these animals.

Turtles are a relatively long-lived species, with lengthy juvenile stages, and have low offspring survivorship (Ernst and Lovich 2009). Studying the demographics of organisms with such characteristics requires long-term studies (Congdon, Dunham, and Van Loben Sels 1994). There is a lack of studies focusing on changes in turtle populations over time, especially within ponds. Turtle traps were set in two ponds in Lyon County, Kansas in 2007 (Nall and Thomas 2009) and again in 2015. Painted Turtles and Red-eared Sliders (*Tracheyms scripta*) inhabited both ponds. Species, sex, and plastron length were recorded for each turtle captured in 2007 and 2015. I examined changes in relative abundance, rations of males, females, and juveniles, and plastron length for both species and Gladfelter and Fence Ponds. Studies such as these offer knowledge on changes in the demographics of this long-lived and globally declining clade of vertebrates.

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CHAPTER 2

MALE RESPONSE TO FEMALE CHEMICAL SIGNALS IN PAINTED TURTLES (*CHRYSEMYS PICTA)*

Abstract

Chemical signals allow animals to communicate clues about sender identity, receptivity to mating, and other information that may benefit both the sender and receiver. Although the use of chemical signals seems probable for chelonians, few studies have focused on this form of communication, and only in a limited number of species. I tested the response of male Painted Turtles *(Chrysemys picta)* to chemical signals exuded by other Painted Turtles. I observed whether male captive Painted Turtles spent more time in close proximity to male or female conspecifics or a control (empty) section of a pool, based only on chemical signals exuded by the stimulus animals. I completed 27 trials and recorded the time male turtles spent in each section of the pool (male, female or empty). The mean amount of time spent in each section of the pool revealed that male Painted Turtles spent the most time in the female section of the pool, followed by the male section and spent the least amount of time in the empty section of the pool. One-way ANOVA and Tukey's test revealed that the only statistically significant difference was the time males spend between the female section and empty sections of the pool, indicating male Painted Turtles use chemical signals exuded by female Painted Turtles. The female and male sections of the pool did not differ significantly in the amount of time males spent in each. Additionally, no significant difference was found in the amount of time a male Painted Turtle spent in the male section of the pool vs. the control section. With many turtle populations declining, it is important to study aspects of their reproduction, including communication. Developing a better understanding of how turtles communicate and their decisions about mating may help improve the success of captive breeding programs for these animals.

Introduction

Animal communication is the sharing of information from a sender to a receiver, with the receiver responding based on the information (Bradbury and Vehrencamp 1998). When information is received by the intended recipient, such as a potential mate, the information is called a signal (Bradbury and Vehrencamp 1998). Information sent in the form of chemicals is referred to as a chemical signal (Wyatt 2003), and is dispersed by diffusion, from high to low concentrations (Bradbury and Vehrencamp 1998). Chemical signals allow the sender to share information efficiently. Chemical signals are often energetically inexpensive to produce (Bradbury and Vehrencamp 1998), especially when exudates double as chemical signals (Mason 1992; Mason and Parker 2010). Chemical signals are also beneficial communication tools, as they are able to be received after the sender is gone and may be detected over long distances (Mason 1992). Chemical signals may be especially useful when visibility is low and the animal cannot rely on sight (Bulova 1997), for example in dark or murky water (Mason 1992). Painted Turtles inhabit a wide variety of aquatic ecosystems including swamps, ditches, and sloughs (Harding 1997) and prefer habitats with mud bottoms (Collins, Collins, and Taggart 2010).

Phylogenetically, chemical signals predate all other communication methods throughout the kingdom Animalia (Bradbury and Vehrencamp 1998; Wyatt 2003) and remain essential for correspondence. Turtles have well-developed olfactory and vomeronasal systems (Ehrenfeld and Ehrenfeld 1973; Halpern 1992) and the ability to detect odors in an underwater environment (Koch, Carr, and Ehrenfeld 1969; Shoji and

Kurihara 1991). With these abilities, chemical signals may be an important form of communication in a variety of turtle species (Ehrenfeld and Ehrenfeld 1973; Lewis et al. 2007). Reptiles use olfaction to locate prey, predators, and mates (Mason 1992), but many factors of olfaction and chemical signals are understudied in reptiles, particularly turtles (Mason and Parker 2010). Given the paucity of empirical studies on chemical communication in chelonians, researching olfaction could offer new insights on communication in turtles. I investigated whether male Painted Turtles (*Chrysemys picta)* respond to possible chemical signals produced by female Painted Turtles.

Turtles use olfaction in both terrestrial and aquatic environments (Legler 1960; Mahmoud 1967; Graham, Georges, and McElhinney 1996). Graham, Georges, and McElhinney (1996) evaluated the Eastern Long-necked Turtle's (*Chelodina longicollis*) ability to orient itself with olfaction above water. Graham, Georges, and McElhinney (1996) created a Y-shaped maze and filled one arm with swamp water and organic materials from the species' home range and the other arm with distilled water. The Eastern Long-Necked Turtles differentiated the scents from the two arms and navigated towards the arm with swamp mud (Graham, Georges, and McElninney 1996). Chemical signals also facilitate territorial behaviors in some tortoise species; for example, feces from Desert Tortoises (*Gopherus agassizii*) exude chemical signals and allow male and female Desert Tortoises to avoid using burrows that contain feces from the same sex (Bulova 1997). Additionally, Rose (1970) discovered that male Texas Tortoises (*Gopherus berlandieri)* became aggressive towards each other once aromatic secretions from their mental glands were secreted.

Communication is necessary in sexually reproducing animals (Bradbury and Vehrencamp 1998). Successful reproduction of turtles requires males and females to be able to find and identify each other (Bradbury and Vehrencamp 1998), and recognizing potential mates of the correct species is a primary purpose of chemical signals in turtles (Poschadel, Meyer-Lucht, and Plath 2006). Turtles have a few potential locations for producing chemical signals, such as inguinal and axillary glands. Painted Turtles, among others, have Rathke's glands between the carapace and plastron (Ehrenfeld and Ehrenfeld 1973). These glands secrete aromatic chemicals into the environment, which may act as chemical signals (Legler 1960; Mason 1992; Lewis et al. 2007). However, the anatomy of these glands for Painted Turtles has not been investigated (Plummer and Trauth 2009), and the product they secrete has not been defined in this species. Male turtles may also distinguish females of the same species by chemical signals released from a female's cloaca (Mason 1992; Mason and Parker 2010). Males Suwannee Cooters, *Chrysemys concinna suwanniensis* (Jackson and Davis 1972), Florida Red-bellied Cooters, *Pseudomys nelsoni* (Kramer and Fritz 1989), and Common Musk Turtles, *Sternotherus odoratus* (Mahmoud 1967) use their snout to nudge the cloaca of a female, presumably detecting chemical signals exuded by the female (Mason and Parker 2010). Additionally, male Ornate Box Turtles (*Terrapene ornata ornata*) sniff and bite at the posterior end of females before mating (Legler 1960).

Two studies have investigated turtles' response to chemical signals. Lewis et al. (2007) observed the response of male Musk Turtles *Sternotherus odoratus* to chemical signals of female conspecifics. Lewis et al. (2007) placed male Musk Turtles in a tank divided into two sections each measuring 49 x 36 x 34 cm with a basking spot in the middle. One side of the tank contained water from an enclosure with a female conspecific and the other side of the tank contained distilled water. Lewis et al. (2007) found that male Musk Turtles chose to spend more time in the tank that previously contained the female Musk Turtle over the side with distilled water. Poschadel, Meyer-Lucht, and Plath (2006) performed a similar study and tested male European Pond Turtles (*Emys orbicularis*) for their response to conspecific chemical signals. Poschadel, Meyer-Lucht, and Plath (2006) filled a 150 x 60 x 30 cm container with water and placed one male European Pond Turtle in the middle of the container. Two inflow tubes allowed water to enter the container; one tube allowed water to flow in from a tank housing a female European Pond Turtle and the other tube allowed water to flow in from a tank filled with distilled water. Male European Pond Turtles chose to spend significantly more time near the inflow tube that allowed in water from the female's tank (Poschadel, Meyer-Lucht, and Plath 2006).

Many studies on turtles from Europe, Asia, and North America illustrate a decline in turtle species throughout the world (Ficetola et al. 2004; Daigle and Jutras 2005; Cheung and Dudgeon 2006), and rarer species are being captive bred in order to increase their numbers. Discovering more about the role of chemical signals in turtle reproduction may help with future conservation and captive breeding efforts (Bradbury and Vehrencamp 1998). Conversely, some turtle species have become established in areas they had not previously occupied. The Red-eared Slider is an invasive species in Taiwan

(Chen 2006) and European wetlands (Cadi et al. 2004; Cadi and Joly, 2004). Cadi and Joly (2003) concluded that the non-native Red-eared Slider is outcompeting the threatened, native, European Pond Turtle, *Emys orbicularis galloitalica* for its preferred basking locations and is thought to compete for nesting sites and food as well. Understanding turtle communication may be useful in eradication efforts of invasive turtle species, for example through the application of pheromone traps (Corkum and Belanger 2007).

I investigated whether captive male Painted Turtles (*Chrysemys picta)* respond to chemical signals secreted by female or male conspecifics, hypothesizing that males would spend significantly more time in close proximity to female's signals.

Methods

Study Area – Painted Turtles were collected from four ponds in Lyon County, Kansas from June to September in 2015. Two of the ponds were located at the F.B. and Rena G. Ross Natural History Reservation located in Americus, Kansas: Fence Pond and Gladfelter Pond (38°30'00.2"N-96°20'15.3"W and 38°29'51.68"N-96°20'12.832"W, respectively). Additionally, two privately owned ponds in Lyon County, Kansas (Emporia and Americus) were also used (38°26'50.4"N-96°17'50.8"W and 38°29'59.5"N-96°19'13.2"W, respectively).

The experiment was carried out in the laboratory at Emporia State University. The room was windowless, and I placed fabric sheets around the florescent lights to create diffused lighting and ensure that the Painted Turtles did not attempt to orient themselves based on the position of the lights (Lewis et al. 2007). I used timers to automatically turn the lights on at 0630 and off again at 2000 (Central Standard Time) to mimic natural photoperiod.

Animal Collection – Collection of Painted Turtles began on 24 June 2015 at the F.B. and Rena G. Ross Natural History Reservation. Collection from the privately owned ponds began on 12 July 2015. I placed two frame net traps (Nall and Thomas 2009) into each pond and baited each trap with canned Jack Mackerel, *Trachurus symmetricus* (Thomas, Nall, and House 2008). Traps were checked and rebaited daily starting at 1500. For each Painted Turtle used in this experiment, I filed a unique notch into its marginal scutes using methods by Cagle (1939) to avoid using test animals more than once during trials. I selected two adult female Painted Turtles and two adult male Painted Turtles to act as stimulus animals. Additional male Painted Turtles were collected throughout the experiment to test the response of male Painted Turtles to chemical signals exuded by conspecifics. Sex of the Painted Turtles was carefully determined. For the purpose of this study, adult females were defined as individuals that lacked elongated foreclaws, had a straight-line plastron length of at least 130mm and a cloacal opening anterior to the rear edge of the carapace (Harding 1997). Adult males were defined as individuals with elongated foreclaws, a plastron length greater than 100mm, and a cloacal opening posterior to the rear edge carapace (Harding 1997; Readel et al. 2008). I recorded species, sex, straight-line plastron length, and notch code on the marginal scutes for each turtle. Painted Turtles were transported to the laboratory and kept singly in a 136-liter plastic tubs. These tubs contained brick basking platforms, and the Painted Turtles were fed Zoo

Med Natural Aquatic Turtle Food, Zoo Med Laboratories, Inc., San Luis Obispo, CA, while they remained in the lab. The tubs contained 6cm of tap water which I cleaned out every third day or whenever the tub became dirty.

Data Collection – After the test animals had been in the lab for 2 weeks, trials began at 1700 Central Standard Time on 1 July 2015. I conducted the trials in a 1.22m diameter plastic kid pool. I used a protractor and tape to divide the pool into three equal sections. I used paint thinner, waterproof spray paint, and polyurethane to make and waterproof the dividing lines. The division line between each section was 2.5 cm wide. The three dividing lines radiating from the center of the pool were labeled as "1", "2", and "3". I placed the test animal in the middle of the pool and I used a random number generator to decide which dividing line the test animal's head was positioned towards at the start of the trial.

I used three black plastic trash bins (43cm x 28cm x 33cm) to contain the stimulus animals within the pool. One trash bin contained a male Painted Turtle, one contained a female, and one remained empty. A soldering iron was used to melt 60 total holes into each trash bin. The holes allowed water and any chemicals produced by the Painted Turtles in the box to flow into the pool, but were small enough to limit the test animal from seeing the stimulus animals inside the trash bin. Bins were kept in place with three bricks on each side, which also helped to prevent the test animals from accessing the area behind the trash bins. The pool contained 12cm of water and the top of the trash bins was 15cm above the top of the water.

Stimulus animals were allowed to adjust to the lab environment for 2 weeks before trials begin. Two adult male Painted Turtles and two adult female Painted Turtles served as stimulus animals and were rotated, with each individual being used in a trial every other day. I allowed all test animals to adjust to lab conditions for at least 48 hours in 136-liter plastic tubs before a trial. At the beginning of each trial test animals adjusted to the pool with the three empty bins for 20 minutes before I added the stimulus animals to the trash bins in the pool. The male stimulus animal was placed into one bin, and the female stimulus animal was placed into another. The third bin remained empty. The experiment began 15 minutes after the stimulus animals were added to the pool. The test animal was considered to be in a new section of the pool once his head and 50% of his carapace was beyond the dividing line. I recorded the test animal’s time spent in each location: female section, male section, or empty section, using the software JWatcher, University of California, Los Angeles, CA, and stop watches. To limit the influence of my presence, I observed the trials from behind a blind made from cardboard. Trials lasted 20 minutes.

After each trial, the Painted Turtles were removed from the pool. I cleaned the pool and bins with alcohol, then rinsed and refilled them with water. The pool sat for 24 hours before being used for another trial. After each trial, I returned the test animals to their original location in the pond. Stimulus animals were returned to their original location at the end of the experiment.

Data Analysis – I performed all data calculations using SAS. I calculated the mean amount of time male Painted Turtles spent in each of the three sections of the pool. Levene's test was used to test for equal variance. I compared the mean time spent in each section of the pool with a one-way ANOVA followed by a Tukey's post-hoc test for mean separation. All statistical tests were conducted at the 0.05 level of significance.

Figure 1-1. Enclosure design featuring a pool divided into three sections, each with a black plastic box.

Results

A total of 27 trials were completed for this study. Out of 20 minutes, male Painted Turtles spent a mean of 9.03 minutes in the section of the pool containing a female Painted Turtle, 6.45 minutes on the side with a male Painted Turtle, and 4.48 minutes in the empty section of the pool.

According to Levene's test, the variances of the three categories were not equal (p = 0.041, W = 3.331), so I performed a square root transformation on the data using the methods of Zar (2010). Levene's test indicated that this data transformation successfully corrected my data for equal variance (p = 0.573, W = 0.562). Using the transformed data, I performed a one-way ANOVA to test for any statistically significant differences between times spent on each side of the pool (Table 2-1). ANOVA illustrated at least one statistical difference between times spent on the male, female, and empty sides [F(2,78) = 6.96 P = 0.0017**]**. Tukey's test revealed a significant difference between the amount of time a male Painted Turtle spent on the female section of the pool and the control section. However, no significant difference was found between the amount of time a male Painted Turtle spent in the female section of the pool and the male section of the pool or between the male section of the pool and the control section.

Table 2-1. Tukey's grouping, sex, mean and standard deviation for the amount of time a male Painted Turtle spent in each section of the pool (n=27).

Tukey's grouping	Sex	Mean (minutes)	Standard deviation
A	Female	9.03	5.58
A B	Male	6.45	4.03
B	Control	4.48	3.65

Discussion

Male Painted Turtles preferred to be in closer proximity to chemical signals exuded by female Painted Turtles over an area that lacked chemical signals. This indicates that male Painted Turtles use chemical signals exuded by females to find mates. No significant difference was found between the amount of time male Painted Turtles spent in the sections of the pool containing another male Painted Turtle vs. female Painted Turtle, indicating male Painted Turtles did not respond differently to chemical signals exuded from male and female Painted Turtles. This may suggest that males can sense chemical signals exuded by other Painted Turtles, but either cannot always distinguish between chemical signals of male and female conspecifics or is not attracted to female turtle chemical signals over male Painted Turtle chemical signals. Because chemical signals may be exuded either actively or passively (Mason 1992), perhaps the stimulus animals were not provoked to exude chemical signals during this experiment. Additionally, the small size of the pool (1.22m diameter) may have resulted in muddling of the chemical secretions of the two sexes. No significant difference was found between the amount of time a male Painted Turtle spent in sections of a pool containing another male Painted Turtle and an empty pool section. Therefore, I did not observe a male preference between male chemical signals and odorless sections of a pool. Male Painted Aquatic turtles are not thought to be territorial (Galbraith, Chandler, and Brooks 1987; Kauffman 1995), so these results are not unexpected.

Differences exist between the enclosures used in the studies focusing on chemical signals with European Pond Turtles and Common Musk Turtles and the enclosure used in this study. The enclosure in the European Pond Turtle study had partial dividers and inflow tubes allowing a constant flow of water (with chemical signals) to flow into the enclosure (Poschadel, Meyer-Lucht, and Plath 2006). The different sections of the pool in the Musk Turtle study were completely closed off from each other, ensuring that chemical signals from one sex did not mingle with chemical signals from another sex. Future studies may benefit from use of a larger pool as a larger volume might limit the potential problem of chemical signals mixing. Additionally, perhaps the concentration of chemical signals was not great enough. Poschadel, Meyer-Lucht, and Plath (2006) allowed their stimulus animals to sit in their enclosures for 24 hours before trials began. Giving the stimulus Painted Turtles more time to produce and accumulate their chemical signals may have produced different results.

Sexually reproducing animals need to communicate to find and identify each other as potential mates (Bradbury and Vehrencamp 1998). Becoming more familiar with communication in turtles is vital in this declining clade of animals. I observed that male Painted Turtles exhibited a preference to the chemical signals exuded by female Painted Turtles than areas lacking chemical signals by conspecifics. This indicates that Painted Turtles may use chemical signals to find potential mates. It is important to note that the lack of a significant preference between male and female chemical signals might have been due to the small dimensions of the test tank or other experimental conditions.

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CHAPTER 3

Changes in Painted Turtle (*Chrysemys picta*) and Red-eared Slider (*Trachemys scripta*) populations in two Kansas ponds from 2007 to 2015

Abstract

Turtles are relatively long-lived organisms, with lengthy juvenile stages (often several years) and low hatchling survivorship. Studying the demographics of organisms with such life histories requires long-term studies as it may take many years to see changes within a population. There are relatively few studies focused on long-term changes in turtle populations, especially within ponds. Turtle traps were set in two ponds in Lyon County, Kansas, in 2007 and again in 2015 at the F.B. and Rena G. Ross Natural History Reservation. Painted Turtles (*Chrysemys picta)* and Red-eared Sliders (*Trachemys scripta)* were collected from both ponds. Chi-square tests detected a significant change in the number of males, females, and juveniles Painted Turtles from 2007 to 2015. Too few Red-eared Sliders were captured to perform a chi-square test. Two-sample t-tests for Painted Turtles showed no significant changes in plastron lengths from 2007 to 2015 from either pond. The lack of change in plastron length is not surprising as conditions such as road density, traffic, and human development did not change in close proximity to the Ross Natural History Reservation between these two years. Interestingly, none of the turtles captured in 2007 were caught again in 2015. Drought and multiple fires at the study site may have caused death or emigration of the turtles originally captured in 2007 and may have contributed to the decrease in juvenile turtles in 2015.

Introduction

Long-term experiments are necessary for researchers studying long-lived organisms, as it may take many years to see changes within a population (Congdon, Dunham, and Van Loben Sels 1994). Long-term studies offer information such as how a population persists over time, how environmental factors influence a population (Gibbons, Green, and Congdon 1983), movements of a species (Arvisais et al. 2002), and changes in demographics (Congdon, Dunham, and Van Loben Sels 1993). Farm ponds are common throughout Kansas; however, studies focusing on turtles in these ponds, and especially how their population changes over time, are rare. I investigated how relative abundance of species, ratios of male, female, and juvenile turtles, and plastron lengths (PL) changed for Painted Turtles (*Chrysemys picta)* and Red-eared Sliders *(Trachemys scripta)* in two ponds in Lyon County, Kansas from 2007 to 2015.

Sex ratios are a vital demographic to study as they can influence population dynamics, especially when too few females are present (Gibbons 1990). Sex ratios for certain turtle species are often slightly skewed (Gibbons 1990). Measured sex ratios of Red-eared Sliders and turtles with similar life histories favor males because they reach sexual maturity sooner than females (Gibbons 1990). Changes in the ratio of males, females, and juveniles in a population can be due to a variety of abiotic and biotic factors, including those that are human-induced (Stickel 1978; Gibbs and Steen 2005; Browne and Hecnar 2007). Gibbs and Steen (2005) synthesized the sex ratios of different turtle species from 165 journal articles and found that the proportion of males grew with road density as females cross roads to nest. Occasionally, the reason for the change in turtle population sex ratios is unclear. From 1945 to 1975, Stickel (1978) studied population changes in box turtles (*Terrapene carolina)* and found that the proportions of female and juvenile box turtles declined, but for unknown reasons. Unnatural, or human-induced changes in sex ratios can harm a turtle population (Gibbons 1990; Browne and Hecnar 2007). When the ratios of males to females is skewed, especially when female numbers drop, the effective reproductive size of the populations may drop, lowering the recruitment in the next generation (Browne and Hecnar 2007). Population stability is often dependent on both adult and juvenile survival (Congdon, Dunham, and Van Loben Sels 1994). Aquatic turtles take a long-time to reach sexual maturity and only a small percentage of young survive (Gibbons 1987), and they require both terrestrial and aquatic environments to live and reproduce. These factors make turtles prone to human-induced environmental changes.

Relative abundance of different turtle species can change over time. Browne and Hecnar (2007) observed changes in turtle assemblages in Point Pelee National Park in Ontario, Canada. Extirpation of the Spotted Turtle (*Clemmys guttata*) and shifts toward populations of Snapping Turtles (*Chelydra serpentina*) and Blanding's Turtles (*Emydiodea blandingii*) occurred over a 30-year period (Browne and Hecnar 2007). Point Pelee National Park is a protected area, but a heavy raccoon population is likely causing the demise of hatchling turtles and turtle eggs (Browne and Hecnar 2007). Anthropomorphic activity can influence species differently, depending on changes in water depth, velocity, and type of substrate (DonnerWright et al. 1999), and human activities may negatively influence turtle populations of certain species (Browne and Hecnar 2007).

Congdon, Dunham, and Van Loben Sels (1994) found that many long-lived species, such as turtles, have co-evolved traits that make it difficult for them to respond to disturbances in their environment. Turtle species have different adaptive responses to environmental changes (Gibbons, Green, and Congdon 1983), so studying a variety of species and a variety of biotic and abiotic factors is necessary to monitor long-term changes for turtles (Gibbons, Green, and Congdon 1983). Long-term studies are required for conservation efforts of declining turtle species (Congdon, Dunham, and Van Loben Sels 1994). Few studies have focused on long-term demographic changes of turtles, especially in farm ponds in Kansas.

I investigated population-level changes in Painted Turtles and Red-eared Sliders in two farm ponds in Lyon County Kansas, specifically, relative abundance, changes in number of male, female and juvenile turtles in a population and changes in plastron length. I compared these data to results available from a 2007 study of the same ponds (House, Nall, and Thomas, 2011).

Table 3-1 Annual precipitation in cm from 2007 to 2015 for Emporia, KS, 66801 (www.ncdc.noaa.gov)

Year	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Annual Precipitation (cm)	99.3	69.8	91.8	119.2	115.9	93.5	71.9	57.5	83.8	70.4	94.7

Methods

Study Area – Turtles were caught in two ponds: Fence Pond and Gladfelter Pond (38°30’00.2”N-96°20’15.3”W and 38°29’51.68”N-96°20’12.832”W, respectively), located at Emporia State University's F.B. and Rena G. Ross Natural History Reservation in Lyon County near Americus, Kansas, USA.

Data Collection – House, Nall, and Thomas (2011) collected turtles from 15 May to 1 October in 2007. I collected turtles from 24 June to 16 August in 2015. In both study periods, the ponds were sampled using rectangular frame net traps (Nichols Net and Twine Inc., Granite City, IL) and floating basking traps (Memphis Net and Twine Co. Inc., Memphis, TN) (House, Nall, and Thomas 2011). Each turtle was marked with unique notches into the marginal scutes using the methods of Cagle (1939) to ensure that the turtles were not counted more than once. Sex was carefully determined by the length of the plastron and secondary sexual characteristics. The plastron was measured as the straight-lined plastron length with dial calipers to the nearest 0.1mm. Adult female Painted Turtles were defined as individuals that lacked elongated foreclaws, had a straight-line plastron length of at least 130mm, and a cloacal opening anterior to the rear edge of the carapace (Harding 1997). Adult male Painted Turtles were defined as individuals with elongated foreclaws, a plastron length greater than 90mm, and a cloaca opening posterior to the rear edge of the carapace (Harding 1997; Readel et al. 2008; House, Nall, and Thomas 2011). Adult female Red-eared Sliders lacked elongated foreclaws and have a straight-line plastron length of at least 160mm (Gibbons and Greene 1990). Adult male Red-eared Sliders had elongated foreclaws, a cloacal opening positioned posterior to the rear edge of the carapace, and a straight-line plastron length of at least 110 mm (House, Nall, and Thomas 2011). For each turtle caught, I recorded species, sex, straight-line length of the plastron, and notch code. I released turtles immediately after recording their information.

Data Analysis – I recorded counts of Painted Turtles and Red-eared Sliders (unique individuals only) for each pond for both 2007 and 2015. Relative abundance, or the proportion of each species in relation to the total number of unique individuals at each pond was also calculated. I used chi-square tests to compare the number of male, female and juvenile Painted Turtles and Red-eared Sliders. Plastron lengths for male, female and juvenile Painted Turtles and Red-eared Sliders of both ponds were recorded. Additionally, a series of two-sample t-tests was used to compare the plastron lengths of male and female Painted Turtles and Red-eared Sliders in 2007 and 2015. All statistical tests were conducted with an alpha of 0.05 using SAS software.

Results

In 2007, 89 Painted Turtles and 16 Red-eared Sliders were captured, and in 2015, 29 Painted Turtles and 17 Red-eared Sliders were captured (Table 3-2). Painted Turtles were more abundant than Red-eared Sliders in both ponds in both 2007 and 2015. Table 3-3 breaks down these numbers by pond and percentage of males, females and juveniles. From 2007 to 2015, the percentage of juveniles caught decreased in Painted Turtles in each pond, and in Red-eared Sliders for Gladfelter Pond (Table 3-3). In 2007, 39 total juvenile turtles were caught in Gladfelter and Fence ponds, which made up 38.02% of Painted Turtles captured and 31.25% of Red-eared Sliders captured. In 2015, only three juvenile turtles were found, making up 6.90% of Painted Turtles captured and 5.88% of Red-eared Sliders captured. I also recorded mean plastron lengths for male, female and juvenile Red-eared Sliders and Painted Turtles for each pond and year (Table 3-4). All turtles captured in 2015 were new captures; no turtles had marginal scute codes identifying with codes given to turtles in 2007.

I performed two chi-square tests for independence to test for significant differences in male, female, and juvenile ratios of Painted Turtles at both ponds between 2007 and 2015. The chi-square test revealed a significant change in the number of female, male and juvenile Painted Turtles in Gladfelter Pond from 2007 to 2015 ($\chi^2 = 6.6212$; df = 2; p = 0.036). The number of male, female, and juvenile Painted Turtles at Fence Pond also changed significantly between 2007 and 2015 ($\chi^2 = 6.43$; df = 2; p = 0.040). More female Painted Turtles were captured than males in Gladfelter Pond in both 2007 and 2015. More female Painted Turtles were captured than males in Fence Pond in 2007, but a strong male bias was observed for 2015. Percentage of juveniles decreased from 2007 to 2015 in both years. Only 25 total Red-eared Sliders were found in Gladfelter Pond and only 7 total Red-eared Sliders were found in the Fence Pond in the 2007 and 2015 trappings, so no chi-square test was conducted for these turtles.

Table 3-2. Number of Painted Turtles and Red-eared Sliders caught at Gladfelter and Fence ponds in 2007 and 2015 (and percent relative abundance).

Pond	Year	Painted Turtles	Red-eared Sliders
Gladfelter	2007	68 (85.00)	12 (15.00)
Gladfelter	2015	17 (54.84)	14 (45.16)
Fence	2007	21 (84.00)	4 (16.00)
Fence	2015	12 (80.80)	3 (20.00)

Table 3-3. Percentage of male, female and juvenile (Juv.) Painted Turtles (PT) and Red-eared Sliders (RES) found in Gladfelter and Fence ponds in Lyon County, KS in 2007 and 2015.

Pond	Year	Male PT	Female PT	Juv. PT	Male RES	Female RES	Juv. RES
Gladfelter	2007	25.0	38.9	38.2	33.3	25.0	41.67
Gladfelter	2015	35.3	58.8	5.9	64.3	35.7	0
Fence	2007	23.8	38.1	38.1	50.0	25.0	25.0
Fence	2015	66.7	25.0	8.3	33.3	33.3	33.3

Table 3-4. Mean plastron lengths (mm) for male, female and juvenile (Juv.) Painted Turtles (PT) and Red-eared Sliders (RES) in Gladfelter and Fence ponds in Lyon County, KS in 2007 and 2015.

Pond	Year	Male PT	Female PT	Juv. PT	PT Tot.	Male RES	Female RES	Juv. RES	RES Tot.
Gladfelter	2007	120.8	147.8	86.3	117.4	187.6	224.7	74.6	149.6
Gladfelter	2015	134.3	152.5	82.6	142.3	181.7	258.1	-	230.9
Fence	2007	135.8	151.0	80.1	120.4	173.2	251.6	85.6	173.1
Fence	2015	109.8	144.3	70.3	125.6	137.4	206.7	42.5	128.9

Additionally, two-sample t-tests were performed to test for differences between the mean plastron lengths for male and female Red-eared Sliders and Painted Turtles for 2007 and 2015 (Table 3.5). T-tests were performed comparing male and female Painted Turtles plastron lengths in both Gladfelter and Fence ponds. No significant differences were found for either. Small sample size prevented meaningful comparison of the plastron length comparisons of Red-eared Sliders from either Gladfelter Pond or Fence Pond.

Table 3-5. Results of the two-sample t-tests comparing plastron lengths between 2007 and 2015 for male and female Painted Turtles at Gladfelter Pond and Fence Pond, Lyon County, KS.

	T	Df	P
Gladfelter Pond			
Male Painted Turtles	-1.37	5	0.23
Female Painted Turtles	-1.27	27	0.22
Fence Pond			
Male Painted Turtles	1.03	5	0.35
Female Painted Turtles	0.63	3	0.57

Discussion

Painted Turtles were more abundant than Red-eared Sliders at both ponds during both years. I did observe a significant difference in the number of male and female, and juvenile Painted Turtles at Gladfelter and Fence ponds from 2007 to 2015. However, low capture numbers of Red-eared Sliders at both Gladfelter Pond and Fence Pond prohibited a chi-square test to determine any changes in the population. Mean plastron lengths of Painted Turtles did not change significantly between years at either pond. Again, too few Red-eared Sliders were caught to perform a meaningful test. The lack of change in plastron lengths are not surprising. Water temperature and food availability (Cagle 1946) and food quality (Gibbons 1967) influence growth rate of turtles. Perhaps these variables remained relatively constant throughout these years. The F.B. and Rena G. Ross Natural History Reservation in Lyon County, KS did not experience any increase in roads during the study period and is surrounded by agricultural fields, a few residential homes, and gravel roads. Additionally, no information on raccoon (*Procyon lotor*) populations or other potential predator populations have been reported for the study area.

Interestingly, none of the turtles caught in 2015 had been previously marked in 2007. Nall and Thomas (2009) captured and marked the majority of turtles within these ponds in 2007 with daily recapture rates exceeding 90% for at least two weeks (Nall and Thomas 2009). With most of the turtle population of 2007 being marked, and none of these individuals being recaptured in 2015, I conclude that most of the turtles that inhabited Gladfelter Pond and Fence Pond in 2015 were immigrants or young individuals less than 7 years of age. It is possible that some of these previously marked individuals may have escaped detection in 2015; however, based on the high previous recapture rates reported in Nall (2011), it seems unlikely that escaping detection would explain the lack of any individuals marked in 2007 in my sampling. The fate of the individuals marked in 2007 is unknown. With all new individuals in 2015, it is not surprising that the ratio of male, female, and juvenile Painted Turtles varied from 2007 and 2015 for both Gladfelter and Fence ponds. Male turtles are more likely to make overland movements to new ponds in search of mates (Thomas and Parker 2000), which may explain the new male bias for Fence Pond in 2015.

The Ross Natural History Reservation experienced drought years and multiple wildfires and prescribed burns between 2007 and 2015, which may have influenced the decrease in juvenile turtles and the emigration of adults. Droughts are known to influence turtle populations by increasing emigration (Gibbons, Green, and Congdon 1983; Cash and Holberton 2005) and affecting egg laying, development, and survival due to a lack of moisture (Gutzke et al. 1987) Drought conditions have repeatedly occurred at my study ponds between the years of turtle collection (Table 3.1). During these years, Fence Pond completely emptied and refilled multiple times. In response to drought, Painted Turtles and Red-eared Sliders are likely to emigrate to other aquatic habitats rather than estivate in the mud (Cash and Holberton 2005), so the turtles marked in 2007 may have emigrated from Fence Pond to one of the more permanent ponds in the area. Gladfelter Pond did not completely empty during this time; however, water levels declined on occasion from 2007 to 2015. Lowering water levels may raise corticosterone levels and cause emigration of Red-eared Sliders (Cash and Holberton 2005). The drought may also explain the reduction in juvenile turtles in 2015. Gibbons, Greene, and Patterson (1982) found that responses to drought varied by turtle species. In some species, fewer females laid eggs in drought years than in non-drought years; in others, more turtles emigrated from the drying water source (Gibbons, Gibbons, Greene, and Patterson 1982). While no significant decline in juvenile Painted Turtles was determined by the chi-square test, the percentage of the population made up by juvenile turtles decreased in both species.

Wild and prescribed fires in July 2012 and April 2015 surrounding the ponds may have also influenced juvenile decline. Multiple fires, including a notable wildfire in July 2012 and a prescribed burn in April 2015, ran through the area, including the land surrounding both Gladfelter Pond and Fence Pond. Hailey (2000) studied how fire affects mortality of the Herman's Tortoise (*Testudo hermanni)* and found their deaths due to fire to vary with season and vegetation type. Fire may also influence population changes in aquatic turtles; however, I am unaware of any empirical studies on the effects of fires on the success of turtle nests. It is possible that egg survival may have decreased due to poor nesting conditions caused by the fire, such as fewer shaded areas, resulting in higher ground temperature and lower humidity. While the number of adults may have been high, lack of recruitment of offspring could have caused declines (Browne and Hecnar 2007), and population stability is partially dependent on juvenile survival (Congdon, Dunham, and Van Loben Sels 1993; Congdon, Dunham, and Van Loben Sels 1994).

Studying these turtle populations for a longer time period, and perhaps annual trapping, could help test for a correlation between environmental conditions and juvenile turtle decrease. Future turtle trapping, and data collection in the same ponds, would give information on whether the juvenile observed decrease is temporary. Consideration of variables such as temperature, precipitation, and predator abundance might provide insight. With turtles being of great concern in their decline, it is important to study how their populations change over time and to monitor these changes. More research focusing on understudied areas, such as Kansas farms. A long-term study documenting water temperatures, food availability, and food quality over a number of years could offer insight on the consistency of plastron lengths in these ponds.

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CHAPTER 4

CONCLUSIONS

The male Painted Turtle spent significantly more time in the section of the pool containing the female Painted Turtle than in the empty (control) section of the pool, indicating he prefered chemical signals exuded by the female Painted Turtle over an area without chemical signals from conspecifics. No significant difference was found for the amount of time male Painted Turtles spent in sections of the pool containing another male Painted Turtle vs. a female Painted Turtle. This may reveal that male Painted Turtles can detect chemical signals from other Painted Turtles, but either cannot always distinguish between chemical signals of male and female conspecifics or is not attracted to female turtle chemical signals. Chemical signals can be exuded either actively or passively (Mason 1992), so perhaps the stimulus animals were not provoked to exude chemical signals in this experiment. Additionally, the small, 1.22 m diameter pool may have resulted in muddling of the chemical secretions of the two sexes. No significant difference was found between the amount of time a male Painted Turtle spent in sections of a pool containing another male Painted Turtle vs. an empty pool section, revealing that he had no preference between male chemical signals and odorless sections of the pool. These results are not surprising as male Painted Turtles are not known to be territorial (Kauffman 1995). Information regarding mate-finding behavior in turtles is becoming more important as many species of this clade are declining in number.

Painted Turtles were more abundant than Red-eared Sliders at both ponds during both 2007 and 2015. None of the turtles caught in 2007 were caught again in 2015. With most of the turtle population of 2007 being marked (Nall and Thomas 2009) and none of these individuals being recaptured in 2015, new individual turtles must be occupying

Gladfelter Pond and Fence Pond, and turtles caught in 2007 traveled to new ponds or died. A significant change in numbers of male, female, and juvenile Painted from 2007 to 2015 was found for both Gladfelter Pond and Fence Pond. However, low capture numbers of Red-eared Sliders for both ponds prohibited chi-square tests to determine any changes in that population. The reduction in juveniles in both Painted Turtles and Red-eared Sliders may have been due to a variety of events, including drought which is known to cause a decrease in egg laying in female emydid turtles (Gutzke et al. 1987). Wild and prescribed fires in July 2012 and April 2015 surrounding the ponds may have also influenced juvenile decline. Lack of recruitment of offspring could cause future declines (Browne and Hecnar 2007), and population stability is partially dependent on juvenile survival (Congdon, Dunham, and Van Loben Sels 1993; Congdon, Dunham, and Van Loben Sels 1994). Mean plastron lengths did not differ significantly for Painted Turtles at either pond between years. Too few Red-eared Sliders were caught at both Gladfelter and Fence ponds to complete a t-test. Water temperature and food availability (Cagle 1946) and food quality (Gibbons 1967) may influence the growth rate of turtles.

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