Population Structure Analysis of Western Painted Turtles

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Abstract

Changes in the population structure, specifically the age, size and sex composition, of Western painted turtles can be studied to gain insight into the changes that will occur in closely related endangered species. As species with temperature-dependent sex determination (TSD), the sex ratios of new clutches are affected by climate, and this could cause problems for the species as climate changes occur. This study focuses on fluctuations in the population structure of a single population located near Cedar Point Biological Station in Ogallala, Nebraska. Each summer from 2005-2016 turtles were caught using floating basking traps and hoop nets. Results show evidence of changes in the sex ratio occurring after a severe drought. Variation in the size structure of the population suggests an influence from movement behaviors and environmental conditions. Results from this population may be informative to how species are affected by changes in climate.
Introduction

This study evaluated the changes in population structure, or the make-up of the population, for Western painted turtles (*Chrysemys picta*). Understanding the way population structure changes is important in determining how climate change and other factors may affect the populations. These results will be helpful in the conservation of painted turtles and similar turtle species.

Climate change affects many species in different ways. For example, Artic sea ice loss reduces habitat of polar bears; sea level changes will reduce habitat and shift suitable habitat for species. For species in the United States, one major concern is the increase in extreme weather events. This includes an increase in the frequency and duration of drought conditions (Future 2016). Drought is a problem for ecosystems that require large quantities of water such as wetlands and pond communities. Drought conditions can have unexpected effects on the species that reside in these ecosystems. For pond turtles, drought changes the water level and temperature and impacts the movement between ponds affecting the population structure.

Habitat loss also threatens pond turtle species; many populations have been displaced or otherwise adversely affected. Habitat has been lost due to human development; this includes development for housing, agriculture, or various infrastructure. Some pond turtles, such as the endangered spotted turtles (*Clemmys guttata*), inhabit wetlands and streams in addition to ponds. These wetland habitats are becoming hard to find. Some regions of the U.S. retain less than 20% of the wetland acreage they once had (Gibbons 2000). Pond turtles are an important part of the ecosystem. Turtle eggs and hatchlings are a food source for species such as raccoons, garter snakes, large wading birds, foxes, skunks, badger, and others (Ernst 2009). The turtles prey on
insects, snails, and aquatic plants among other things. Thus, pond turtles are a vital part of aquatic ecosystems.

Western painted turtles, *Chrysemys picta* hereafter called painted turtles, are at low risk for extinction. Painted turtles are often studied because they are numerous and can easily be accessed to study. Knowledge about their population dynamics can aid the conservation of closely related endangered species. For example, spotted turtles (*Clemmys guttata*) and Blanding’s turtles (*Emydoidea blandingii*) are members of the same family, Emydidae which includes pond and marsh turtles. Both of these species are on the International Union for Conservation of Nature (IUCN) red list of threatened species. They are threatened by road mortality, collection for trade, and habitat degradation and fragmentation (IUCN 2016). Spotted turtles and Blanding’s turtles are threatened on both a global and local scale, but painted turtles are numerous enough to only be affected on a local scale.

Painted turtles belong to the class Reptilia and do not breathe through their skin like amphibians. Amphibians have cutaneous respiration and breathe through their skin, while reptiles use non-cutaneous respiration. They also have amniotic eggs which have protective layers and can be laid on dry land (Wyneken 2008). Turtle shells are formed from their ribs, and grow each season. Each scute, a bony external plate made of keratin, of the shell grows out from its base. This allows for the replacement of old scutes. Turtles can be aged by looking at the growth lines on a scute, but this method is not as reliable as counting the growth rings in bones (Wyneken 2008). Unlike counting growth rings in bones, counting scute growth lines is possible without killing the turtles; however these growth lines often wear off and make aging difficult. Turtle aging methods are important for determining the age of the turtles and make it possible to examine the population structure in regards to age.
The age of the turtles is closely related to their size. Size structure can be used to represent general age, although age cannot be positively determined. Each season results in growth and sexual development. Male painted turtles mature near four years of age (Schwanz 2010). Females generally reach reproductive maturity after five or six seasons despite their size (Rowe 1992). Males grow faster than females in their first ten years of life, which leads to males being significantly larger than females by age six. Females grow slower, but are significantly heavier than males, even when eggs are excluded from the comparison (Germano 2008). The size of the reproductive female often affects the size and success of their offspring. The larger females will have a bigger egg mass and clutch size, the number of eggs produced at one time. The growth rates of juvenile turtles are higher in larger bodies of water because of the higher habitat quality and food availability. Growth rates are affected by the variability of food and the water temperature; higher water temperatures give variation in food type (Rowe 1992). Growth rates can be an indicator of environmental conditions.

A population also needs to have enough males and females to ensure future generations of painted turtles. Most long term population studies find a sex ratio of 1:1 (Ernst 2009). However, it can vary by population. Many factors affect the sex ratio of a painted turtle population. Temperature is likely to affect the sex ratio of the painted turtles because they exhibit temperature-dependent sex determination (TSD). TSD is a type of environmental sex determination in which the temperatures experienced during embryonic development determine the sex of the offspring. As a species with TSD, it is important to look into the specifics of painted turtles’ sex-determination and what could cause issues for the survival of the species. If environmental conditions change and produce extreme offspring sex ratios, several ecological and evolutionary responses could occur. These turtle populations could reduce the temperature
dependence of sex determination, alter maternal egg-laying behavior or the crucial temperatures of sex determination, or change geographic range (Schwanz 2010).

With TSD, sex is determined by incubation temperature during the middle third of embryonic development. For painted turtles this is normally during July, and hatchlings emerge from their eggs in August or September (Schwanz 2010).

A few factors affect the temperatures eggs are exposed to, and therefore the sex ratio of the clutch. First is the vegetation cover. More vegetation cover leads to a lower nest temperature and less vegetation cover leads to a higher nest temperature (Schwanz 2010). Females may select sites to affect the sex ratio of their clutch. This would involve finding a location with a certain amount of vegetation cover. However, studies have shown that females selected sites that maximized the probability that their eggs would complete development rather than influence the sex ratio of their offspring (Ernst 2009). For example, females chose sites that had a good location and vegetation cover to be relatively safe from predators.

The air temperature also affects the sex ratio of new clutches. In a study by Lisa Schwanz, cooler temperatures led to a higher proportion of males and warmer temperatures caused a lower proportion of males. Thus, higher temperatures caused fewer males to be produced. Overall, the study found that population demography depends more on annual variation in climate and predation than it does on nest-specific biology such as vegetation cover (Schwanz 2010). This information is important to determine what effect climate change will have on turtle populations, and how turtles can adapt to changes. This data can aid conservation of turtle populations and help us to understand changes in the populations.

Extreme temperatures during embryonic development can even cause developmental abnormalities. According to a study, eggs exposed to temperatures over 34.2 degrees Celsius for
60 hours show an apparent increase in abnormal development (Rory 2013). If temperatures increase as a result of climate change, the frequency of hatchlings with abnormalities will also increase. These abnormalities generally reduce fitness, the ability to survive and reproduce (Rory 2013). This is one reason it is important to study the effects of temperature changes on turtle development. It is also important because these temperatures may be seen in their natural habitats instead of a lab experiment. If the climate changes enough to severely alter hatchling development, it would cause issues for the species.

Previous work has been done on the population structure of western pond turtles in California due to concerns about decreasing turtle populations and that populations would be adult-biased unable to replace themselves with offspring (Germano 2001). Germano (2001) provided evidence to suggest these concerns were not merited. Since turtles are long-lived, populations may survive periods of low recruitment by greater juvenile survivorship during favorable environmental conditions. Second, growth rates can increase due to habitat quality leading to large juveniles which may be miss-identified as adults. Germano (2001) concluded that the turtle populations are surviving, but are considerably smaller than in the past. Overall, the loss of habitat affected the turtle populations, but they were adapting. Similar concerns have been expresses for reptiles in the rest of the Great Plains (Future 2016).

The goal of this study was to analyze the changing population structure in a population of painted turtles near Ogallala, Nebraska. To reach this goal, the objectives were to examine the change in male to female sex ratio, to study the change in size structure, and to address what might be causing these changes with temperature and precipitation data.
Methods

The study population resides in Yellow Pond which is a few miles north of Lake Ogallala in Western Nebraska (Figure 1). A mark-recapture study of the turtle population began twelve years ago and is still ongoing. Painted turtles are trapped and tagged for one week each summer (between June and July) at this location.

My research team used both basking traps and hoop-nets to trap the painted turtles. The basking traps were made of a topless wire cage with a ring of plastic pipes around the top (Figure 2). The plastic pipes allowed the trap to float, and wire ramps allowed the turtles to climb onto the trap to bask. Then the turtles jump back into the water on the inside of the plastic pipes and become trapped. Ten basking traps were set up throughout the pond. Researchers have been using basking traps for a while now.

According to a study done by Catherine Ream, basking traps provide the most information per unit of effort (Ream 1966). One issue found with using basking traps was sampling juveniles; however, my study caught many juvenile turtles in basking traps. Ream’s study suggested that the most efficient and accurate estimation of population structure comes from a
combination of basking traps and hand captures. My study supplemented the use of basking traps with baited hoop nets.

In my study, three hoop nets were baited with sardines and set up in different areas of the pond. The hoop nets were made of netting around wire hoops. The net funnels allowed the turtles to swim in, but not out. In Ream’s study, baited hoop nets yielded mostly males and relatively few juveniles (Ream 1966). Overall, the combination of basking traps and hoop nets should yield an accurate representation of the population structure.

The traps were checked once a day during the week of sampling. The traps were reset after the turtles were collected into buckets. Each turtle was scanned for the presence of Passive Integrated Transponder (PIT) tags (Gibbons 2004). PIT tags are small electronic tags that are injected into the turtles and can later be scanned to give the ID of the turtle. The turtles that had no PIT tags were injected with PIT tags, and then a unique combination of holes were drilled into their shells to identify them. All the turtles were then weighed with a spring scale.

Next, measurements were taken on the size of the turtles. Using a caliper, the length and width of the top of the shell, the carapace, were measured to the nearest millimeter. The length and width of the bottom side of the shell, the plastron, were also measured. The connection between carapace and plastron, called the bridge, was measured as well. The sex of each turtle was recorded. After all measurements were taken, the turtles were released back into the pond.

All data from 2005 to 2016 was compiled into a database. A COUNTIFS function in Excel was used to tally the number of males and females in the population each year. Then the number of males was divided by the number of females to get a male to female (M:F) sex ratio.
The change in the sex ratio each year was graphed. Significance was tested using a simple Chi square analysis with the null hypothesis that the sex ratio would stay the same over time (Lane 2007).

Size classes were created to divide the population into size categories. These classes were chosen by examining both the natural breaks in the carapace length and the quartiles in the long term database. The first three size ranges were determined by the values of the first, second and third quartiles (0 to 95mm: size class 1, 95 to 130mm: size class 3, and 130 to 165mm; size class 3) of carapace length. The size range of sexually mature females was found by looking at data collected during 2007-2008. During these years my research team documented the presence of eggs in female turtles. This, along with the value for the third quartile, was used to create a division for sexually mature turtles (165 to 200mm: size class 4). The largest turtle caught had a carapace length of 200mm so it was used as the upper bound.

The number of turtles in each size class each year was counted. Then the proportion of the population in each size class over time was graphed. Chi square analysis was used to test the null hypothesis that the proportion in each size class remains constant (Lane 2007).

After analyzing the change in sex ratios and in size distribution over time, I considered what might be causing these changes in the population. Temperature and precipitation were regarded as factors that may influence the population structure of the painted turtles. Data was found on the National Oceanic and Atmospheric Administration (NOAA) website (Climate 2016). Their data was used to look for a relationship between the climate and the changes in population structure.

I used linear regression with two factors to test this relationship (Lane 2007). For example, the percent male was used as the response variable with the temperature and
precipitation as predicting variables to determine the effect of temperature and precipitation on the sex ratio or size distribution in the population. Lastly, the data was tested for a lag effect of 3 or 4 years to test the effect of temperatures during embryonic development.

The Palmer Hydrologic Drought Index (PHDI) was also used to test the effect climate change may have on the population. The PHDI shows hydrological drought and wet conditions to accurately reflect groundwater conditions, reservoir levels and other values (Heim 2013). A more negative PHDI means worse drought conditions. The PHDI uses an average of data from the past four years to get the PHDI for a year. For example, the 2006 index is influenced by data from 2002-2006. The relationship between PHDI and the sex ratio of the population was tested using linear regression with the PHDI and the proportion of males captured. A lag effect was again tested to account for the time between incubation and sampling for a generation of turtles.

Results

My study found that the male to female ratio tended to change over time (p=.097, Figure 4). There were always more females than males but the difference between the sexes was somewhat greater during certain years. The ratio of males to females ranged from .35 to .96.

![Number of Males and Females Sampled](image)

*Figure 4. The number of male and female turtles caught each year at the pond. The ratio of males to females tended to change over time (chi-square, p = 0.097).*
The proportion of both males (p<.001) and females (p<.001) in each size class changed over time. The change in distribution throughout the size classes for both males (Figure 5) and females (Figure 6) was statistically significant. The graphs also show the difference in the size class distribution for males and females. There are more females in the larger size classes and more males in the smaller size classes.

**Figure 6. The proportion of female turtles in each size class changed over time (p < 0.001).**

**Figure 5. The proportion of male turtles in each size class changed over time (p < 0.001).**

We found no relationship between the proportion of males and the temperature or precipitation with a lag of 0, 1, 2, 3, or 4 years (p>>0.05). Single variable linear regressions
were also tested for the proportion of males or females in a size class versus the temperature or precipitation with a lag of 0, 1, 2, 3, or 4 years (p>>0.05).

Two-variable linear regressions were tested with the proportion of males or females in each size class versus the temperature and precipitation with a lag of 0, 1, 2, 3, or 4 years (p>>0.05). Two of the relationships between the proportion of females in a size class with the temperature and precipitation were found to be statistically significant. The proportion of females in size class 2 correlated with the temperature and precipitation with no lag [intercept= -3.38 (SE: 1.03), temperature slope= 0.049 (SE: 0.014), precipitation slope= -0.012 (SE: 0.019), p=0.015]. The proportion of females in size class 4 correlated with the temperature and precipitation with a three year lag [intercept= -2.69 (SE: 1.05), temperature slope= 0.04 (SE: 0.014), precipitation slope= 0.057 (SE: 0.027), p=0.031].

Next, the data was tested for a relationship with the Palmer Hydrologic Drought Index (PHDI). The proportion of males captured increases as the PHDI increased (p=0.02, Figure 7).

![Figure 7. The proportion of males captured was lower during drought conditions (p = 0.020)](image-url)
Discussion

Female turtles are generally larger than males, and I documented similar differences in size class distribution for the males and females in my study (Germano 2001). Females were more likely to be in size classes 3 or 4 while the males were in size class 2 or 3. The changes in the distribution for both sexes might have been affected by drought conditions. In the future, we hope to test this idea against our data.

Two potential influences of temperature and precipitation on the proportion of females in a size class were the effects of TSD and the effects of environmental conditions on movements. The first relationship was between size class 2 and the temperature and precipitation with no lag. The relationship showed that an increase in temperature and decrease in precipitation suggested an increase in the proportion of females in size class 2. This may have been caused by the current water levels, rather than changes in reproductive output. Although it does support the idea of fewer males being produced during high temperatures since size class 2 represents fairly young females (Schwanz 2010). Current water levels can affect which individuals are present in the pond. For example, turtles may move to other ponds if the water is too low, or males may disperse to a more favorable location to find females. Each gender may migrate differently depending on environmental conditions and the time of year.

The second significant correlation found was between size class 4 and the temperature and precipitation with a three year lag. The relationship showed that an increase in temperature and precipitation indicated a higher proportion of females in size class 4. This was likely caused by the growth of females to reproductive maturity. The changes may have also been affected by movement between ponds and the suitability of the pond for egg laying.
The observed change in the male to female ratio may be explained by our finding that the proportion of males captured correlated to the Palmer Hydrologic Drought Index (PHDI). The drought years of 2002-2005 may have created the ratios seen in 2005-2010, in which we observed a much lower male to female ratio. Also, the drought conditions in 2012 might explain the larger number of females in 2015. Our results match the expectation for more females to be produced during periods with warmer nest temperatures (Schwanz 2010). No results were found with the temperature and precipitation data, but the PHDI, which merges them with other data, was able to find a correlation with the proportion of males. This may because the eggs are affected by more than simply temperature during their embryonic development. For example, both the rate at which the soil conducts heat and the level of humidity play a role in the hatching rate of the eggs (Parrott 2010). The PHDI uses more information about the changes in weather conditions than the basic temperature and precipitation data, which allows for a more accurate look at the relationship to the changing sex ratio. For these reasons, PHDI is recommended for future research.

The relationship found with the proportion of males and the PHDI shows that the drought conditions were most likely affecting sex determination during embryonic development. The warmer temperatures should cause more females to be produced, which we observed four years later. The underproduction of males may not have shown up yet. Males from previous years are still counted in the graph, and males grow faster and live longer than females. The effect might be even more dramatic if we could separate out the turtles by age. This would allow us to more accurately measure the effect drought is having on the population structure.

Our research has implications for understanding how species might respond to changes in long-term climate conditions. Nebraska is predicted to experience increasing temperatures and an
increasing frequency, severity, and duration of droughts due to climate change (Wilhite 2016). Since we have seen a relationship between drought and the proportion of males in the population, this increase in drought conditions will probably cause a lower ratio of males in the turtle populations. This change may not have an impact on large populations, but smaller populations are more at risk. As the painted turtles appear to be affected by drought conditions, related endangered species may too. A large portion of the U.S. is expected to have an increase in dry conditions and in temperature (Future 2016). Based on our results, this could present additional challenges for endangered turtle populations.

Wildlife managers may want to perform periodic checks of the sex ratio and the productivity of the population. It may be difficult to track changes in the sex ratio because turtles are long-lived, but it would be important to make sure there are still enough females laying successful clutches. Tagging females with trackers would help with this. Overall, the management of endangered turtle populations will need to continue, and changes to their management might be needed to ensure the survival of the species.

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Images

Basking trap: http://suretrap.com/i/840879.jpg

Hoop net: http://cdn.memphisnet.net/images/120.gif

Yellow pond: photo taken by Ellen Dolph.