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Notes on the reproductive ecology of the rough-footed mud turtle (*Kinosternon hirtipes*) in Texas, USA

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Abstract. *Kinosternon hirtipes* is among the least-studied North American turtles and little is known concerning reproduction. In the United States, *K. hirtipes* occurs at < 10 sites within a single creek drainage in Presidio County, Texas where it is considered a threatened species. We investigated the reproductive ecology of one of these populations at Plata Wetland Complex in 2007. We captured nine female *K. hirtipes* in wire mesh traps and hoop nets baited with fish. Eggs were obtained by injecting females with oxytocin. We recovered 19 eggs from six females captured in May and June. The smallest female that produced eggs was about 7.1 years old. Mean (± 1SD) clutch size, egg length, egg width, egg mass, and clutch mass were 3.1 ± 1.4 eggs, 28.7 ± 1.4 mm, 17.5 ± 0.9, 5.3 ± 0.6 g, and 17.5 ± 0.8 g, respectively. Relative egg mass and relative clutch mass were 0.035 and 0.011, respectively. There was a significant, positive linear relationship between female carapace length (CL) and egg width. The relationship between CL and relative egg mass was negative, and approached statistical significance. Relationships between CL and clutch size, egg length, and egg mass were not significant. Reproductive attributes of *K. hirtipes* in Texas are similar to those reported from a population in Mexico.

Keywords. Clutch size, egg size, *Kinosternon hirtipes*, reproduction, threatened species, Texas.

The rough-footed mud turtle (*Kinosternon hirtipes*) is a highly variable species comprising six recognized subspecies distributed from west Texas, USA, southwards into Chihuahua, Mexico, and south and east on the Mexican Plateau to the Chapala, Zapotlán, San Juanico, Pátzcuaro, and Valle de México basins (Iverson, 1981; Legler and Vogt, 2013). *Kinosternon hirtipes murrayi* is the largest subspecies (straight-line carapace length [CL] to 196 mm; Smith et al., 2015) and has the most extensive distribution, occurring from west Texas and Chihuahua, south into northern Jalisco, northern Michoacan, and eastern Estado de Mexico (Iverson, 1981; Legler and Vogt, 2013). The northernmost populations of *K. hirtipes* (sensu lato) occur in Presidio County, Texas where < 10 small, isolates are known from the Alamito Creek drainage (Ernst and Lovich, 2009; Platt and Medlock, 2015). *Kinosternon hirtipes* is considered a threatened species by Texas Parks and Wildlife Department (2013) owing to a limited geographic distribution within the state and on-going habitat degradation.

Kinosternon hirtipes ranks among the least-studied North American turtles and many aspects of its natural history remain poorly known (Lovich and Ennen, 2013). In particular, there is a notable paucity of information on

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reproductive ecology. Iverson et al. (1991) investigated reproduction among a population in Chihuahua, Mexico, and laboratory studies indicate that hatchling sex is determined by incubation temperature (Ewert et al., 2004); otherwise the reproductive ecology of *K. hirtipes* has gone unreported. Importantly, published reports of reproduction among the remnant *K. hirtipes* populations in Texas are lacking, although such natural history data are critical for designing effective conservation measures (Dayton, 2003). We here report on the reproductive ecology of one of the few known *K. hirtipes* populations in Texas.

Our study was conducted at Plata Wetland Complex (PWC; elevation = 1125 m), located on a private ranch in the Alamito Creek drainage approximately 56 km SE of Marfa in Presidio County, Texas. PWC consists of four livestock tanks (ponds): Railroad (612 m^2), Crotalus (900 m^2), Turner One (2520 m^2), and Turner Two (3780 m^2). Railroad Tank is fed by an artesian spring and linked to Crotalus Tank by a shallow drainage ditch about 244 m long. Turner One is located approximately 190 m from Railroad Tank and water is supplied by rainfall and a wind-driven pump. Turner Two is approximately 245 m from Turner Tank One and reliant on rainfall for water. Because Railroad and Crotalus Tanks are spring fed, water levels remain relatively stable throughout the year with a maximum depth of about 1.5 m. Water depth in Turner Tanks One and Two varies depending on seasonal rainfall (less so at Turner One); maximum depth in each is about 1.2 m. The environmental characteristics of the study site are described in greater detail elsewhere (Wilde and Platt, 2011; Platt et al., 2016). Regional climate is characterized by mild winters (rarely < 0 °C) and hot summers (> 40 °C) with highly variable annual rainfall (mean ca. 370 mm) (Powell, 1998).

We trapped turtles at PWC from April through mid-July, and September 2007 as part of a larger population study of *K. hirtipes* conducted in the Alamito Creek drainage (2007-2010). We captured turtles using a combination of wire mesh funnel traps (1.0 m long × 50 cm diameter; mesh = 12.5 mm) and hoop nets (2.5 m long × 1.0 m diameter; mesh = 25 mm). Traps and hoop nets were baited with sardines (packed in oil or water) or fresh carcasses of locally captured sunfish (*Lepomis*). Wire mesh traps were set from mid-morning to early evening (ca. 1000-2030 hr) and checked at intervals of 1-2 hours. Hoop nets were deployed in mid-morning (ca. 1030) on one day and checked the following morning. Each captured turtle was permanently marked by shell notching (Cagle, 1939) and then measured (CL and plastron length [PL]) with dial (± 0.1 mm) or tree calipers (± 1.0 mm) depending on body size. Body mass (BM) was determined with spring scales (± 1.0 g). Turtles with a CL ≥ 100 mm were sexed using external secondary sexual characteristics (Iverson 1985b). We were unable to reliably determine the sex of turtles below this size threshold.

Male and juvenile turtles were processed in the field and released, while females were returned to the lab and held in plastic wading pools for 21-24 days to insure that oviducal eggs were fully shelled. We then induced oviposition by injecting oxytocin into the pectoral muscles at a dosage of 2.0 units/100 g of body mass (Ewert and Legler, 1978). Following the injection, each female was placed in a 38 liter tub half-filled with water and fitted with a wire-mesh grate positioned 5 cm above the bottom; this allowed eggs to fall through and prevented accidental trampling by the female (Platt et al., 2008; Legler and Vogt, 2013). A second injection was administered within 24 hours to insure deposition of the complete clutch. Most eggs were removed from the tub 10-20 minutes after deposition, although a few remained underwater for somewhat longer; all eggs were recovered < 1 hour after being deposited. Eggs were then weighed on an Ohaus triple beam balance (± 0.1 g), and measured (length and width) with dial calipers (± 0.1 mm). Following Iverson et al. (1991) we calculated relative clutch mass (RCM) [clutch mass / (gravid female body mass — clutch mass) × 100] and relative egg mass (REM) [RCM/clutch size]. Female turtles were released at their respective capture sites 24-48 hours after oviposition. We used linear regression to explore relationships between female body size and clutch and egg attributes. Statistical analyses were performed by program JMP (version 3.2, SAS Institute, Cary, North Carolina, USA). General statistical references are from Zar (1996). Mean values are presented as ± 1SD, and results were considered significant at $P \leq 0.05$.

We captured 87 turtles (25 males, nine females, and 53 juveniles) at PWC. We treated the females (mean CL = 142 ± 15 mm; range = 121-163 mm) captured in May-June with oxytocin, and recovered 19 eggs from six females (CL = 132-159 mm) in late June (Table 1). Based on von Bertalanffy growth models (Fabens, 1965) developed for this population (Smith, 2016), the smallest female in our sample that produced eggs was estimated to be 7.1 years old. There was a significant positive linear relationship between egg width (EW) and CL ($R = 0.91$; $F_{1,5} = 20.72$; $P = 0.0104$; Fig. 1). The relationship between CL and relative egg mass (REM) was negative (REM = -0.0002CL + 0.04) and approached statistical significance ($R = 0.76$; $F_{1,5} = 5.52$; $P = 0.0785$). The relationships between CL and clutch size ($R = 0.03$; $P = 0.94$), egg length ($R = 0.02$; $P = 0.96$), and egg mass ($R = 0.68$; $P = 0.13$) were not significant.

Our data provide the only information on the reproductive ecology of *K. hirtipes* outside of Mexico (Iverson

Table 1. Attributes of clutches obtained from six female *Kinosternon hirtipes* collected in Presidio County, Texas, USA. *See text for discussion of potential methodological biases.

Attribute	n	Mean ± 1 SD	Range
Clutch size*	6	3.1 ± 1.4	1–5
Egg length (mm)	19	28.7 ± 1.4	24.7–30.8
Egg width (mm)	19	17.5 ± 0.9	15.0–18.7
Egg mass (g)*	19	5.3 ± 0.6	3.6–6.4
Mean clutch mass (g)*	6	17.5 ± 8.8	5.3–29.1
Relative clutch mass*	6	0.035 ± 0.019	0.009–0.061
Relative egg mass*	6	0.011 ± 0.002	0.008–0.015

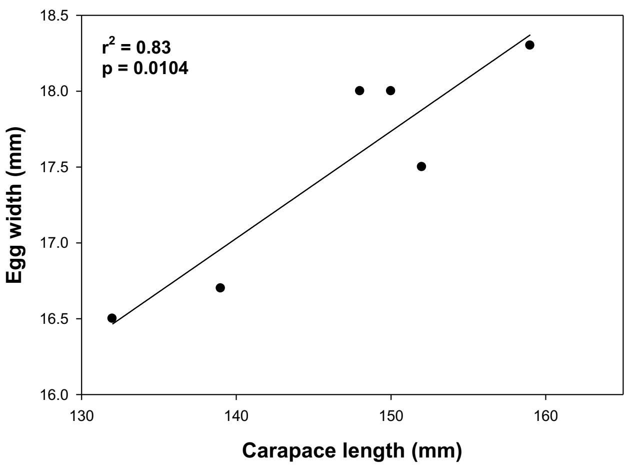

Fig. 1. Relationship between carapace length and egg width in a sample (n = 6) of female *Kinosternon hirtipes murrayi* from Presidio County, Texas, USA.

et al., 1991). The clutch size (mean and range) we report for *K. hirtipes* in Texas is almost identical to the clutch size estimated from corpora lutea counts in Chihuahua, Mexico (3.0 ± 0.8 eggs; range = 1-5 eggs; n = 34; Iverson et al. 1991). However, it should be noted that egg retention can occur after oxytocin injections (Congdon and Gibbons, 1985) and without taking radiographs of females (Gibbons and Greene, 1979), we cannot be certain that females deposited a complete clutch. Therefore, the clutch size we report is best considered a conservative estimate. That said, Tucker (2007) found that clutch size in a group of female red-eared sliders (*Trachemys scripta elegans*) treated with oxytocin was statistically equivalent to clutch size in natural nests. Although Iverson et al. (1991) found a positive correlation between clutch size and CL in *K. hirtipes*, no such relationship was evident in our study, possibly owing to the small number of females we sampled. Because *K. hirtipes* deposits multiple clutches during a single reproductive season (Iverson et al., 1991), CL may be a more suitable predictor of total annual fecundity rather than individual clutch size.

The values we report for egg mass (EM), egg length (EL), and egg width (EW) are similar to those from a much larger sample of *K. hirtipes* in Mexico (EM = 4.8 ± 1.0 g; range = 3.7-6.5 g; n = 11; EL = 28.8 ± 1.8 mm; range = 24.1-33.2 mm; n = 74; EW = 16.3 ± 0.8 mm; range = 14.6-18.5; n = 74; Iverson et al., 1991). Because females in our study deposited eggs directly into the water and these remained submerged for varying (but usually brief) periods, it is possible some eggs absorbed water and increased in mass (e.g., Wilgenbusch and Gantz, 2000). However, any increase in mass was probably minimal as most eggs were retrieved < 20 minutes after deposition. Clutch mass (CM) and relative clutch mass (RCM) were also similar to values reported by Iverson et al. (1991) among *K. hirtipes* in Mexico (CM = 14.4 ± 4.3 g; range = 6.7-29.4 g; n = 28; RCM = 0.071 ± 0.014; range = 0.048-0.105; n = 28). The negative relationship between REM and body size reported by Iverson et al. (1991) was likewise evident in our data, although of marginal statistical significance. This relationship suggests a decreased investment in each egg with increasing maternal body size (Iverson et al., 1991).

Similar to our results, Iverson et al. (1991) found a significant positive relationship between female CL and mean egg width. This relationship is not unexpected if egg width is determined by the pelvic aperture diameter which in turn scales to body size (Congdon and Gibbons, 1987). However, evidence for pelvic aperture constraints on egg size in kinosternids is conflicting (Macip-Rios et al., 2013). Some studies suggest egg width is determined by pelvic morphology (Wilkinson and Gibbons, 2005; Macip-Rios et al., 2012, 2013), while others found no evidence for such constraints (Iverson, 1991; Macip-Ríos et al., 2009; Lovich et al., 2012; Macip-Rios et al., 2013). Larger female *K. hirtipes* are producing larger eggs and according to Iverson et al. (1991), this increase is achieved by increases in egg width rather than length, which is probably constrained by oviduct length.

The two smallest mature females with corpora lutea found by Iverson et al. (1991) in Mexico had CLs of 99.4 and 97.4 mm, a body size very similar to the smallest females exhibiting secondary sexual characteristics in our study population. However, the smallest female from which we recovered eggs was considerably larger (CL = 132 mm). Despite this difference between the two populations, growth models suggest sexual maturity is attained at approximately the same age in Texas (7-8 years) and Mexico (6-8 years; Iverson et al., 1991). Obviously given our small sample size of reproductive females, further study is necessary to fully address this question.

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