A Population Demographic of Midland Painted Turtles (*Chrysemys picta marginata*) in Conrad Balliet Family Nature Preserve Pond

Leah Vogt

Wittenberg University

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Abstract

The Painted turtle (*Chrysemys picta*) is a widespread North American species, and its subspecies found in Ohio is the Midland Painted turtle (*Chrysemys picta marginata*). While it is generally a widespread species and less at risk than other freshwater turtle species, understanding different local populations of the species can help us better understand the species as a whole, as well as factors that may play a role in conserving other freshwater turtle species. I conducted a mark-recapture study over the course of two separate study periods to assess the Midland Painted population in Conrad Balliet Family Nature Preserve's Pond, a local pond in Clark County, Ohio. Of the turtles recorded, 28 individual turtles were captured, and there was a 1.33:1 ratio of male turtles to female turtles and a 0.19:1 ratio of juveniles to adult turtles. Males tended to be in higher abundance in traps when there were few females and accounted for more recaptures than females. The estimated population size for the pond is 30 Midland Painted turtles. Males were smaller in size than females, in carapace length and width, plastron length and width, shell height, and weight. This information, and further studies on the pond's population, can serve to inform conservation methods for this species and other applicable freshwater turtle species as is relevant.

Introduction

The Painted Turtle, *Chrysemys picta,* is a species that can be found throughout the United States and is the only turtle species of North America to have a natural range across the whole continent (Ernst and Lovich 2009). While the species primarily inhabits freshwater systems, it can also be found in brackish water. However, they tend to be in higher abundance in shallow wetlands with slow moving water that are heavily vegetated and have areas for the turtles to bask, such as swamps, marshes, and ponds (Van Dijk 2011). As of 2011, the IUCN lists the Painted Turtle as a species of least concern, meaning for the near future the species as a whole is unlikely to be threatened. While the species still faces threats, largely anthropogenic, that can affect population numbers, it is less impacted by them than other freshwater turtle species (Van Dijk 2011). Therefore, with other freshwater turtle species experiencing population decreases, the Painted turtle's population stability could serve as a population model for understanding factors that may play a role in freshwater turtle population persistence to be applied to help imperiled freshwater species.

Because of its abundance the painted turtle is widely studied (Litzgus and Smith 2010). However, separation between populations of the same species can result in differing natural histories, population trends, and life history characteristics. The clearest difference in separated populations is in the 4 different subspecies of the Painted turtle: the Eastern Painted (*Chrysemys picta picta*), the Western Painted (*Chrysemys picta bellii*), the Southern Painted (*Chrysemys picta dorsalis*), and the Midland Painted (*Chrysemys picta marginata*). They each have their own area of distribution (Figure 1), and this has shown varying differences between the 4 subspecies in individuals' size and coloring (Agha et al. 2018; Ernst and Lovich 2009). The subspecies distribution was affected by the Wisconsin glacial period, in which the 3 distinct populations of

Painted turtles (*C.p. picta*, *C.p. dorsalis*, and *C.p. bellii*) were isolated from one another in different regions of the country. When the glaciers receded, these distinct populations spread northward into their current ranges (Bleakney 1958). The fourth subspecies of the Midland Painted is thought to have originated from the hybridization of the Western Painted and Southern Painted when they encountered one another during this northward dispersal, while the Eastern Painted turtle was isolated by the Appalachian Mountain range. This Midland Painted subspecies then colonized a range that was not yet colonized by a species of Painted turtle, which consisted of the upper South, Midwest, and some Northeastern parts of the United States, as well as parts of southern Canada. This subspecies is the one found in Ohio, and the one covered in this study (Bleakney 1958; Ernst and Lovich 2009; Starkey et al. 2003).

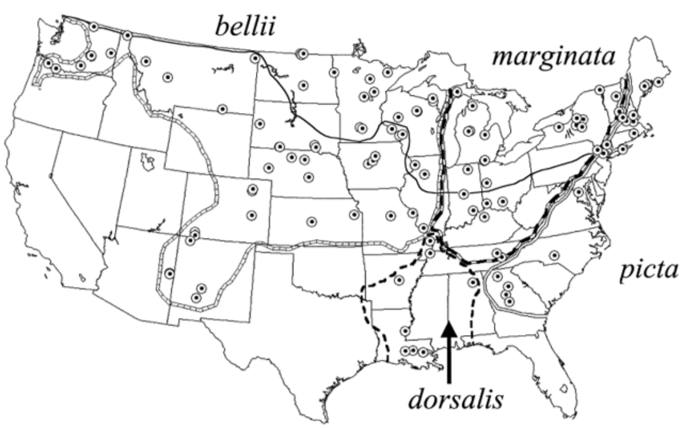

Figure 1. Distribution of 4 different subspecies of *Chrysemys picta*: *C.p. bellii, C.p. dorsalis, C.p. marginata*, and *C.p. picta.*

(Ernst and Lovich 2009)

The differences caused by population separation continue further within the subspecies as well. Drought can skew populations of Western Painted turtles toward female, as well as cause a slower growth rate (Powell et al. 2023). More specifically for Midland Painted turtles, differences in clutch size and body size have occurred in Michigan Midland painted turtles according to different freshwater habitat, and likely based on diet (Frazer et al. 1993). Furthermore, proximity to roads had the potential to skew Midland Painted populations towards more males, due to females' nesting in the gravel on roadsides and increasing their chances of roadside mortality (Dupuis-Desormeaux et al. 2017; Dupuis-Desormeaux et al. 2019). These differing aspects can change our understanding about the species, as they may deviate from known demographic parameters of the species. Therefore, population demographic studies of species are used as a way of understanding these factors about local populations, which add to our understanding of the species as a whole (Hanscom et al. 2020; Knoerr et al. 2021).

One of the ways demographic data can be collected is through mark-recapture techniques. For a mark-recapture study, at least 2 sessions are conducted. In the first capture period, all animals caught are marked via some identifiable means (Ryan 2018). In the case of turtles, notches in marginal scutes on the carapace, toe clips, and PIT (Passive Integrated Transponder) tags are some of the ways in which to mark captured individuals (Dodd 2016). These animals are then released back into the population. Follow-up capture sessions involve identifying the number of marked individuals from a random sample of caught individuals (Ryan 2018). The data on captured and marked individuals can be used assess growth in individuals, average size of individual in the population, sex ratio, estimated recruitment, and population estimates (Hanscom et al. 2020). Because the Painted turtle is well studied, information on population dynamics of local metapopulations of this species can be important to understanding how the species' populations trend remain stable and inform possible conservation methods for other freshwater turtle species, as well as this species should it be at risk at some point in the future (Hanscom et al. 2020). This paper describes the population breakdown of sex, age class, and body size for the Midland Painted turtle population of a local pond in Clark County, Ohio based on a mark-recapture study conducted on the pond.

Methods

We used a mark recapture study to record Midland Painted Turtle numbers in the pond at Conrad Balliet Family Nature Preserve, a local pond in Clark County, Ohio. Over the course of two separate study periods, 21 September 2022 to 6 October 2022 and 28 March 2023 to 15 April 2023, Promar 520 baited hoop traps were placed at separate locations along the pond, 4 in the fall and 6 in the spring (Figure 1). They were baited with wet cat food or sardines, with the bait being reused twice, and replaced after the second reuse. The traps were placed at multiple locations around the pond to try to provide coverage of the whole pond and not bias the study towards the possible turtle population in one part of the pond. The additional 2 traps for the spring session were added to provide coverage for areas of the pond that the original fall session did not cover. The traps were checked daily and were set and checked multiple times a week. Upon checking the traps, each turtle was processed on site. When taken out of the funnel traps, each turtle was placed in a cloth snake bag and in-situ field work and data collection was done on each turtle individually. While in the bag, the turtle was also scanned for a PIT tag to establish whether it was a recapture, and the turtle's mass was measured using an appropriate Pesola scale. The mass of the bag was recorded and subtracted from the overall mass of the bag and turtle to find the mass of the turtle alone.

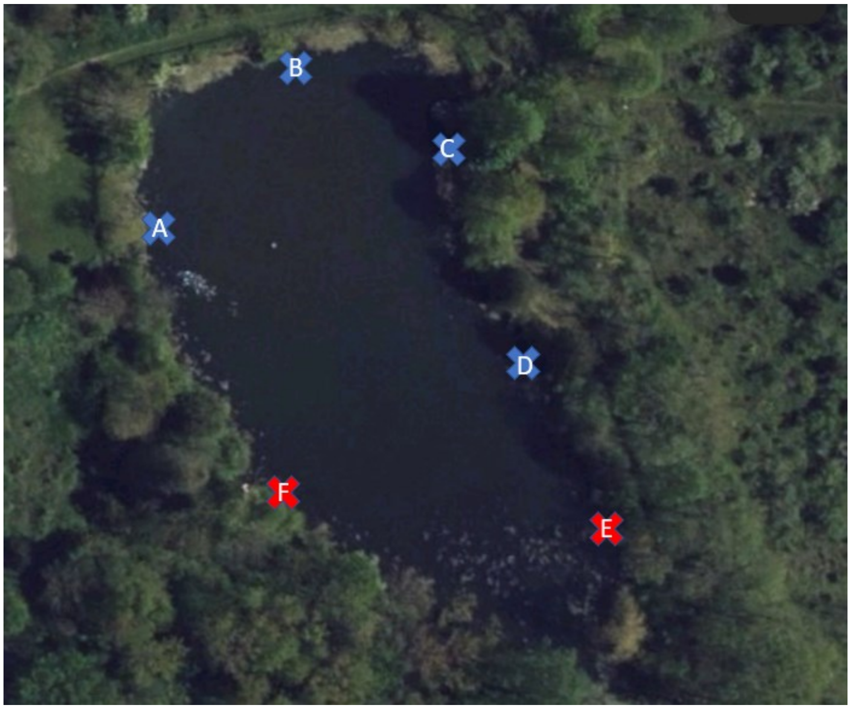

Figure 2. Representation of the pond at Conrad Balliet Family Nature Preserve and the placement different funnels traps for turtle trapping. Blue denotes traps used in during the September/October study period, and red denotes traps added during the March/April study period. Traps were assigned a letter to keep track of turtle movements.

The turtle was then removed from its bag and its shell was assessed for any prior notches on the marginal scutes of the carapace to check for the possible recapture of a turtle that was not PIT tagged. A PIT (Passive Integrated Transponder) tag has a unique 15-digit number, which can be used for identification of recaptured turtles. Species and sex of the turtle were identified. For Midland Painted turtles, sex identification was based on secondary sexual characteristics, specifically cloaca position, claw length, tail width/length, and plastron concavity, with males having a lower cloaca position on the tail, longer claws, wider and longer tails, and slight concavity to their plastron (Ernst and Lovich 2009; Gamble 2006). The turtles from the first study period had to be re-identified for sex upon recapture, due to the dependence on plastron concavity for sex determination used in the first study, which is less distinguishable in painted turtles than other freshwater turtle species (Rowe 1997). Because of this, turtles from the first study period that were not recaptured in the second study period and did not have their sex confirmed based on the secondary sexual characteristics, had their sex determined from comparison of body measurements to the other sex-known turtles.

After species and sex identification, the carapace length and width, the plastron length and width, and the shell height for the turtle was measured in millimeters using calipers. Based on the measured carapace length, the age class of the turtle was identified. Age class for the subspecies was based on minimum size at sexual maturity, which is a carapace length of 145mm for females and 90mm for males (Hughes et al. 2016). Turtles that reached the minimums for carapace length for each sex were classified as adults, while turtles below these minimums respective to sex were classified as juveniles.

If the turtle had no scute marks, its shell was scute marked with a file according to the 1-2-4-7 scale as an ID (Figure 3). The shells were also marked in white paint marker with their scute ID number as a more temporary identification and one observable without trapping. If the turtle had no PIT tag, a PIT tag was injected, unless the turtle was very weak and lethargic or there was inclement weather, and we were trying to limit the turtle's exposure to it. This process was repeated for each turtle per trap. All turtles captured were measured and marked, but only the data on Midland Painted turtles were used for this study.

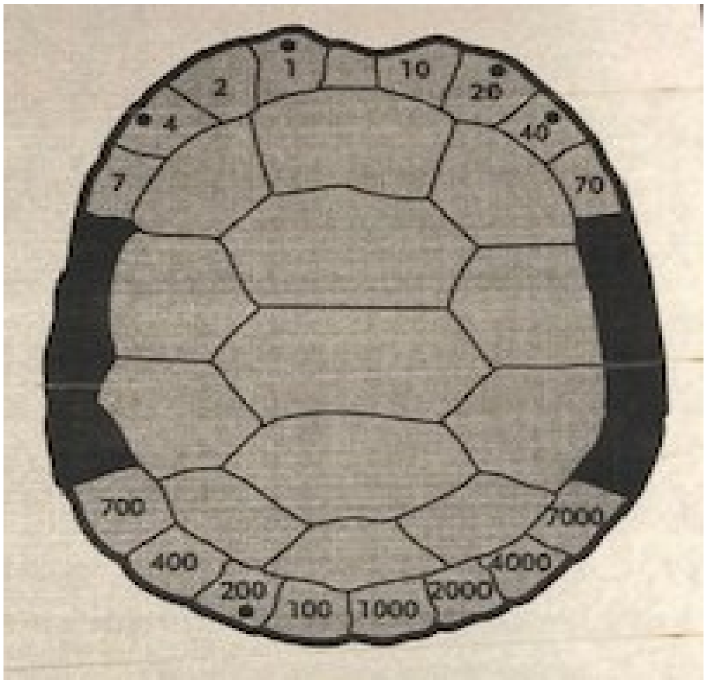

Figure 3. Marking System of the Marginal Scutes on the Carapace. This marking system is used to denote a turtle's ID. The 1-2-4-7 system allows for up to 9999 unique possible identifications. A turtle with these marks would be labelled as 265.

(Buhlmann et al. 2008)

Statistical analysis was performed on all body measurements for differences between male and female turtles, as well as differences between ratios of male versus female turtles, using t-tests and chi-square goodness of fit tests respectively. T-tests were used to determine significant differences in the averages of the measurement data between males and females, and the chi-square goodness of fit test was used to determine if the data collected during the study on population levels was representative of the population as a whole. Only the data for turtles with a confirmed correct sex were analyzed to avoid discrepancies and possible bias within the data. From the mark-recapture data, the overall population size was estimated using the Schnabel Model. Under the Schnabel model, we make the mark-recapture assumptions that: (1) the population is closed and therefore no births/deaths/immigration/emigration are occuring during the study period, (2) the population does not change size, (3) all samples are random and not biased, (4) recapture of individuals is not affected by marking, and (5) marks are not lost, gained, or overlooked (Ryan 2018). The RStudio function "Schnabel" was used to calculate the population estimate, N, using the equation:

$N = \sum(C_i*M_i)/ \sum R_i$

C_i = captured individuals in a sample

M_i = marked individuals in a sample

R_i = recaptured individuals in a sample

The function "Schnabel" was also used in RStudio to calculate a 95% confidence interval was calculated using a Poisson distribution.

Results

Population Size and Demographic

There was a total of 58 capture events of Midland Painted turtles at Conrad Balliet Family Nature Preserve Pond throughout the 2 study periods: 28 individual turtles and 30 recaptures. Of the 28 individual turtles, 9 were adult females, 13 were adult males, 6 were juveniles (Figure 4). Of the juveniles, one was identified as male, while the other 5 were identified as female. Seven of the capture events, 3 initial captures and 4 recaptures, were of turtles that could not have their sex confirmed due to not being recaptured during the second study period. Based on assumptions made from comparison of body measurements, two of these turtles were classified as juvenile females and one was classified as an adult male. However, because their sex and age class were assumed and not confirmed, they were not included in the data analysis to avoid potential bias. Therefore, the known population of turtles was 9 adult females, 12 adult males, 3 juvenile females, and 1 juvenile male. The juvenile to adult ratio of Midland Painted turtles was 0.19:1, and the adult male to adult female ratio was 1.33:1. There were equal numbers of individual adult males and adult females captured ($\chi^2_1 = 0.429$; $p > 0.05$).

On days turtles were caught, males tended to be more prominent in the traps than females (Figure 5). Adult males also made up the majority of the recaptures, and the ratio of adult males to adult females of Midland Painted turtles recaptured was 3:1. Overall, there were more males than females recaptured ($\chi^2_1 = 5.69$; $p < 0.05$; Figure 6). Based on the capture, recapture and marked numbers from Table 2, the Schnabel model estimated the population size of Midland Painted turtles in the pond to be 30.27 turtles, with a 95% confidence interval of 22.15 to 45.40 Midland Painted turtles.

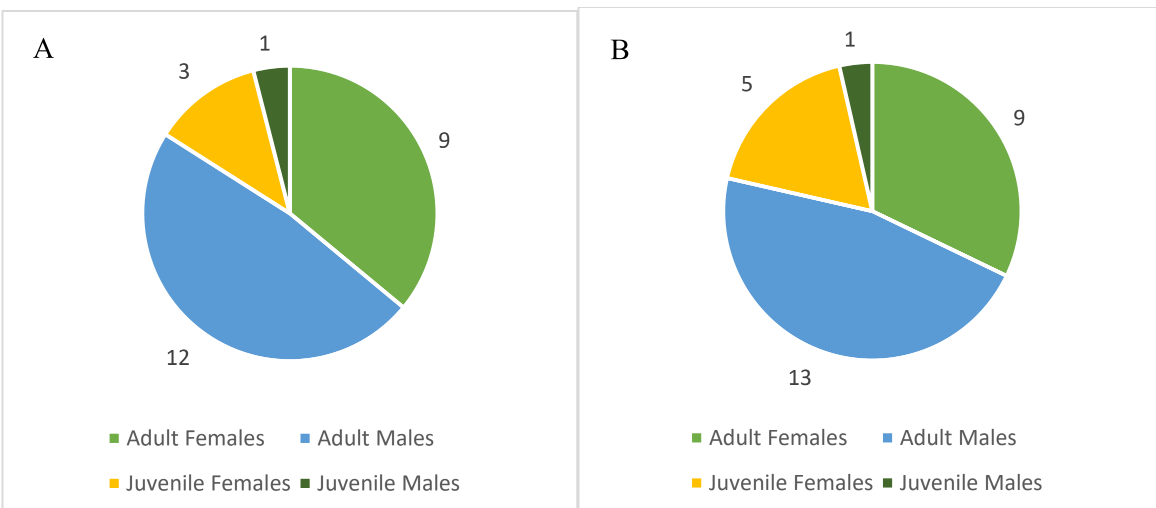

Figure 4. Population Representation of the Sex and Age Class of Captured Turtles. (A) Of the turtles with confirmed sex turtles, 12 were adult males, 9 were adult females, 3 were juvenile females, and 1 was a juvenile male. (N = 25) (B) Of the total, including turtles with assumed sex, 13 were adult male, 9 were adult female, 5 were juvenile female, and 1 was a juvenile male. (N = 28)

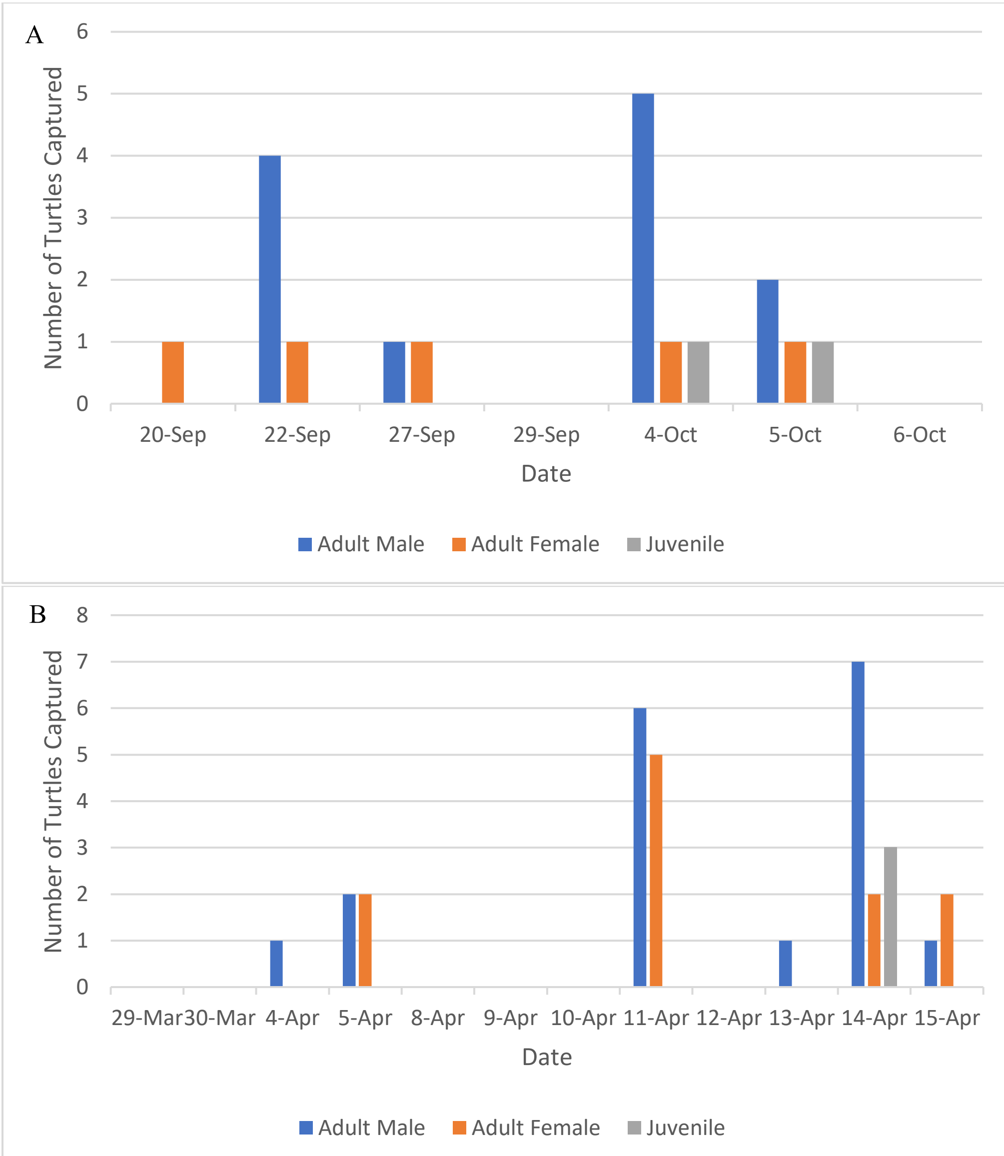

Figure 5. Representation of the breakdown of the sex of turtles caught on days traps were checked during (A) fall period and (B) spring period. Includes initial captures and recaptures and excludes the 7 capture events from which the turtles did not have a confirmed sex or age class. (N = 51)

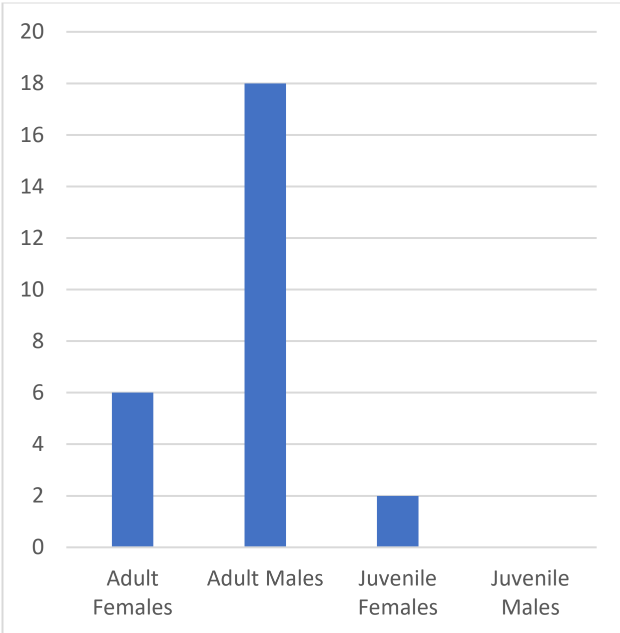

Figure 6. Recapture numbers of adult males, adult females, juvenile females, and juvenile males. Excludes the 4 recaptures where the sex and age class of the turtles were not confirmed. (N = 26)

Sexual Dimorphism

Adult female Midland Painted turtles were larger than adult males in multiple body measurements: carapace length ($t = 2.09$, $df = 19$, $p < 0.001$; Table 1, Figure 7); carapace width ($t = 2.09$, $df = 19$, $p < 0.001$); plastron length ($t = 2.09$, $df = 19$, $p < 0.001$); plastron width ($t = 2.09$, $df = 19$, $p < 0.001$); shell height ($t = 2.09$, $df = 19$, $p < 0.001$); and mass ($t = 2.09$, $df = 19$, $p < 0.001$).

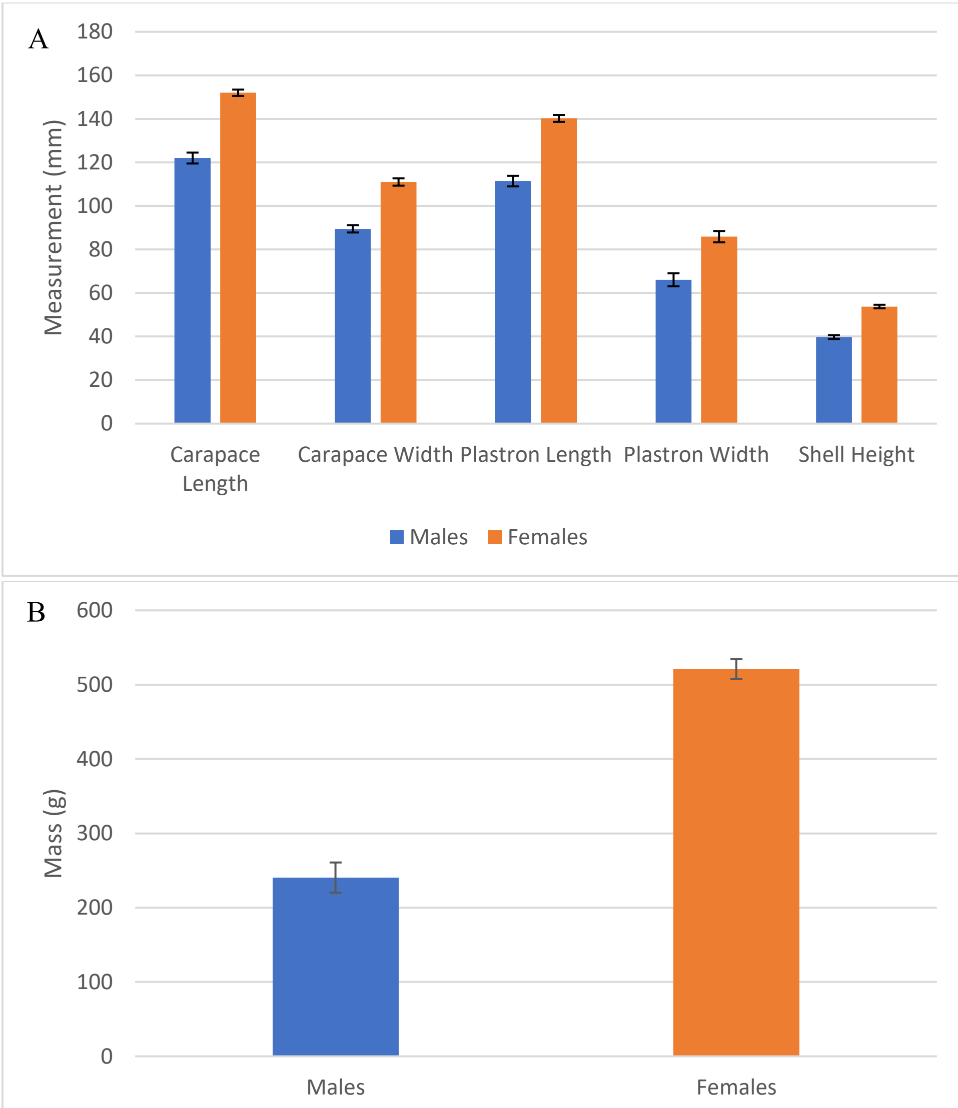

Figure 7. Average body measurements between adult male and female Midland Painted turtles with standard error bars. (A) Represents shell measurements (B) Represents mass (N=21)

Body Size	Females	Males
Carapace Length (mm)	152 ± 1.45 (N = 9)	121.15 ± 2.46 (N = 12)
Carapace Width (mm)	111 ± 1.24 (N = 9)	88.92 ± 1.66 (N = 12)
Plastron Length (mm)	140.22 ± 1.59 (N = 9)	110.23 ± 2.53 (N = 12)
Plastron Width (mm)	85.88 ± 2.59 (N = 9)	66.08 ± 2.76 (N = 12)
Shell Height (mm)	53.78 ± 0.83 (N = 9)	39.75 ± 0.90 (N = 12)
Mass (g)	520.89 ± 13.42 (N = 9)	240.42 ± 20.40 (N = 12)

Table 1. Average body size measurements for adult male and adult female Midland Painted turtles. Standard error included. (N = 21)

i	C_i	R_i	U_i	M_i
20-Sep	1	0	1	0
22-Sep	6	0	6	1
27-Sep	3	1	2	7
4-Oct	10	4	6	9
5-Oct	6	4	2	15
4-Apr	1	1	0	17
5-Apr	4	1	3	17
11-Apr	11	7	4	20
13-Apr	1	1	0	24
14-Apr	12	8	4	24
15-Apr	3	3	0	28

Table 2. Representation of Midland Painted turtles during the capture sessions. i = capture session date, C_i = number of individuals captured at the ith date, R_i = number of recaptured individuals at the ith date, U_i = number of animals marked for the first time at the ith date, and M_i = number of animals marked in the population prior to the ith date. The Schnabel model uses C_i, R_i, and M_i to estimate population.

(Ryan 2018)

Discussion

The Midland Painted turtles of the pond at Conrad Balliet Family Nature Preserve showed sexual dimorphism in all body measurements, with females being larger than males in carapace length/width, plastron length/width, shell height, and body mass. This is consistent with known population parameters about Painted turtles (Ernst and Lovich 2009; Litzgus and Smith 2010). One likely reason that sexual dimorphism occurs in this species is that the males do not engage in aggressive behavior during mating season, similar to many species of aquatic turtles, and therefore, a bigger size is not favored in order to compete with other males. In contrast, the size dimorphism with smaller males is likely due to one of three current hypotheses: (1) females being larger having larger clutch sizes and/or increased clutch laying, (2) easier and greater mobility from a smaller size allows for males to more easily reach seek out and find a mate, or (3) males are smaller because they spend more energy trying to find a mate than on growth. All of these are possible hypotheses as to why adult male turtles may be smaller than adult female turtles, and they are not mutually exclusive of one another (Agha et al. 2018; Berry and Shine 1980).

The population of Midland Painted turtles in the pond was slightly skewed towards adult males, though there was no significant difference between the abundance of the sexes. However, while 6 juveniles were captured in the pond, 5 of them were classified as juvenile because they did not reach the size of sexual maturity that was associated with their sex. It is possible that some of the juvenile turtles that were confirmed as female were sexually mature but fell short of the average age of sexual maturity for females being 145mm and thus were classified as juveniles, when they could have been adults. Therefore, it is possible that there are more adult female turtles in the population. The sex ratio being slightly towards males, as well as the recapture rate for males being much higher than for females, could indicate a trapping bias for males with the hoop traps. Female *Chrysemys picta* are capable of escaping hoop net traps, with one study indicating that within 24 hours, 80% of the females placed into traps escaped (Frazer et al. 1990). This could mean that female Midland Painted turtles in the population are simply escaping before the traps are checked, and therefore why more males were recorded. Furthermore, males may also be more attracted to traps if there is a female present, especially during spring and fall mating periods (Frazer et al. 1990; Gamble 2006). The fact that many days during this study had several males captured with few females captured support this possibility. Any or each of these factors could influence the sex ratio of captured turtles.

Despite the slight skew towards male Midland Painted turtles in the population, because there is not a significant difference between the adult males and adult females, this pond represents a fairly healthy population ratio of male turtles to female turtles with a healthy population ratio being a ratio of 1:1 for males and females (Dupuis-Desormeaux et al. 2017).

Because of this, we can assume that there are no factors affecting this pond that would significantly skew population numbers, such as drought, which has shown to skew Painted turtle populations in favor of females, or proximity to roads/increased risk of roadside mortality, which has shown to skew Painted turtle populations in favor of males (Dupuis-Desormeaux et al. 2017; Powell et al. 2023). The lack of skewed sex ratio for this pond supports the idea of separating wetlands from roadsides, when possible, as the road closest to the pond at Conrad Balliet Family Nature Preserve being distant from the pond and having little traffic. This has the likelihood of decreasing the risk of road mortality of turtles, specifically females who may venture near them when nesting. Separating wetlands from roads could also help with recruitment levels in ponds, as more mature/nesting females would survive to produce more offspring.

While it is not uncommon for freshwater turtle species to have lower numbers of juveniles, because juveniles tend to have lower survivorship than adults, the low numbers of juveniles could be an indication of low levels of recruitment (Rowe and Dalgarn 2010). This is important to track because while adult numbers could suggest a healthy population of a species of turtle, lack of juveniles adding to the population would lead to a sudden decrease of the turtle population in the future when the adults begin to die. Juveniles tend to inhabit shallower waters, while adults tend to inhabit the deeper waters (Rowe and Dalgarn 2010). Since all the traps were set near the shore and in shallower waters, it would make sense that more juveniles would be caught by our trapping method, but that was not the case, which could be an indication of lower numbers of juveniles in the pond. However, other studies on freshwater turtle studies also show lower proportions of juveniles caught, likely because they tend to be more cautious in their movements (Litzgus and Mousseau 2004). Juveniles are more at risk of predation because they tend to keep to the shallow waters, and likely have developed a sense for cover to protect them

(Rowe and Dalgarn 2010). The fact that the traps were close to shore and in shallow waters, but had no cover provided to them likely affected juvenile movements. Therefore, they may not have risked the open area for a chance of possible food and instead chose to remain under cover, and thus this could be a sampling bias for our method.

The possible lack of juveniles could possibly be remedied by the inclusion of nursery ponds near the larger pond, something not found at the pond at Conrad Balliet Family Nature Preserve. Nursery ponds are smaller, shallower, and more heavily vegetated ponds that are more suitable for smaller turtles than larger ones, and juveniles tend to thrive in them. Once old enough, the juveniles then move into the larger adjacent body of water to continue their adult life. The nursery ponds tend to provide good habitat and protection for juvenile turtles, thus allowing them better survivorship, and increasing recruitment into the overall population (Hughes et al. 2016). More data would need to be recorded for the population at Conrad Balliet Family Nature Preserve Pond in order to ascertain whether recruitment levels are truly low and nursery ponds would be a good addition.

Continued monitoring of this population over time will allow for more insight into survivorship and recruitment within the population. Understanding survivorship of the pond will help us to know if this pond has the factors in it that produce long longevity in Midland Painted turtles, which could be translated into conservation actions in implementing factors that increase longevity in turtles in areas where populations are decreasing/dying off. Understanding recruitment shows how juvenile survivorship numbers are and how many general Midland Painted turtles are being added to the population every year. As juvenile turtles of all species tend to have a high mortality rates and low survivorship, knowing factors that may increase juvenile survivorship can be a potential benefit for most freshwater turtle species when considering habitat parameters which promote better recruitment. Follow-up studies should take these aspects into consideration, as data is gathered over longer periods of time to better bolster the knowledge of the population of the pond at Conrad Balliet Family Nature Preserve Pond.

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