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Population ecology and home range of the Mexican Rough-footed Mud Turtle (*Kinosternon hirtipes murrayi*) in Central Mexico

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Abstract. Population ecology and demographic data are fundamental for species management and conservation planning. For Mexican kinosternid turtles there is a need for basic natural history and population ecology data. The Rough-footed Mud Turtle (*Kinosternon hirtipes murrayi*) is one of the lesser-studied species, even though it is broadly distributed, occurring from Western Texas to Central Mexico. We conducted a study on the species in Michoacán, Mexico for two years. Basic population parameters were estimated, and telemetry was used to measure home range size and movements of males and females. Population size in a 1.42-hectare wetland was calculated to be 301 (± SE 5.89) individuals, mainly adults. The adult sex ratio was skewed toward males (3.1:1). Female home range size was larger than that of males, and males moved larger distances between relocation events. The radio-tracked individuals did not leave the water during winter months and during the dry season. Habitat degradation due to eutrophication may be affecting population survivorship and recruitment.

Keywords. Mexican Rough-footed Mud Turtle, *Kinosternon hirtipes murrayi*, population ecology, home range, México.

INTRODUCTION

Knowledge of demographic characteristics, home range size, and movement patterns are important for designing conservation and management strategies for species (Gibbs and Amato, 2000; Primack, 2012). Demographic characteristics include: population size, abundance, sex ratio, population structure, survivorship, and the contribution of these parameters to populations dynamics through time (Caswell, 2001). To measure any of these population characteristics accurately requires long-term data or a high rate of recaptures to estimate these parameters accurately (Lemos-Espinal et al., 2005; Molina-Zuluaga et al., 2013). However, because turtles are long-lived organisms, collection of demographic data presents a challenge because their lifespan can reach several decades (Crouse et al., 1987; Edmonds and Brooks, 1996; Enneson and Litzgus, 2008) and recaptures can be sparse (Chao, 1989). On the other hand, measuring home range size and movement patterns requires more detailed studies where individuals are followed through space and time (Hays, 1992; Godley et al., 2002; Pérez-Pérez et al., 2017). Typical home range and movement studies are conducted using radiotelemetry so that individuals can be located repeatedly (Cochran, 1980; Singer and Blakenhol, 2015). When long term studies are coupled with radiotelemetry, clearer patterns of habitat use, migration, resource use, and seasonal patterns like aestivation can

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be detected. Despite the importance of long-term and detailed studies of turtle populations, most of the information that exists is on species within the United States (Iverson, 1991; Rouane et al., 2008; Enneson and Litzgus, 2008; Lovich and Ennen, 2013).

Outside of the US, long-term and detailed studies with turtles have been largely neglected. For example, Mexico has the second most diverse turtle fauna in the world (Rhodin et al., 2017), yet long-term mark-recapture and radiotelemetry studies are few to non-existent (Legler and Vogt, 2013). Only recently, biologists have started generating this kind of data on Mexican turtle species (Macip-Ríos et al., 2009; 2011; Vázquez-Gómez et al., 2016; Pérez-Pérez et al., 2017). Mud turtles (Kinosternidae) have been a particular focus, as they are the most diverse turtle lineage in Mexico (Legler and Vogt, 2013). One of those species, the Mexican Rough-Footed Mud Turtle (*Kinosternon hirtipes*) is broadly distributed from the Big Bend region in a few localities in Western Texas (Platt and Medlock, 2015) to central Michoacán in the Mexican Transvolcanic Belt (Iverson, 1992). Throughout this range, five *K. hirtipes* subspecies are recognized: *K. h. hirtipes* in the Valley of Mexico; *K. h. megacephalum* (extinct) in Viesca, Coahuila; *K. h. magdalense* in the Magdalena River basin, Michoacán; *K. h. chapalense* in Chapala Lake and Zapotlán Lake, Jalisco; *K. h. tarascense* in Patzcuaro Lake, Michoacán; and *K. h. murrayi*, from the Big Bend region of Texas to the highlands of Michoacán (Iverson, 1981).

Despite their wide distribution in Mexico, most ecological information on *K. hirtipes* is from Iverson (1981; 1985), who described sexual size dimorphism, morphological differences, and basic distributional patterns among the subspecies. Some information also exists on *K. h. murrayi*. For example, Iverson et al. (1991) described the growth and reproduction of this subspecies in Chihuahua, Platt et al. (2016a) described their diet, Platt et al. (2016b) also described the reproductive ecology in their northern distribution limit in Texas, Platt and Medlock (2015) studied aestivation behavior, and Smith et al. (2015; 2018) reported a new body size record and nesting behavior. In general, these studies demonstrate that *K. h. murrayi* exhibits more morphological variation than other subspecies, has wide variation in body size, and is sexually dimorphic, with males typically being larger than females (Glass and Hartweg, 1951; Iverson, 1985).

Our aim was to generate additional ecological information on *K. h. murrayi* using capture-mark-recapture methods and radiotelemetry in a wetland near Morelia, Michoacán, México. Our specific objectives were to describe basic population ecology parameters, home range, and movement patterns of *K. h. murrayi* in a human-modified landscape.

MATERIALS AND METHODS

Study Site

This study was carried out near Morelia, Michoacán, México in irrigation canals that come from a wetland called "La Mintzita" (13°38'N, 101°16'E). "La Mintzita" is a natural spring that is associated with a 57-hectare (ha) wetland recognized by RAMSAR (The Ramsar Convention of Wetlands, 2014). This wetland has been managed by humans since pre-Columbian time and still maintains significant biological diversity including the presence of several species of fishes (some of them endemic like *Zoogeneticus quitzeoensis*, *Skiffia lermae*, *Yuriria alta*, among others), waterfowl, amphibians, reptiles, and various mammals (Marin-Togo and Blanco-García, 2012).

The two main irrigation canals at "La Mintzita" are fed by the most northern part of the wetland. These canals are used by local people to irrigate corn fields and manage runoff during the wet season when water levels are high. The canals have a low current and flow northeast for 2 km before joining a tributary of the Cuitzeo Lake basin that is also known as the "Rio Grande" of Morelia. Compared to other tributaries of the "Rio Grande", the irrigation canals studied here are less polluted and degraded. The total area sampled has a coverage area of 14266 m^2.

Trapping protocol

This study was conducted during two wet seasons, from May-December 2016 and June-November 2017. We placed traps for one night, at least two times per month. We used two fyke nets and 9-12 minnow traps (Promar, Garden, Ca.). Traps were baited with fresh fish and placed in the irrigation canals from approximately 17:00 h to 10:00 h the following day. For each trapping session, traps were placed in the same place in 2016, but had to be changed in 2017 due to an unusually high level of water at the beginning of the season and a dramatic increase in invasive aquatic plants, *Elodea* sp. and *Eichhornia crassipes*. Our sampling effort for the first season (May-December 2016) was 2142 trap hours using 12 minnow traps plus two fyke nets, and 1836 trap hours in the second sampling season (June-November 2017) using 9 minnow traps plus two fyke nets.

Turtle measuring protocol

All captured turtles were marked using the shell-notch code system from Cagle (1939), then measured. We measured body mass (BM) to the nearest gram using a spring scale (1 g). Morphological characters, including straight-line carapace length (CL), straight-line plastron length (PL), carapace width (CW), and carapace height (CH) were measured to the nearest 0.01 mm using dial calipers.

Males were identified by using secondary sexual characteristics: long and bulky tail, a developed spine at the end of the tail, a prominent notch in the hind lobe of the plastron, and had a concave plastron (Iverson, 1999). Females were identified by their size and absence of the characteristics used to identify males. Females had a plastron that covered all body parts. According to Iverson et al. (1991), female *K. hirtipes* are sexually mature at 95-100 mm CL and males of this size are hard to differentiate from females. Age classes were divided into four categories for our population structure analysis: hatchlings/yearlings (less than 50 mm CL), immature (50-90 mm CL), adults (95-140 mm CL), and asymptotic adults or old adults (larger than 140 mm CL). Captured adult females were brought to the laboratory for take X-ray photographs to determine reproductive status. Females stayed two or three days in the laboratory and were then returned to the field.

Radio telemetry protocol

We equipped 11 turtles (six females in 2016 and five males in 2017) with radio transmitters (Models: TXE-315G Telenax, Ciudad del Carmen, Quintana Roo; PR 99 Wildlife Materials, Murphysboro, IL, and R1900 series from Advanced Telemetry Systems, Isanti, MN) that weighed less than 30 g. A Yagi antenna and two different receivers (Telenax R1000 and Advanced Telemetry Systems R2000) were used to locate individuals. Turtle relocations were recorded to the nearest 3 m with a handheld GPS (Garmin eTrex 10). Individuals were relocated at least twice per month during the study period.

Statistical analyses

Due to the low recapture rate in our data set, population size was estimated with a heterogeneity estimator (Mh) following Chao (1989). This analysis was done with the CARE1 package in R (Chao and Yang, 2003). A Chi-squared test was used to test if the sex ratio was significantly different from 1:1 (Zar, 1999). Differences in body size and other morphological measurements between males and females were tested with a Student's t-test. Parametric assumptions for normality and homogeneity were tested using Shapiro-Wilks and Bartlett tests (Zar, 1999). Statistical analyses were run in JMP v5.0.1 (SAS Institute, 2002).

Locations were originally collected in decimal degrees, then transformed to the UTM coordinate system using Earth Point (Clark, 2018) for subsequent analysis. Home range size was estimated using two methods, the minimum convex polygon (MCP) and kernel density (KD). Fifty percent kernel density estimates were calculated to remove the influence of outliers and a smoothing parameter (h) was calculated using least squares cross validation (LSCV). Home range sizes were calculated in the adehabitat package in R (Calenge, 2006; R Development Core Team, 2008). Total distances moved between relocations and estimated daily movements were calculated by hand using the location-sequenced UTM coordinates. We did not compare male and female home range sizes because of unequal sample sizes. For all analyses, we used $\alpha = 0.05$.

RESULTS

A total of 96 *K. h. murrayi* turtles were marked during both sampling seasons and 18 were recaptured. Eighty-eight of these turtles were captured only once, nine were captured twice, two were captured three times, and two were captured four times. Estimated population size using the Mh model was 301 (± SE 5.89) individuals (lower confidence interval (CI) = 297.7 – upper CI = 305.1). Based on the area sampled (surface water coverage), turtle density was estimated as 211 turtles/ha. The 96 turtles captured included 69 males, 22 females, three immature juveniles, and two hatchlings/yearlings. The adult sex ratio was significantly biased toward males 3.1:1 ($\chi^2 = 24$, $P < 0.0001$).

Average straight-line carapace length, (± SD), for males was 118.1 (13.91) mm and 121.31 (15.37) mm for females, but they were not significantly different ($t_{89} = 0.87$, $P = 0.38$). Females (49.60 ± 6.78 mm) had greater CH (43.12 ± 4.9 mm; $t_{89} = 4.14$, $P < 0.0001$), longer PL (females = 110.92 ± 15.13 mm; males = 97.52 ± 8.33, $t_{89} = 3.96$,

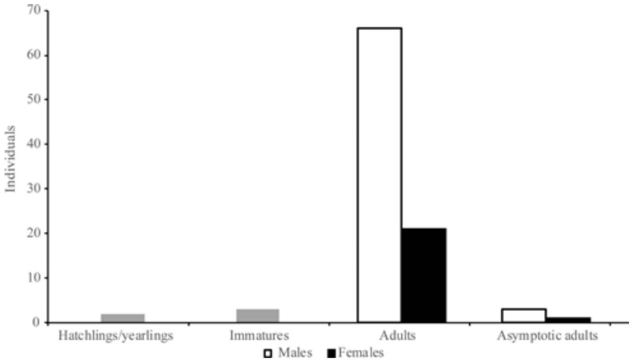

Fig. 1. Population structure of the *Kinosternon hirtipes murrayi* population at "La Mintzita" wetland. Grey bars are immature individuals, including hatchlings and immatures.

Table 1. Home range area for *Kinosternon hirtipes* males and females calculated by MCP and 50% kernel estimates. Home ranges are given in hectares.

Turtle ID	Locations per individual	Sex	MCP home range	50% kernel home range
5	24	Female	0.14	0.02
7	21	Female	0.28	0.08
9	19	Female	18.91	8.50
15	25	Female	0.33	0.05
16	23	Female	2.33	3.18
17	22	Female	0.90	0.34
82	6	Male	0.02	0.60
92	5	Male	0.00	0.56
93	5	Male	0.02	0.28
94	5	Male	0.001	0.92
95	5	Male	0.07	1.40

P = 0.0005), and greater BM than males (females = 313 ± 113.80 g; males = 237 ± 63.49 g; $t_{89} = 2.99$, P = 0.006).

Ten females were brought to the lab to take radiographs. Only two females have eggs on oviduct. One with five (CL = 117.6 mm) and the other with six eggs (CL = 136 mm). Both females were collected during September 2016. Egg were measured on the radiographs. Eggs length averaged 27.18 mm in length and 15.25 in width in the five-eggs clutch, and egg length averaged 28.32 mm in length and 16.50 mm in width in the six-egg clutch.

Relocations ranged from 25 to 19 for females and from 6 to 6 for males. Combined relocations mean was 15.54 (± SD 9.08). Female home range size varied from 0.14 – 18.91 ha based on the MCP estimates and 0.02 – 8.53 hectares for KD estimation (Table 1). Mean home range size, (± SD), for females was 3.81 (7.43) ha for MCP method and 2.02 (3.39) ha for KD. Home range size for males varied from near 0 – 0.072 ha with the MCP method and 0.28 – 1.40 ha with the KD method (Table 1). Average home range size, (± SD), for males was 0.024 (0.028) ha with the MCP method and 0.75 (0.42) ha when estimated with KD.

Average distances moved, (± SD), between relocations by females was 57.76 (123.27) m and 73.83 (109.82) m for males (Table 1). Average estimated daily movements, (± SD), for females were 6.20 (13.09) m per day and 4.89 (7.38) m per day for males.

DISCUSSION

Estimated population size was similar to other population estimates for kinosternids such as *K. oaxacae* (Vazquez-Gómez et al., 2017), *K. integrum* (Macip-Ríos et al., 2009), and *K. sonoriense* (Hulse, 1982), although areas sampled varied. Compared with the abundance (detectability) of data for the same subspecies presented by Iverson et al. (1991) for a population in Chihuahua (604 captures, but no population size information), and Platt et al. (2016b) (87 marked turtles and 2.4:1 sex ratio), our data on sex ratios are generally the same. Male-biased sex ratios have also been reported before in other kinosternids such as *Sternotherus odoratus* (Smith and Iverson, 2002), *K. sonoriense* (Stone et al., 2015), *K. leucostomum* (Ceballos et al., 2016), and other populations of *K. hirtipes* such as those in Texas (Platt et al., 2016b). According to Gibbons and Lovich (1990) a skewed sex ratio could be caused because one sex reaches sexual maturity earlier (generally are smaller in size), which could affect population structure.

The population structure observed in our study (many adults and very few hatchlings and immature individuals) also agrees with data reported in other kinosternid studies (Frazer, 1991; Iverson, 1991; van Loben Sels et al., 1997; Macip-Ríos et al., 2011). This may be an artifact of trapping techniques because it is hypothesized that hatchlings and yearlings have a low catchability rate, or the mesh of traps may allow them to escape (Ceballos et al., 2016). However, Macip-Ríos et al. (2018) did capture a large proportion of hatchlings and immatures with the same traps and bait in a *Kinosternon creaseri* population in the Yucatan Peninsula. Also, Vázquez-Gómez et al. (2016) found hatchlings using the same trapping protocol in a *K. oaxacae* population. Thus, our results may represent a close approximation to the true population structure.

There are several reasons that could explain biased sex ratios and the absence of hatchlings and yearlings in population structure. According to Smith and Iverson, (2002), differential mortality among the sexes could influence sex ratios, while climate change and habitat degradation could change incubation temperatures that also affect sex ratio (Eisenberg et al., 2017). Moreover, different habitat usage could also affect these results. Nevertheless, as we mentioned before, we used the same trapping protocol as in previous studies (Macip-Ríos et al., 2011; Vázquez-Gómez et al. 2016; Macip-Ríos et al., 2018) where we were able to capture hatchling and yearling individuals. Because of this, we presume that the low capture rate of hatchling and yearlings could be attributed to low recruitment, which could be related to the male-biased sex ratio.

The overall recapture rate was very low at 19%. This could indicate two things; turtles move extensively in the study area, or turtle catchability is affected by the trapping protocol. Even though this trapping protocol has been successful in other mud turtle populations, the "La Mintzita" population could have been affected by the dramatic changes in water levels and abundance of invasive plants. Because of these changes to the habitat, we were unable to set up the traps in one of the irrigation canals during the last sample season because the thick aquatic vegetation prevented traps from sinking and there were very few places with "open water" to place the traps. There is evidence that turtles inhabit eutrophic habitats (Iverson, 1999; Germano, 2010); however, when we forced our traps into water full of *Elodea* sp. and *Eichhornia crassipes* no turtles were captured.

Contrary to a general pattern among kinosternids, where males are generally larger than females (Ceballos and Iverson, 2014), in the "La Mintzita" population females appear to be larger than males. For *K. hirtipes,* similar sexual size dimorphism results have been reported for other populations (Carmen and Verde basins) and subspecies such *K. h. tarascense*, *K. h. magdalense*, *K. h. megacephalum*, and *K. h. chapalense* (Iverson, 1985). Furthermore, our largest male (CL = 147.5 mm) was smaller than the 195 mm CL male reported by Smith et al. (2015). Our largest female was 177 mm in CL, which is smaller than record sizes previously reported (Ernst and Lovich, 2009). Our result contrasts with data from other studies of *K. hirtipes* in Presidio, Texas (Platt et al., 2016b) and the Santa Maria River in Chihuahua (Iverson et al., 1991), where males were also larger than females in CL. Our data are comparable to those presented by Iverson et al. (1991) who reported that females have a larger plastron than males.

Apparently, variation in sexual size dimorphism is common in *K. hirtipes*, which contrasts with other kinosternids like *K. subrubrum*, *K. bauri* (Lovich and Lamb, 1995) *K. integrum* (Macip-Ríos et al., 2009), *K. scorpioides* (Forero-Medina et al., 2007), and *K. sonoriense* (Stone et al., 2015). Body size differences between males and females have been interpreted as a result of sexual selection interacting with natural selection (Wilbur and Morin, 1988; Gibbons and Lovich, 1990). These differences could also be driven by minimum size required for mating and reproduction (Iverson, 1985), differential survivorship, and habitat selection, which could affect growth patterns (Huey, 1982). According to Iverson et al. (1991), *K. hirtipes* males grow faster and larger than females during their first five years; during this time, males start showing secondary sexual characteristics, while females start showing their secondary sexual characteristics at from 6-8 years old.

Home range results indicated that *K. hirtipes* did not show signs of aestivation, as previously described (Iverson, 1981). "La Mintzita" turtles exclusively lived in aquatic habitats, with the exception of two individuals that moved to other irrigation channel at 700 m straight-line distance. Male and female home ranges slightly overlap, and even though home range sizes are not comparable due to unequal sample sizes, females seem to have a larger home range. Larger home range size in females could be explained by the need to find suitable nesting sites (Pérez-Pérez et al., 2017). However, the habitat degradation previously noted could force females to move more in the landscape to find suitable food resources and nesting sites. We acknowledge that our relocations could be insufficient since home range and movement standard deviations are close or large than mean values; however, our data still provide an estimate of overall home range of this population for an under-studied turtle, but more research is needed to determine differences in habitat use and movement habits between males and females.

During our two-year observations in the "La Mintzita" habitat, we identified habitat degradation by water reduction and saturation of *Elodea* sp. and *Eichhornia crassipes*, that could contribute to decline turtle abundance. Females in this population are still breeding, as evidenced by the observation of eggs in X-radiographs taken from females collected in both sampling seasons. However, due to the skewed male sex ratio, the reproductive potential of the population may be limited (Le Galliard et al., 2005). This is a conservation concern since this *K. h. murrayi* population represents one of the southernmost locations of this subspecies.

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