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Comparing Semi-Urban and Forest Populations of the Jalisco Mud Turtle (*Kinosternon chimalhuaca*)

Jose Garrido
Central Washington University, garridot@cwu.edu

COMPARING SEMI-URBAN AND FOREST POPULATIONS OF THE JALISCO MUD TURTLE (*KINOSTERNON CHIMALHUACA*)

A Thesis

Presented to

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Master of Science

Biology

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by

José Reynaldo Garrido

March 2021

Central Washington University
Graduate Studies

We hereby approve the thesis of

José Reynaldo Garrido

Candidate for the degree of Master of Science

APPROVED FOR THE GRADUATE FACULTY

March 2, 2021 ______________________
Dr. Daniel Beck, Committee Chair

March 2, 2021 ______________________
Dr. Alison Scoville

March 3, 2021 ______________________
Dr. Lixing Sun

____
Dean of Graduate Studies

ABSTRACT

COMPARING SEMI-URBAN AND FOREST

POPULATIONS OF THE JALISCO MUD TURTLE

(*KINOSTERNON CHIMALHUACA*)

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Mud turtles (family Kinosternidae) are primarily threatened by climate change, overexploitation, and land development. To survive in increasingly urbanized and arid regions, mud turtles often inhabit man-made water sources such as cattle troughs and irrigation ditches. These bodies of water are critical in urban habitat where they may offer some of the last remaining refugia; however, the effect of these conditions on population structure is poorly understood. The Jalisco mud turtle (*Kinosternon chimalhuaca*) was described in 1997 from a small range south of Puerto Vallarta, Mexico. Since its description, critical ecological research has remained largely nonexistent, hindering effective conservation and management. Recent satellite imagery surrounding our study sites in have shown the loss of lowland deciduous forest and an increase in human activity through deforestation and fragmentation with a dramatic increase in population and tourism. Our research reports the first comparative analysis of *K. chimalhuaca*'s populations from a pristine forested arroyo habitat in the Chamela-Cuixmala Biosphere Reserve, and a semi-urban habitat in a nearby small town. In July and December of 2019, our team surveyed a small ~1km irrigation ditch in the middle of town and astonishingly captured, marked and measured 226 turtles, estimating a population of 741 ± 132 individuals. Similar trapping effort in the forest habitat surrounding the Chamela field station yielded 12 turtles, added to a collection of 25 prior opportunistic captures. In town, turtles exhibited a female-biased ratio (1:2.16; 68% female; N = 174), contrasting the male-biased forest population (2.63:1; 28% female; N = 29). The carapace length (CL) of forest males were more bimodally distributed and significantly larger ($p = 0.036$) than their semi-urban counterparts. Forest females were also significantly larger than female turtles from the town ($p = 0.012$). The findings of this preliminary dataset warrant further investigation into the driving factors supporting abundant semi-urban populations and the effects of human-turtle interactions in the face of global turtle declines.

ACKNOWLEDGEMENTS

I am exceptionally grateful for the innumerable amounts of assistance I have received from many people and organizations throughout this project. Most of all I want to thank my advisor, Daniel Beck, for his unwavering support and guidance since the first day we met. Through the most difficult circumstances, he was always my biggest advocate, and I could not have navigated them without his tireless encouragement and wisdom. Thank you to my other committee members Alison Scoville and Lixing Sun for their exceptional advice and insightful knowledge. Thank you to Taggert Butterfield for his guidance and assistance measuring turtles late into the night. Thank you to Nicholas DeHollander and students from CWU's SOBRE Mexico Program for their field assistance. Thank you to Kathrine Renton and the workers at the Universidad Nacional Autónoma de México's Estación de Biología Chamela for access and provisions at my forest study site. Thank you to Doña Eva and the entire Robles family for sharing their home, food and wonderful conversion, as well as aiding my success in travel, access, field collections. Thank you to Rodrigo Macip-Ríos for his assistance in permitting, editing, and preliminary field data. Thank you to Akrem Dawed for his help in coding and advice in using low-cost biotechnology. A huge thank you to the entire CWU Biology department and graduate cohort for their endless solidarity and inspiration. Thank you to my partner, Elizabeth, and our dogs for their constant uplifting support and encouragement. Thank you to my parents for their many years of love and guidance. This project was made possible by funding through Central Washington University, the National Science Foundation, the Washington State Distinguished Fellowship Award, Sigma Xi, Holohil LLC, and the Kittitas Audubon Society.

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CHAPTER I

GENERAL INTRODUCTION

Turtles are considered the world's most endangered vertebrates, threatening 61% species with extinction in this century (TTWG 2017). Mud turtles (family Kinosternidae) living in arid regions face a particularly high risk from mounting pressures of climate change, land development and overexploitation (Butler et al. 2016). The decline of kinosternids is a serious detriment to terrestrial and aquatic ecosystems where they perform significant ecological roles in insect control, soil bioturbation, and seed dispersion (Lovich et al. 2018). However, the conservation and management of several kinosternids in Mexico are significantly hindered by a lack of records and ecological research (Legler & Vogt 2013). While human populations continue to grow exponentially, few studies examine how land development effects mud turtle population structure. Investigating how turtle populations respond to urbanization requires demographic data from varied and novel habitats and should be a top priority for informing conservation measures and combating future extinctions.

Land development and habitat loss are some of the most significant local factors in mud turtle declines (Butler et al. 2016; Stanford et al. 2020). Since 1980, the human population of Puerto Vallarta has increased from 65,000 to over half a million, not including the four million tourists who visit the city every year (UN 2019). This sharp rise in human population and land development has resulted in enormous biodiversity losses throughout the region, with effects radiating into our study site at the Chamela-Cuixmala Biosphere Reserve (140 km south) (Flores-Casas & Ortega-Huerta 2019). This is a devastating loss for the region, as the surrounding neotropical dry forest biome is Earth's most endangered tropical ecosystem and internationally recognized for high endemism and species richness (Janzen 1988; Ceballos & Garcia 1995). Recent satellite imagery surrounding our study sites has shown the loss of lowland deciduous forest and an increase in human activity from land development, fragmentation and the rise in population and tourism radiating from cities like Puerto Vallarta (Bautista & López-Caloca 2009; 2011). Although eight of the 16 Mexican *Kinosternon* species inhabit the Pacific coastal plains, many areas still have no published records of freshwater turtles due to a lack of exploration and recorded local knowledge (Legler & Vogt 2013; TTGW 2017; Lopez-Luna 2018). In this region, three new *Kinosternon* species have been recently described including *K. chimalhuaca* (Berry et al. 1997), *K. vogti* (Lopez-Luna et al. 2018), and *K. cora* (Loc-Barragan 2020) — all threatened by the growing pressures associated with their proximity to human development. Since *K. vogti* was discovered in the urban drainages around Puerto Vallarta, it has never been recorded in a natural habitat. Mud turtles rely on man-made water sources such as cattle troughs and irrigation ditches to survive in the increasingly urbanized and arid regions they inhabit (Carr 1952). These bodies of water are critical in urban landscapes where they may offer some of the last remaining refugia. Although poorly studied, these conditions can have either a positive or negative influence on turtle populations across taxa (Spinks et al. 2003; Rizkalla & Swihart 2006; Elston et al. 2014; Ryan et al. 2014; French et al. 2018). Mud turtles exhibit impressive behavioral and physiological plasticity, but many ecologists agree that such resilience may only delay, and not prevent, local extinctions (Schlesinger et al. 1990; Butler et al. 2016).

Human development plays a significant role in local turtle declines; however, the widespread effects of climate change endangers populations internationally. While most animals are threatened by rising global temperatures, the effect on mud turtles can be seen and predicted directly. This is because, like many turtles, kinosternids exhibit temperature-dependent sex determination (TSD), meaning hatchling sex is determined in the egg by its surrounding temperature (Vogt & Bull 1982). Sex determination outcomes vary among taxa, but in all mud turtles, eggs incubated at warmer temperatures (during the middle-third stage of incubation) produce exclusively female offspring, and >34˚C often result in mortality. Models have predicted that climate change has the potential to heavily skew sex ratios in the next 30 to 50 years with temperature increases of <2˚C (Janzen 1994). Furthermore, an increase of ≥4˚C (predicted within this century) could lead to entirely female offspring and mass nest mortality. Three traits exist by which turtles may adapt to thermal changes: nest-choice, nesting phenology, and altering their pivotal temperature (T_{piv}), or the temperature at which sex ratios are 1:1 (Valenzuela & Lance 2004). Mud turtles in Mexico often travel <150 m to nesting and estivation sites, limiting the thermal availability of nest-site choice as a method of adaptation (Morales-Vereja & Vogt 1997; Pérez-Pérez 2017; Montiel-Ugalde 2018). Kinosternids in the seasonally dry forests reproduce during the short rainy season (July-September), limiting adaptation through nesting period phenology and behavior change. Geographical shifts in the T_{piv} have been recorded in some turtle species (this has not been established in kinosternids); however, most biologists agree that the rate of projected environmental changes will likely surpass the limits of turtles' biological adaptability (Valenzuela & Lance 2004; Butler 2016). Furthermore, aquatic habitats near human development often experience increased local temperatures through urban heat island effect which increases the rate of seasonal evaporation, diminishes water quality, and reduces aquatic biodiversity (Manley 1958; Johnson 2013). With limited methods for adapting to a rapidly changing environment, studying the effects of climate change and urbanization on mud turtle populations are critical to inform conservation measures and prevent future extinctions.

The Jalisco mud turtle (*K. chimalhuaca*) was described in 1997 in a small range south of Puerto Vallarta, having been previously mistaken for the widespread Mexican mud turtle (*K. integrum*). Since its description, critical ecological research from its full range of occupied habitats have remained largely nonexistent, limiting comprehensive conservation assessments and management plans. My research reports the first comparative analysis of *K. chimalhuaca*'s populations from two unstudied habitats: a pristine forest arroyo and a semi-urban ditch. I explored the effects of urbanization by sampling turtles from a semi-urban site in a perennial, man-made canal predominantly exposed and bordered by concrete, gravel, and grasses. In contrast, the forest habitat we sampled is characterized by a network of ephemeral arroyos with some flowing, shallow pools and shaded by a dense canopy in the Chamela-Cuixmala Biosphere Reserve. I predicted that we would find significant differences in population structure (abundance, sex ratio, body size, and body size distribution) between semi-urban and forest habitats. Comparing turtles from novel and contrasting habitats provides insight into the effects of urbanization and will help develop strategies to maintain the health and biological integrity of mud turtle populations in the face of declines by human-caused habitat alteration.

CHAPTER II

LITERATURE REVIEW

More than half (55%) of the world's human population now lives in urban areas (UN 2020). This number is expected to increase to 68% by 2050 in combination with a population increase of up to 2.5 billion people, totaling over 10 billion. This proportion is even higher in North and Central America where over 80% of the population lives in urbanized areas. Although some argue that human migration to cities removes pressure from natural ecosystems, population growth necessitates continuous resources, development, and expansion of urban areas (Shen 2005; French 2018). This poses a growing threat to maintaining biological diversity in sensitive and endangered natural ecosystems. Responses to fragmentation and urbanization have shown mixed effects in several vertebrate taxa, often negatively impacting native and specialist species, but sometimes favoring generalist and invasive species (Fahrig 2003; Rodda & Tyrell 2008). In reptiles, the outcomes of urbanization are typically harmful; they include, decreased abundance, species richness, gene flow, and survivorship (Henderson et al. 2009; Banville & Bateman 2012; Hunt et al. 2013; Beninde et al. 2016; Sullivan et al. 2017). However, in the metropolitan area of Charlotte, North Carolina different species of semi-aquatic turtles show mixed survivorship (Eskew et al. 2010). Yellowbelly sliders (*Trachemys scripta*) and common snapping turtles (*Chelydra serpentina*) show high survivorship (73–92.5% and 91.4–99.4%, respectively), whereas eastern mud turtles (*Kinosternon subrubrum*) show uncharacteristically low survivorship (58.6–73.6%). Urban land development will continue to adversely affect turtle biodiversity in most ecosystems. But with a broad range of responses, evaluating which species and populations are at highest risk is essential for determining the most effective management and conservation strategies.

Urban water sources can be critical refugia for turtles in developed habitats, but the impacts on populations structure are complex and vary according to life history, habitat complexity, and stability. Two important characteristics associated with increases in turtle abundance are regional drought intensity and proportions of females (for turtles with TSD), both of which are positively correlated with urbanization and climate change (Leng et al. 2015; Valenzuela et al. 2019). For semi-aquatic turtles who only estivate during the driest years, such as painted turtles (*Chrysemys picta*), perennial urban water sources may not always pose a significant advantage for overall survivorship (Bowne 2008). Man-made aquatic habitats can drastically reduce abundance in some populations of *C. picta* (Minton 1968), whereas similar conditions cause other populations to increase or remain stable (Rizkalla 2006). Bowne et al. (2018) found that across 11 states, there was a significant positive relationship between urbanization and proportions of mature female painted turtles, which may be caused by limited thermal refuge and urban heat island effects. Habitat stability also plays an important role in turtle populations, although some species appear resilient to periodic disturbance in man-made habitats (Paul & Meyer 2001; Roe et al. 2011; Plummer et al. 2008). In an urban population of softshell turtles (*Apalone spinifera*), individuals emigrated downstream during periods of habitat disturbance, and quickly recolonize after stabilization, with insignificant decreases in survival rates (Plummer & Mills 2008).

The vast majority of publications regarding urbanization focus on presence or abundance and seldom examine turtles' specific response to common urban habitat characteristics such as limited thermal refuge, altered resource availability, and diminished water quality (French 2018). Future investigations would have greater significance by narrowing the scope of research on urban habitats to investigate the specific drivers of a population-level response. Anthropogenic contamination of waterways can be documented in remote areas far from human development or decades after disturbance events. This has the potential of exposing even protected turtle populations to a wide variety of long-term health effects. In three federally protected California habitats, 57 pesticides were detected in western pond turtle (*Emys marmorata*) populations found locally from historic mines and downwind from urban and agricultural areas (Meyer et al. 2016). In a contaminated Virginia river, high mercury levels were detected in four turtle species (*P. rubriventris, C. picta, S. odoratus,* and *C. serpentina*) depending on diet preference (Bergeron et al (2007). High mercury levels have been linked to reduced hatchling success and altered behavior in snapping turtles (Hopkins et al. 2013). Failey et al. (2007) also determined that semi-aquatic turtles inhabiting golf course ponds had significant delays in reaching sexual maturity. Studies that focus on deciphering the various population-level effects posed by specific habitat conditions and contaminants could play an important role in determining the health and integrity of semi-aquatic turtle populations.

Research has rarely investigated the effects of perennial urban aquatic habitats on turtles in arid regions who estivate during long periods of drought. In one study, Roe et al. (2011) documented that eastern long-necked turtles (*C. longicollis*) flourished in suburban perennial ponds, documenting three times more abundance, five times faster growth rates, a female-skewed sex ratio, and similar survivorship compared to wild populations from ephemeral wetlands. Suburban *C. longicollis* were benefitted by foregoing the long-term estivation typically essential to survive the long dry season in naturally ephemeral water sources. Conversely, among several populations of Sonoran mud turtles (*Kinosternon sonoriense*), density was significantly higher in intermittent water sources, although permanent water bodies boasted significantly larger body sizes (Stanila 2009). Permanent and intermittent aquatic habitats supported both male-biased and female-biased populations, but habitat structure was the main driver of abundance and density. Further demographic research should be conducted from various habitat structures to investigate the response of population densities from sex ratios in combination with habitat structure.

Turtles with TSD may experience higher abundance in response to increased temperatures, but this does not necessarily reflect the health or stability of the population. A generalized model predicted that along a thermal gradient, TSD can independently cause female-skew turtle populations to result in peak turtle abundances; but population size drastically decreases when warmer temperatures create male-limited populations or high egg mortality (Boyle et al. 2014). However, the population-level effects of TSD are rarely independent, and often conflated with other factors that significantly drive abundance such as habitat structure and resource availability. Nonetheless, reporting population sex ratios, and their changes over time, may signal a persisting response to local temperature changes, and help predict a local population crash from male-limiting feminization. Consideration of how local sex ratios and habitat structure may drastically skew abundance is especially important when using these populations to infer the conservation status of rare, unstudied, and new species with limited records. In the initial description and type locality of the Jalisco mud turtle (*Kinosternon chimalhuaca*), a dense population of 81 turtles are reported living in “a clear pond located 30 m southeast of Mexico Highway 80” and from two other exposed pools along the highway (Berry et al. 1997). Although sex ratios were not reported, Berry et al.’s other analyses suggest these populations were heavily female-skewed. Being the only published record of *K. chimalhuaca* populations, this was only demographic reference used to establish the conservation status of *K. chimalhuaca* as “least concern” (IUCN 2007). However, depending on the effects of perennial water sources on the resource availability, and local temperature or exposure on the sex ratios in these populations, Berry et al.'s recorded abundance may represent an overestimate of typical population size when compared to forest populations living in the densely covered ephemeral arroyos (which represent a large portion of its range). This does not suggest the conservation status is necessarily inaccurate, but it could be enhanced by including sex ratios and abundance data from other habitat types to represent the full range of occupied habitats. Characterizing the relative densities in various habitat types across *K. chimalhuaca*'s range, and the factors most prominently influencing population density, will lead to more comprehensive estimates of population health and better inform future conservation and management strategies.

CHAPTER III

JOURNAL ARTICLE

Comparing Semi-urban and Forest Populations of the Jalisco Mud Turtle (*Kinosternon chimalhuaca*)

José R. Garrido[1], Taggert Butterfield[2], Alison G. Scoville[1], Rodrigo Macip-Ríos[2], Daniel D. Beck[1]*

1. Department of Biological Sciences, Central Washington University, 400 E. University Ave, Ellensburg, WA 98926, USA
2. Escuela Nacional de Estudios Superiores, Unidad Morelia, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro 8701, Morelia, Michoacán 58341, México

*Corresponding author; email: josereygarrido@gmail.com

Mud turtles (family Kinosternidae) are primarily threatened by climate change, overexploitation, and land development (Butler et al. 2016). To survive in increasingly urbanized and arid regions, mud turtles often inhabit man-made water sources such as cattle troughs and irrigation ditches (López-Luna et al. 2018). These bodies of water are critical in urban habitats where they may offer some of the last remaining refugia. Although poorly studied, these conditions can have either a positive or negative influence on turtle abundance across taxa (Spinks et al. 2003; Elston et al. 2014). Mud turtles exhibit impressive behavioral and physiological plasticity, but many ecologists agree that such resilience may only delay, and not prevent, local extinctions (Schlesinger et al. 1990).

Our research reports the first comparative demographic analysis of two Jalisco mud turtle (*Kinosternon chimalhuaca*) populations: one from a small town's central irrigation ditch, and another from a nearby pristine neotropical deciduous forest arroyo. Since the initial description of *K. chimalhuaca* over twenty years ago (Berry et al. 1997), critical ecological research has remained largely nonexistent while human pressures continue to grow. At the northern extent of *K. chimalhuaca*'s small range, the human population of Puerto Vallarta, Mexico has increased from 65,000 to over half a million since 1980, not including the four million tourists who visit the city each year (UN 2019). Satellite images of our nearby study sites shows fragmentation, a loss of lowland deciduous forest, and an increase in human activity caused by the sharp rise in population and tourism radiating from nearby cities like Puerto Vallarta (Bautista and López-Caloca 2009). We explore the effects of urbanization by sampling turtles from a semi-urban site in a perennial, man-made canal predominantly bordered by concrete, gravel, and grasses. The canal is generally exposed, except for under sparse vegetation and in sections flowing under the town's streets (Fig. 1). In contrast, the forest habitat we sampled is characterized by a network of ephemeral arroyos with many shallow pools and shaded by a dense canopy in the Chamela-Cuixmala Biosphere Reserve (19.4984°N, -105.0443°W) (Fig. 1). We predicted that we would find significant differences in *K. chimalhuaca* population structure (abundance, sex ratio, mean body size, and size class distribution) between the semi-urban and forested habitats. Comparing turtles from such contrasting habitats may provide insight into the effects of urbanization and for developing strategies to maintain the health and biological integrity of mud turtle populations in the face of human-caused habitat alteration.

Methods.— From 2015–2018 we opportunistically captured and measured *K. chimalhuaca* in several arroyos of the Chamela Forest, before conducting targeted trapping at both the town and forest site in 2019. Although *K. chimalhuaca* is the only freshwater turtle species present in the region, identification was confirmed by the presence of a reduced plastron and contact of the auxiliary and inguinal scutes (Berry et al. 1997). We sampled turtles from both populations during the night using partially submerged hoop-nets baited with punctured tuna or sardine cans (Moll and Legler 1971). In the Chamela Forest, trapping efforts comprised four traps set for two nights during both July and December of 2019, totaling 16 trap nights. In town, during both July and December 2019, two traps were set the first night followed by four traps the next night for a total of 12 trap nights. During each capture, sex and weight were recorded before measuring straight-line carapace length (CL), straight-line carapace width (CW), plastron length (PL), plastron width (PW), and shell height (SH) with digital calipers to the nearest 0.1 millimeters (Carr 1952). We confidently identified sex only in turtles typically >90 mm; thus, only they were included in our analyses. Turtles were given individual identification numbers using a modified marginal scute notch numbering system (Cagle 1939).

We used the statistical computing environment R 4.0.2 (R Core Team 2020) to test for differences in *K. chimalhuaca* population characteristics between the two habitats. The Rcapture 1.4-3 package was implemented to calculate our population abundance estimates and reported with standard error (Rivest and Baillargeon 2019). The differences in adult sex ratios were calculated in a Chi-squared test with an alpha of 0.05. To test for normality in body size distribution we used a Shapiro-Wilks test. To transform our unbalanced and nonparametric data, measurements were processed using an aligned rank transformation in the ARTool 0.10.7 package and compared using a two-way factorial ANOVA with an alpha of 0.05 (Kay and Wobbrock 2020). The use of an ANOVA allowed us to test for interactions, and examine the main effect of site without the effect of sex ratios to bias each populations' mean body size. Measurement values were averaged in our analyses for turtles who were captured more than once.

FIG. 1. An arroyo sampled in the forest habitat from the Chamela-Cuixmala Biosphere Reserve (left) and the exposed semi-urban habitat sampled from the nearby town's central irrigation canal (right).

Results; Abundance.— In four years of mixed sampling effort in the Chamela Forest, we marked a total of 37 turtles, with four recaptures. Forest trapping efforts from 16 trap nights in July and December 2019 yielded 12 turtles, while the remaining 25 captures were opportunistically encountered from 2015–2018. Recapture data in the forest were not sufficient to calculate a precise population estimate. In a 400 m reach of the town ditch, 12 trap nights from July and December 2019 yielded 224 captures of 206 individuals. During two nights of sampling in July, 44 turtles were captured in the first night and 94 the second night with five recaptures. In two nights of trapping in December, the first night yielded 28 turtles with nine recaptured from July. The second night yielded 68 turtles with six additional recaptures from July and no recaptures from the previous night. No turtle was captured more than twice during the entire sample period.

A total of 17 recaptures among all sampling events estimates 741 ± 132 turtles occupy the ditch in town.

Sex Ratios.— Sex ratios differed significantly between forest and the town populations. In town, turtles exhibited a female-biased sex ratio (1:2.16; 68% female; N = 174) that significantly different from an expected 1:1 sex ratio (χ^2 = 12.18; P < 0.0005). This contrasted the male-biased sex ratios in the forest (2.63:1; 28% female; N = 29) which also differed significantly from a 1:1 sex ratio (χ^2 = 5.83; P < 0.016) (FIG. 2).

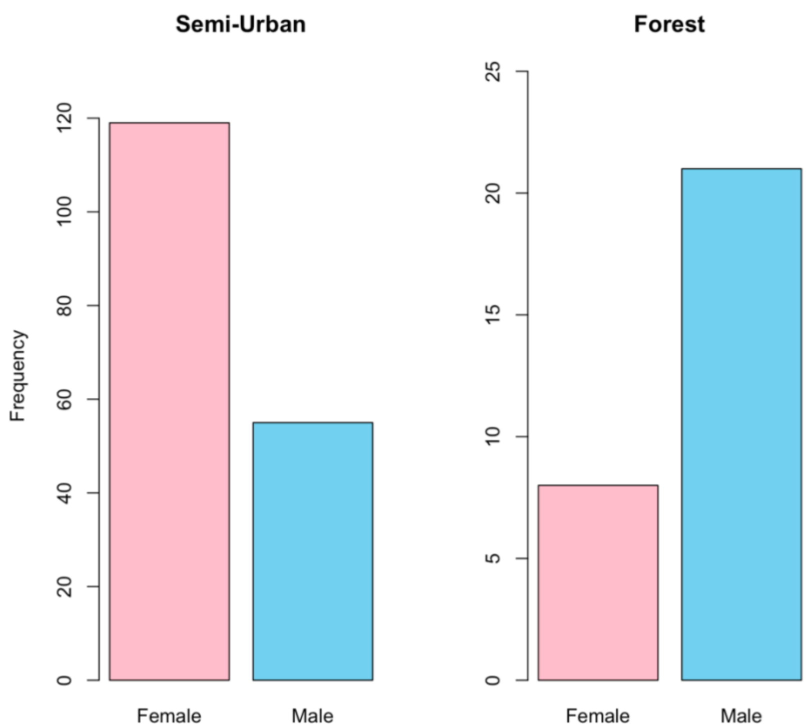

FIG. 2. Frequency of male and female *K. chimalhuaca* from a semi-urban and forest habitat.

Size.—The mean CL of male turtles from the Chamela Forest ($\bar{x}$ = 132.2; SE = 4.3; N = 23) was larger than males from the town ($\bar{x}$ = 123.5; SE = 2.1; N = 63). (TABLE 1; FIG. 3). Forest females ($\bar{x}$ = 120.0; SE = 4.1, N = 9) were also, on average, larger than semi-urban females ($\bar{x}$ = 113.5;

SE = 0.8, N = 134) (TABLE 1; FIG. 3). The larger size of forest turtles was statistically significant for both males (t = 2.719; df = 209; P = 0.036) and females (t = 3.100; df = 209; P = 0.011) in a post-hoc comparison of a two-way factorial ANOVA. Sexual dimorphism was confirmed where males were significantly larger than females in both populations (F = 36.9 ; df = 209; P < 0.00001; Table 1). No interaction between site and sex were detected (F = 0.012; df = 209; P = 0.91). Body size class distributions of forest turtles differed significantly from a normal distribution in both males (W = 0.87; N = 26; P = 0.006) and females (W = 0.78; N = 10; P = 0.01; FIG. 3) in a Shapiro-Wilks test. In contrast, turtles in town showed size class distributions that conform to normality for both males (W = 1; N = 64; P = 0.97) and females (W = 0.98; N = 134; P = 0.53; FIG. 3).

TABLE 1. Weight (g) and measurement (mm) averages with standard error reported for each sex and population with straight-line carapace length (CL), straight-line carapace width (CW), plastron length (PL), plastron width (PW), and shell height (SH).

	N	Weight	CL	CW	PL	PW	SH
Forest Male	23	296 ± 29	132.2 ± 4.3	83.1 ± 2.2	101.6 ± 2.7	64.7 ±1.5	44.4 ± 1.7
Forest Female	9	251 ± 25	120.0 ± 4.1	81.5 ± 2.9	101.7 ± 3.9	63.5 ± 2.6	44.1 ± 2.1
Semi-urban Male	63	254 ± 11	123.5 ± 2.1	78.4 ± 1.1	97.9 ± 1.3	64.2 ± 0.8	41.7 ± 0.7
Semi-urban Female	134	225 ± 5	113.5 ± 0.8	75.9 ± 0.5	97.1 ± 0.6	63.6 ± 0.4	42.1 ± 0.4

Some forest males were substantially larger than other adults (FIGS. 3 and 4); these larger individuals ranged from 153.3–177.6 CL ($\bar{x}$ = 162.5; $\sigma_{\bar{x}}$ = 2.9, N = 6) compared to the more abundant group of smaller wild males (CL < 131.5) ($\bar{x}$ = 122.8; $\sigma_{\bar{x}}$ = 1.8, N = 18). The CL of the largest forest male (580 g, CL = 170, PL = 128, PW = 79, MW = 100, SH = 57) exceed the previously recorded maximum CL for males of 157 mm (Berry et al 1997). However, the largest individual females came from the town site, with the largest female (334 g, CL = 140, PL = 108, PW = 72, MW = 88, SH = 48) exceeding the previously reported maximum CL of 127 (Berry et al. 1997).

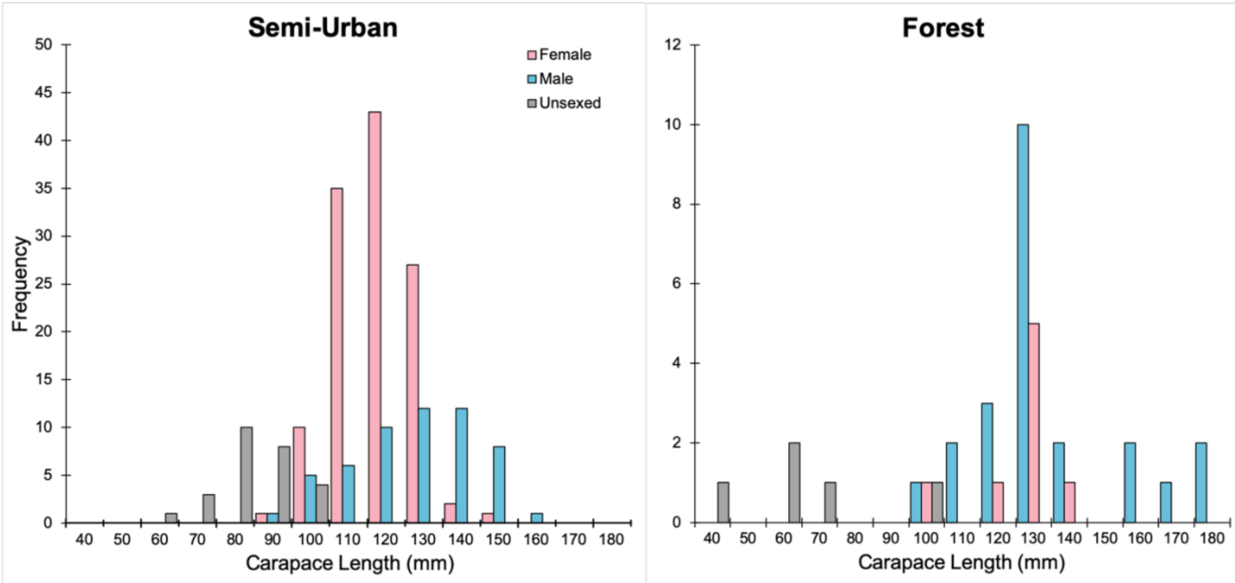

FIG. 3. Size class distribution of males (blue), females (pink), and unsexed juveniles (grey) from semi-urban and forest habitats at first capture. X-axis values are at the upper limits of each 10 mm bin.

FIG. 4. Two typical adult males from the Chamela Forest demonstrating the differences between small (above) and large males (below).

Discussion.— Jalisco mud turtles in town had a much greater population density than those in the forest. Although the town ditch is less than a kilometer in length, the highest density of turtles was concentrated in deeper water (>1 m) and closest to the town's center. Nutrient loading from communal greywater drains in town may feed turtles directly, or act to support and concentrate food resources such as the abundant populations of *Poecilia* fish we observed — a high-calorie food source for opportunistically carnivorous mud turtles. Moreover, the perennial irrigation ditch in town allows turtles to benefit from potentially year-round resource availability and either delay or entirely avoid estivation. In contrast, mud turtles from the forest leave their ephemeral arroyos during the dry season and spend the next several months underground in a period of estivation until the summer rains return. These differences in habitat structure and food resources are likely what is driving differences in population size between forest and semi-urban populations. Both forest and town habitats in our study differ from the nearby type locality of *K. chimalhuaca*: "…a clear pond located 30 m southeast of Mexico Highway 80." Here, Berry et al. (1997) recorded a dense population of mud turtles in an exposed one-acre pond, capturing 81 individuals in ten hours of trapping. This supports the notion that population dynamics for *K. chimalhuaca* are highly variable and tied to habitat features.

Mud turtles in the forest showed a strongly male-biased sex ratio, whereas turtles in town showed the opposite pattern — with females outnumbering males two to one. This difference may be a result of the limited forest sample size, or due to the effect of temperature-dependent sex determination (TSD) during incubation. In kinosternid turtles, eggs incubated at high temperatures produce almost entirely female offspring, whereas the sex of turtles incubated at lower temperatures depends on each species' fixed expression pattern (TSD Ia or II) (Vogt and Flores-Villela 1992; Butler et al. 2016). The expression of TSD in Kinosternidae is highly variable and has not been explicitly reported for *K. chimalhuaca*. However, its sister neotropical species (*K. hirtipes* and *K. sonoriense*) exhibit highly polarized patterns of TSD Ia with a narrow transitional range of temperature (TRT) near ~29°C, with lower temperatures producing exclusively males (Ewert et al. 2004; Iverson et al. 2013, Pereira et al. 2017). This suggests that the male-biased sex ratios of the forest population may result from cooler nesting temperatures influenced by a denser canopy cover surrounding the arroyos. In contrast, the female-biased sex ratio in town suggests that exposed conditions (Janzen 1994) or urban heat island effect (Manley 1958, Bowne et al. 2018) may be responsible for increased nesting temperatures and, in turn, a greater proportion of females; however, sex-biases in mortality were not addressed. Although a female-skewed population potentially increase abundance, the growing population must also be without male-limitation and supported by sufficient resources and habitat structure (Stanila 2009; Boyle et al. 2014). More research is needed on *K. chimalhuaca*'s specific TRT and the thermal landscape to fully explain hatching sex ratios and provide a more complete understanding of how human-modified habitats may influence turtle populations with TSD.

The population size structures, as represented by body size class distributions, differed significantly between turtles from the forest and the town (FIG. 3). Mean body size of both males and females were larger in the forest than in town. Forest males also had a broader distribution of body sizes, while the female sample was not sufficient to confidently assess body size-class distribution. Forest males were bimodally-divided, forming distinct large and small groups with nearly all "large" males exceeding the previously recorded maximum CL for the species (FIG. 4; Berry et al. 1997). The presence of these large males is largely responsible for the size difference we observed between semi-urban and forest males. As with population size (above), differences in mud turtle population structure between semi-urban and forest populations are likely a result of differences in habitat structure and resource availability. The forested habitat is more shaded by the tree canopy and water availability is more strongly influenced by the dry season. The semi-urban habitat, on the other hand, presents more consistent water resources in the form of the town ditch, and potentially higher environmental temperatures from lower vegetation cover or heat island effect, which may affect sex ratios. Differences in mean body size may be attributed to differences in survivorship or growth curves, where habitats that support older or larger turtles may positively skew mean body size. These factors (and others) likely combine to create the differences we observed in *Kinosternon chimalhuaca* population structure between forested and semi-urban habitats. Additional surveys from Chamela and other forest populations, along with replicates from additional semi-urban populations, are needed to determine whether our observations are part of a general pattern whereby semi-urban mud turtle populations maintain high population densities and show sex ratios skewed in favor of females.

Understanding how the structure of turtle populations varies among habitat types, and especially how human altered habitats may influence rare and threatened turtle populations, is critical in the face of global turtle declines. Since its description in 1997, ecological research on *K. chimalhuaca* has remained largely nonexistent and has certainly not been conducted within its full range of occupied habitats (Berry et al. 1997). The turtles in this study were surveyed as part of a preliminary investigation and although the differences between populations are demonstrable, additional sampling from other areas is needed. The relevance of our study would be greatly increased through replication with other turtle species, as well as the inclusion of more diverse and outlying habitats. We enlisted community members to help us trap, mark and measure our semi-urban turtle population. As partners in the study, the townspeople became eager to learn more about and protect their resident turtles. In a rapidly urbanizing region, such buy-in from local stakeholders is crucial to developing greater awareness and appreciation, as well as helping to ensure the continued conservation of this endemic species. Community outreach also provides a valuable opportunity to build conservation partnerships that can determine the success of long-term prospects for maintaining viable populations for species in peril. We hope our discoveries will inform a broader understanding of the effect of semi-urban habitats on turtle populations and also underscore the importance of involving community stakeholders as partners in meeting conservation objectives.

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CHAPTER IV

CONCLUSION

I used population density, sex ratios, and class size distribution, and size distribution as metrics to compare the effects of urbanization on the population structure of Jalisco mud turtles (*K. chimalahuca*) from two previously unstudied habitats: a perennial semi-urban ditch and an ephemeral forest arroyo. My initial hypotheses of finding significant differences in the demographics of turtle populations from these contrasting habitats was supported in all calculations. Population density was drastically higher in the town's irrigation ditch where 226 turtles were captured (17 recaptured), estimating a population of 741±132 individuals. In contrast, similar trapping effort in the forest habitat yielded only a dozen individuals which were added to a collection of 25 prior observations, with four recaptures. Sex ratios were very different between the two habitats: nearly two females for every male in town, and three males for every female in the forest. In town, a higher female proportion, combined with the benefits of a year-round water source, are likely important factors contributing to increased abundance. In the forest, mean carapace lengths of male and female adults were significantly larger than their semi-urban counterparts ($p = 0.036$ and $p = 0.012$, respectively), and forest males were more bimodally distributed, with larger size classes more strongly represented than in town.

To my best knowledge, this is the first research to examine the demographic comparison of a kinosternid in urbanized habitats. This is also the first report of Jalisco mud turtle populations from either an undisturbed forested arroyo or a semi-urban irrigation ditch. Moving forward, this project would greatly benefit with increased sampling in the forest, as well as the inclusion of more turtle habitat types. Future research is planned in collaboration with local students and community members to explore how diet, temperature, and movement contribute to the differences I detected in population structure. My continued research will support a more holistic understanding on the effects of urbanized habitats on mud turtle populations and express the importance of involving community stakeholders to sustainably meet conservation objectives. The significant differences I found between semi-urban and forest provide critical new data for the Jalisco mud turtle and will inform future conservation strategies as to how mud turtles population in Mexico respond to urbanization.

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