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Reproductive ecology and natural history of *Kinosternon herrerai* (Testudines: Kinosternidae) at the center of its distribution

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Abstract

Reproductive ecology and natural history of *Kinosternon herrerai* (Testudines: Kinosternidae) at the center of its distribution. Mexico harbors 10 endemic species of mud turtles of genus *Kinosternon,* but natural history information is lacking for most of them. Herein we describe some generalities of the reproductive ecology and natural history of one population of the Mexican endemic, Herrera's mud turtle, *K. herrerai* from Hidalgo, Mexico. Females and males were similar in body mass and carapace length. Additionally, larger and heavier turtles moved more than smaller and lighter ones. Clutch size varied from one to six eggs, with an average of 3.4 ± 1.7, and egg laying occurred at environmental temperatures between 15 and 21°C ($\bar{x}$ = 18.6°C). Total nesting time (from selection of nesting site to oviposition) lasted ca. 37 min, and the incubation period was 75 days *in situ.* These results enhance our understanding of natural history aspects of *Kinosternon* mud turtles, which are necessary to carry out conservation actions to preserve all of its populations.

Keywords: Clutch size, Hidalgo, Movement rate, Mud Turtles, Reproduction.

Resumen

Ecología reproductiva e historia natural de *Kinosternon herrerai* (Testudines: Kinosternidae) en el centro de su distribución. México alberga 10 especies endémicas de tortugas de barro del género *Kinosternon*, pero hace falta información de historia natural para la mayoría de ellas. Aquí describimos algunas generalidades de la ecología reproductiva e historia natural de una población de la tortuga Casquito de Herrera, endémica mexicana, *K. herrerai* en Hidalgo, México. Las hembras y los machos fueron similares en masa corporal y longitud del carapacho, además, las tortugas más

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grandes y pesadas se movieron más que aquellas más pequeñas y livianas. El tamaño de la puesta varió de uno a seis huevos, con un promedio de 3.4 ± 1.7, y la puesta de huevos ocurrió a temperaturas ambientales entre 15 and 21°C ($\bar{x}$ = 18.6°C). El tiempo total de anidamiento (desde la selección del nido hasta la ovoposición) duró ca. 37 min y el periodo de incubación fue de 75 días *in situ*. Estos resultados mejoran nuestra comprensión de los aspectos de historia natural de las tortugas de barro *Kinosternon*, que son necesarios para llevar a cabo acciones de conservación para preservar todas sus poblaciones.

Palabras clave: Hidalgo, Reproducción, Tamaño de puesta, Tasa de movimiento, Tortugas de barro.

Resumo

Ecologia reprodutiva e história natural de *Kinosternon herrerai* (Testudines: Kinosternidae) no centro de sua distribuição. O México abriga 10 espécies endêmicas de tartarugas-de-lama do gênero *Kinosternon*, mas faltam informações sobre a história natural da maioria delas. Aqui, descrevemos algumas generalidades da ecologia reprodutiva e da história natural de uma população de Hidalgo da tartaruga-de-lama-de-herrera, *K. herrerai*, endêmica do México. As fêmeas e os machos eram semelhantes em termos de massa corporal e comprimento da carapaça. Além disso, as tartarugas maiores e mais pesadas se movimentavam mais do que as menores e mais leves. O tamanho da ninhada variou de um a seis ovos, com uma média de 3,4 ± 1,7, e a postura dos ovos ocorreu em temperaturas ambientais entre 15 e 21°C ($\bar{x}$ = 18,6°C). O tempo total de nidificação (desde a seleção do local de nidificação até a ovipostura) durou cerca de 37 min, e o período de incubação foi de 75 dias *in situ.* Esses resultados aumentam nossa compreensão dos aspectos da história natural das espécies de *Kinosternon,* que são necessários para realizar ações de conservação para preservar todas as suas populações.

Palavras-chave: Hidalgo, Reprodução, Tamanho da ninhada, Tartarugas-de-lama, Taxa de movimento.

Introduction

Mud turtles of the genus *Kinosternon* have their greatest taxonomic diversity and endemism in Mexico; however, there are important information gaps in the understanding of their natural history and ecology (Pritchard and Trebbau 1984, Macip-Ríos *et al.* 2009, 2015, Legler and Vogt 2013). In Mexico this genus is represented by 17 species (Iverson 1991a, López-Luna *et al.* 2018, Loc-Barragán *et al.* 2020, TTWG 2021, Berriozabal-Islas *et al.* 2023, Ramírez-Bautista *et al.* 2023) and nine subspecies (Iverson 1985, Legler and Vogt 2013, Berriozabal-Islas *et al.* 2023). *Kinosternon* species inhabit humid, arid, and dry tropical environments from the United States of America to Argentina (Legler and Vogt 2013). All species are aquatic, but many spend significant amounts of time in terrestrial habitats.

Most *Kinosternon* species are omnivorous, feeding on insects, fish, carrion, and plant matter (Legler and Vogt 2013). Within the genus, studies of systematics, distribution, biogeography (Iverson 1985, Legler and Vogt 2013), demography (Iverson 1991b, Macip-Ríos *et al.* 2011), and conservation (Macip-Ríos *et al.* 2015, Berriozabal-Islas *et al.* 2020, 2023) predominate. Of the 17 species found in Mexico, 10 (59%) are endemic (Berriozabal *et al.* 2023, Ramírez-Bautista *et al.* 2023). Population ecology has only been studied in some of these endemics including *Kinosternon abaxillare* Baur, 1925 (Reyes-Grajales *et al.* 2021), *K. alamose* Berry and Legler, 1980 (Iverson 1989), *K. chimalhuaca* Berry, Seidel, and Iverson, 1997 (Butterfield *et al.* 2020), *K. creaseri* Hartweg, 1934 (Macip-Ríos *et al.* 2018), *K. oaxacae* Berry and Iverson, 1980 (Vázquez-Gómez *et al.* 2015, 2016), and *K. vogti* López-Luna, Cupul-Magaña, Escobedo-Galván, González-Hernández, Centenero-Alcalá, Rangel-Mendoza, Ramírez-Ramírez, and Cazares-Hernández, 2018 (Rosales-Martínez *et al.* 2022). Behavior and reproduction have been extensively studied only in *K. integrum* (Iverson 1999, Macip-Ríos *et al.* 2009, 2011). Thus, basic biological information is lacking for most species, including the widely distributed Mexican endemic, Herrera's mud turtle, *Kinosternon herrerai* Stejneger, 1925.

Herrera's mud turtle, *Kinosternon herrerai* (Figure 1) is considered to be aquatic (Legler and Vogt 2013, Berlant and Stayton 2017). The reduced plastron size in this species (Figure 1B) suggests that it is an inhabitant of more permanent water bodies (Berry 1977, but see Iverson 1991c). It occurs in the eastern states of Tamaulipas, Veracruz, San Luis Potosí, Hidalgo, and Puebla (Figure 2; TTWG 2021), with records of introduced individuals in the state of Mexico (Legler and Vogt 2013). Data from northern and southern populations (Legler and Vogt 2013) have shown that individuals are both diurnal and nocturnal (Aguirre-León and Aquino-Cruz 2004), they reproduce (courtship and copulation) underwater (Carr and Mast 1988) during the dry season (July and August) and produce multiple clutches from two to four eggs (Carr and Mast 1988). Although descriptive data of Iverson (1991c) might indicate that *K. herrerai* has male-biased sexual size dimorphism, detailed studies of populations at the extremes of its distribution have shown differing patterns. In northern populations, males are larger than females, and both tend to be more frugivorous (Carr and Mast 1988), while in southern populations, sexes are similar in body size, but males are heavier than females, and both tend to be more carnivorous (Aguirre-León and Aquino-Cruz 2004). Populations in the central part of the distribution have not been extensively studied, probably because of rarity and small population sizes. Therefore, additional information about populations in this region would be a valuable contribution to the knowledge of this widely distributed *Kinosternon* species and to the generation of national and international conservation efforts. This study describes some general aspects of the ecology (local movements, sex ratio, reproductive period, clutch size) of one population of *K. herrerai* from Hidalgo, Mexico.

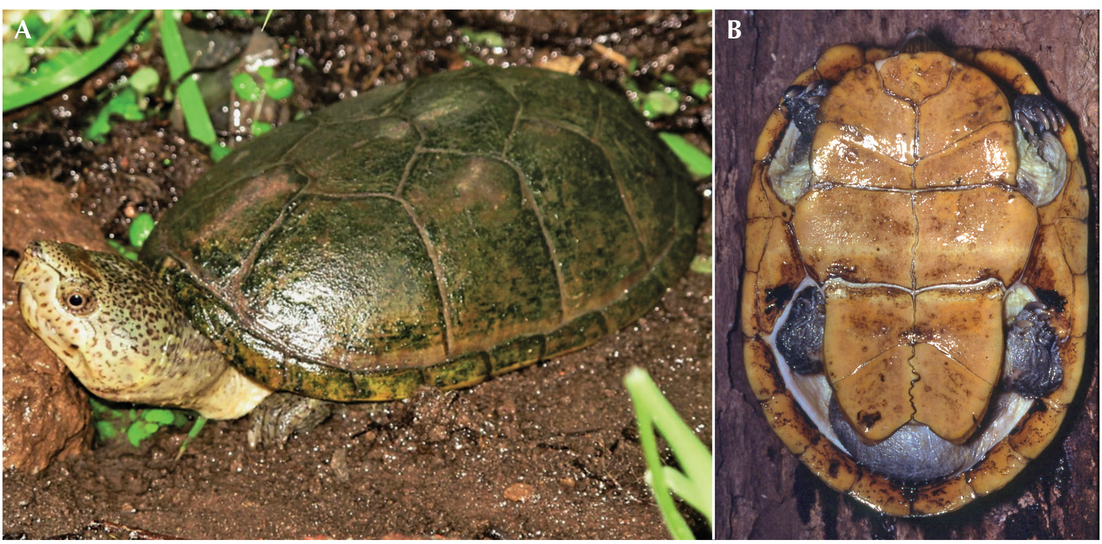

Figure 1. (**A**) Adult female *Kinosternon herrerai* from the municipality of Acatlán, Hidalgo, Mexico (photo by authors). (**B**) A plastral view of adult male from Veracruz, Mexico (photo by John Iverson).

Materials and Methods

The study was carried out in three ponds (Santa Rosa, El Transformador, and Totoapita Canutillo), separated from each other between 300 m to 400 m a.s.l., within the municipality of Acatlán, Hidalgo, Mexico (Figure 2). The study area is located at 2120 m a.s.l., where the main vegetation type is xeric scrubland (Pavón and Mesa-Sánchez 2009). The three ponds were from 0.20 m to 1.5 m depth and 35 m long by 13 m wide, and they were permanent water bodied whose level fluctuated depending on the seasonality of precipitation and the irrigation system of nearby field crops.

We sampled three days each month for one year (from October 2008–October 2009), using the same sampling effort (one person for each site). Each sampling period was from 07:00 to 11:00 h and from 17:00 to 19:00 h. The sampling design was developed based on the species' activity cycle (Carr and Mast 1988, Aguirre-León and Aquino-Cruz 2004, Legler and Vogt 2013). We visually located and hand captured each turtle and recorded the following data: date, carapace length (CL in mm, taken to the nearest 0.01 mm using a digital caliper, measured parallel to the mid-plastral plane; Iverson and Lewis 2018), and body mass (BM in g, measured with an electronic portable scale). Measurements

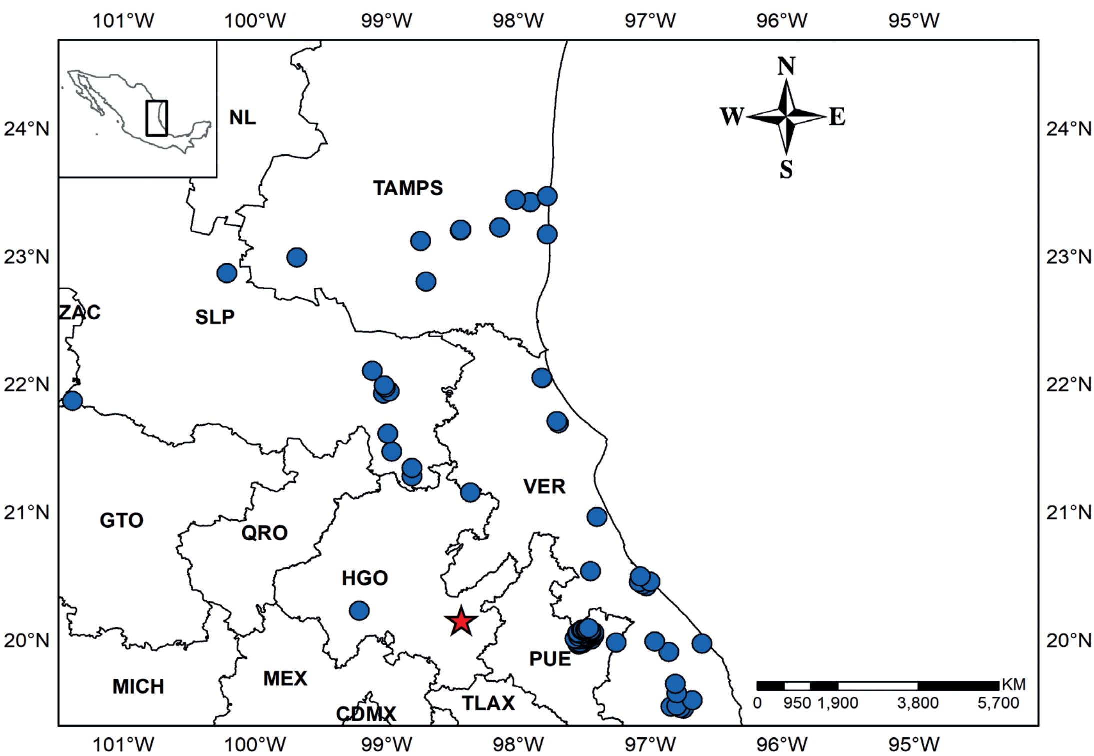

Figure 2. The known distribution range of *Kinosternon herrerai* in Mexico. The blue dots are records obtained from GBIF (2023) datasets, which consider specimens recorded by governmental national institutions and those deposited in national and international scientific collections. The red star is the municipality of Acatlán, where the three studied ponds are located. Mexican states shown are Nuevo León (NL), Tamaulipas (TAMPS), Zacatecas (ZAC), San Luis Potosí (SLP), Guanajuato (GTO), Querétaro (QRO), Hidalgo (HGO), Michoacán (MICH), Estado de México (MEX), Ciudad de México (CDMX), Tlaxcala (TLAX), Puebla (PUE), and Veracruz (VER).

are reported as mean ± 1 SE. The distance between exact capture and recapture sites (measured with a tape measure in m) was used to calculate the movement rate of each turtle. Sex and age class were also determined for each turtle. Sex was determined by secondary sexual characters such as the elongated tail, concave plastron, and proportionally larger head for males, compared with shorter tails, a flat plastron, and smaller head for females. For both sexes, age class was assigned as hatchling (16.2–21.0 mm CL), juvenile (52.0–110.6 mm CL), and adult (111.5–140.4 mm CL) following Legler and Vogt (2013). Turtles were marked using indelible ink. We painted a number (sequential) on the carapace of each individual; the number was permanent enough to last for at least one year of study. The reproductive period was determined by observations of specific events, including courtship and mating, egg laying, and the first detection of hatchlings in the studied ponds (Carr and Mast 1988). When oviposition was observed, we measured the environmental temperature of the nest (at ground level) using a Miller-Weber rapid-registering thermometer (± 0.2°C).

To determine whether the sex ratio was biased, we carried out a chi-square analysis. We performed Mann-Whitney U tests to evaluate intersexual differences in CL and BM. A Kruskal-Wallis test was carried out to compare female CL and BM among ponds. Spearman correlations were calculated to test for a relationship between CL and BM with movement rate. To evaluate whether movement rate differed among ponds, we used a one-way analysis of variance. We considered results significant at $p \leq 0.05$. Statistical analyses were performed in Statistica 10.0 (Statsoft Inc).

Results

The total of 40 different individual turtles were captured in the three ponds a total of 24 times. This number included 27 adults, seven juveniles, and six hatchlings. For two ponds with adequate sample size, the sex ratio was 1:2.5 female-biased ($\chi^2 = 4.9$, $p = 0.03$). Females and males were similar in BM and CL both within and among ponds (Table 1). When data were pooled, female BM was 438.4 ± 14.6 g (range 340–530) and CL was 126.4 ± 2.2 mm (range 112–140) ($N = 17$); for males BM was 426.5 ± 16.1 g (range 345–503) and CL was 126.9 ± 2.24 mm (range 113–140) ($N = 10$). No differences were detected between the sexes for BM ($Z = 0.33$, $p = 0.74$) or CL ($Z = -0.18$, $p = 0.86$).

Based on 24 recaptures and 40 total captures, movement rate was similar among the three ponds ($F_{2, 61} = 0.61$, $p = 0.85$, $N = 64$) when considering all individuals of both sexes; however, movement rate was positively correlated with CL (Figure 3A) and BM (Figure 3B). Hence, larger and heavier turtles moved more than smaller and lighter turtles. No turtles were observed to move between ponds during our one-year study period.

The reproductive period occurred from early spring (March) to early fall (October). During this time, we observed mature females ($N = 5$) initiate intrasexual fighting behavior (female-female) using their bodies, forelimb claws, and heads. Once the fighting ended, a nearby male pursued, subdued, and copulated with the winning female. Within the study period (October 2008 to May 2009) we found five clutches, three around the Santa Rosa pond, and two nearby Totoapita Canutillo pond. Clutch size varied from one to six eggs, with an average of 3.4 ± 1.7. Egg laying occurred in nests composed of moist soil (mud), and wet grass and weeds, where environmental temperatures varied between 15 and 21°C ($\bar{x} = 18.6$ °C). Total nesting time (from selection of nesting site to oviposition) lasted ca. 37 min and the incubation period was 75 days *in situ.*

Discussion

The results of this study increase our knowledge of the natural history of Herrera's mud turtle, *Kinosternon herrerai* (Legler and

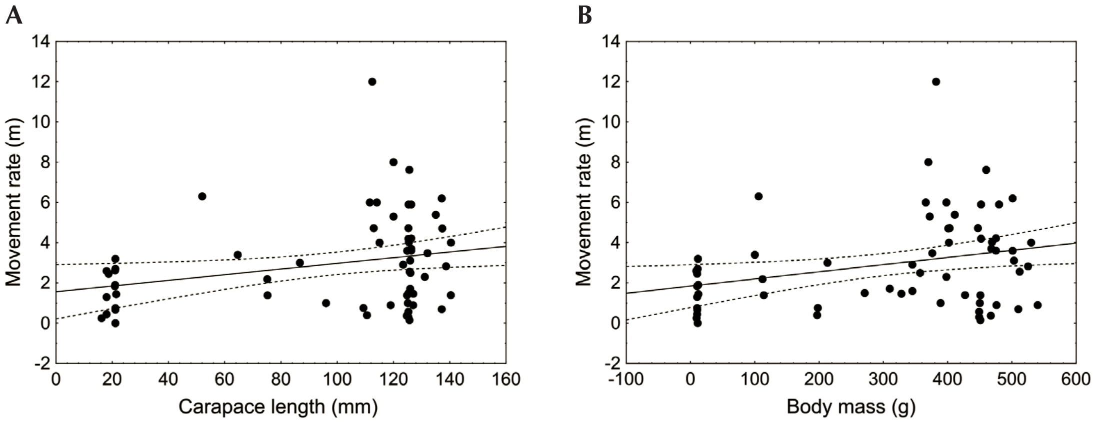

Figure 3. Relationship between carapace length (**A**; r = 0.25, *p* = 0.04, *N* = 64) and body mass (**B**; r = 0.27, *p* = 0.03, *N* = 64) with movement rate of *Kinosternon herrerai* from southeastern Hidalgo, Mexico. Dashed lines represent 95% confidence intervals.

Vogt 2013). Prior to oviposition (March), the population in our study area (considering all three ponds together) consisted of only 10 adult males, 17 adult females, seven juveniles, and six hatchlings. This is a small population size compared with other studies of Mexican turtles (Macip-Ríos *et al.* 2011, Vázquez-Gómez *et al.* 2016, Reyes-Grajales *et al.* 2021). We attribute this result in part to the lack of trapping, since mud turtles are generally collected using baited hoop traps; however, we were seeing the same few individuals multiple times suggesting that the number of turtles in each pond is quite small (mean recapture rate: 60%).

Our results provide evidence of the typical female-biased sex ratio of kinosternids (Vázquez-Gómez *et al.* 2016, Reyes-Grajales *et al.* 2021, De la Cruz-Merlo *et al.* 2022). In general, the sex ratio could be explained by an equilibrium of population size through fecundity (Stearns 1992) and by regulation of nest environmental temperature during embryonic development (Pough *et al.* 2001, Macip-Ríos *et al.* 2009). Considering population structure, the number of individuals found in the three ponds indicates a reasonably stable population, as occurs in other *Kinosternon* species (Macip-Ríos *et al.* 2011) but further study is needed to support this conclusion.

In this study, nests experienced a range of environmental temperatures between 15°C to 21°C, which are related to the production of female-biased clutches (Ewert *et al.* 2004). We did not find intersexual differences in body size (CL) and body mass, which is a pattern more similar to southern populations of *K. herrerai* (Aguirre-León and Aquino-Cruz 2004), but contrast with that found in northern populations of this species (Carr and Mast 1988). In most species of the genus *Kinosternon,* males are larger than females (Berry and Shine 1980, Iverson 1999, Macip-Ríos *et al.* 2009, Ceballos and Iverson 2014), most likely because they defend territories against other males (Macip-Ríos *et al.* 2009). However, there is evidence of species with female biased sexual size dimorphism (Iverson 1985, De la Cruz-Merlo *et al.* 2022). The results found here could be explained by little or no competition among males for resources (i.e., space, food, and mates) along with competition among females for mates; or they could be due to the effect of small sample size. A closely related taxon, *K. creaseri* did not show differences in body size between males and females (Macip-Ríos *et al.* 2018).

In this population of *K. herrerai,* the reproductive activity period for both sexes is

Table 1. Carapace length (CL, in mm) and body mass (BM, in g) of females and males of *Kinosternon herrerai* from each sampled pond in Hidalgo, Mexico. Mann-Whitney U test parameters are shown for within sexes and among ponds (males only) comparisons. Kruskal-Wallis test parameters are also shown for comparisons of females among ponds.

Trait / Sex	Pond									
	Santa Rosa		El Transformador		Totoapita Canutillo		Kruskal-Wallis		U Mann-Whitney	
	Female	Male	Female	Male	Female	Male	H	P	Z	P
	N = 4	*N* = 7	*N* = 9	*N* = 3	*N* = 4	-				
CL	131.1 ± 3.4	127.4 ± 3.3	126.3 ± 3.0	126 ± 0.5	122.0 ± 5.9	-	1.96	0.38	0.00	1
	(125.1–138.7)	(113.0–140.4)	(112.5–140.4)	(125.3–127.0)	(111.5–137.1)	-				
	Z = 0.66, *p* = 0.51		*Z* = 0.0, *p* = 1.0							
BM	474.5 ± 26.8	411.0 ± 20.4	423.6 ± 20.2	462.7 ± 7.1	435.8 ± 32.8	-	2.2	0.33	1.60	0.11
	(411.0–525.0)	(345.0–503.0)	(340–530)	(452–476)	(366.0–510.0)	-				
	Z = 1.61, *p* = 0.11		*Z* = -1.11, *p* = 0.27							

long (March-October). During this time females might produce up to three (maybe more) clutches of a small number of eggs (3.4 ± 1.7), similar to the reported clutch size for other populations of the same species (3.7 eggs; Legler and Vogt 2013) and another congeneric species *K. integrum* (4 eggs; Iverson 1999). This reproductive period is longer compared to the other species such as *K. integrum,* (July–October; Macip-Ríos *et al.* 2009), *K. oaxacae* (Vázquez-Gómez *et al.* 2015), and *K. hirtipes* (Wagler, 1830) (De la Cruz-Merlo *et al.* 2022). In general, turtles of the genus *Kinosternon* have small clutch sizes, which are related to body size (Macip-Ríos *et al.* 2017, Heston *et al.* 2022).

Reproductive behavior (i.e., courtship, nesting, and oviposition) occurred in and/or on the shore of the ponds, where movement rate is determined by size (CL and mass) rather than sex, with larger individuals moving longer distances, and more frequently than smaller individuals. Although we did not observe movement almong ponds, given the proximity of the ponds in the study area, we consider them part of the same population. Movement rate within the ponds could be related to searching for mates and food, but thermal ecology also could have a role in movement rate and patterns of turtles (Parlin *et al.* 2017).

Most species of the genus *Kinosternon* require a specific type of water source (e.g., ponds, streams, or lakes) to live and reproduce. *K. herrerai* appears to be more aquatic than some congeners and therefore less likely to move between suitable aquatic habitats. Unfortunately, turtles from this population inhabit degraded ponds, which are potentially polluted with heavy metals and pesticides. Hence, conservation efforts should include rehabilitation of the aquatic habitats and protection of these and other ponds. Given that *K. herrerai* is a Mexican endemic species, it might be subject to trade in the illegal specialty reptile trade. The small numbers of turtles in our study ponds could easily be decimated by illegal trapping. The species has an international and national conservation status of Near Threatened (NT; van Dijk *et al.* 2007) and is subject to Special Protection (Pr; SEMARNAT 2010). Conservation actions should be taken to preserve all populations, especially those near their altitudinal limit as in the present study.

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