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Notes on the reproductive ecology of the Oaxaca Mud Turtle (*Kinosternon oaxacae*) in the vicinity of Mazunte, Mexico

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Abstract. *Kinosternon oaxacae* is one of the least known species of Mexican turtles. We conducted a preliminary survey from August to November 2013 in three localities on the coast of Oaxaca with the objective to increase and complement the knowledge of the reproductive ecology of *Kinosternon oaxacae*, including field data on nesting season, clutch size, egg size, and relative clutch mass. We found 21 females with eggs in their oviducts. With this sample we determined that the reproductive season extends from early May to early November. Clutch size averaged two eggs (± 1), and egg size averaged 30.61 (± 1.89) mm in length, 17.11 (± 0.69) mm in width, and 7.64 (± 0.57) g in mass. Relative clutch mass (clutch mass/gravid body mass) was 0.046 (± 0.023), and the smallest gravid female was 118.8 mm carapace length. No correlations of reproductive traits were found with body size, and no variation in reproductive traits or body size was detected among localities. Comparisons with related (*scorpioides* group) species were made.

Keywords. Egg size, clutch size, nesting season, Oaxaca, relative clutch mass.

Mexico is the second richest country in turtle species in the world (Legler and Vogt, 2013; van Dijk et al., 2014). From the 61 known taxa of Mexican non-marine turtles (Legler and Vogt, 2013; Flores-Villela and Garcia-Vazquez, 2014; van Dijk et al., 2014), 57% are endemic. Unfortunately the general knowledge of the Mexican turtle fauna is relative meager, and according to Legler and Vogt (2013), *Kinosternon oaxacae* "is one of the least known species in Mexico". There are only a few published data available, including its description (Berry and Iverson, 1980) and some preliminary natural history data (Iverson, 1986). This species is known from 44 specimens from only 15 localities from the Ometepec River Basin near Guerrero to the western limits of Isthmus of Tehuantepec (Iverson, 1986; Carr, 1993; Legler and Vogt, 2013).

Reproductive data of *K. oaxacae* came from a sample of 21 individuals examined by Iverson (1986). The available information can be summarized as follows: vitelogenesis extends from April to July and mating and ovulation occur ("probably") in June or/and July. Data from females for a natural reproductive event (oviductal eggs or corpora lutea) are absent of the literature, and nesting season 'probably' begins in mid July, but it is not known when it ends. Estimated clutch size could be between two to five eggs, and no data about egg size is known whatsoever (Iverson, 1986).

For species with limited distributions as such *K. oaxacae* (21120 km^2 aprox.), quality data are necessary to develop proper conservation strategies (Klemens, 2000; Primack, 2012). The coast of Oaxaca is an economically poor region with high environmental impact due to tour-

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Table 1. Variation in mean reproductive traits in *Kinosternon oaxacae* across the three localities in the vicinity of Mazunte, México. RCM = relative clutch mass (= clutch mass/gravid females body mass). n = sample size. Numbers in parenthesis are standard deviations.

Locallity (n)	Clutch Size	Egg Length (mm)	Egg Width (mm)	Egg Mass (g)	RCM
San Roque (11)	1.8 (0.87)	30.07 (1.72)	17.20 (0.82)	7.62 (0.46)	0.048 (0.02)
Escobilla (8)	2.0 (1.06)	31.56 (2.07)	17.06 (0.62)	7.73 (0.74)	0.040 (0.02)
El Aguacate (2)	2.5 (2.12)	29.75 (0.07)	16.85 (0.10)	7.11 (0.01)	0.060 (0.04)

ism (supposedly environmental friendly), meteorological phenomena (hurricanes, tropical storms, etc.), and non-sustainable land management policies. The accelerated development of other activities, such cattle ranching, agriculture, and unorganized urban growth could impact the future of this scarcely known endemic species.

The aim of this study was to increase/complement the knowledge of the reproductive ecology of *Kinosternon oaxacae,* including field data on nesting season, clutch size, egg size, and relative clutch mass. For that we conducted a preliminary survey from August to November 2013 in the vicinity of Mazunte, Oaxaca, at the localities of El Aguacate (15°43'18"N, 96°23'15"W; 13 surveys), Escobilla (15°43'55.77"N, 96°43'59.55"W; 14 surveys), and San Roque (15°46'43"N, 96°27'33"W; 12 surveys), NAD 27 datum. We consider a survey as one day of fieldwork from 9:00 to 17:00 h. We used fyke net traps and hand captures to gather females with a minimum carapace length (CL) of 90 mm as described as the minimum size of reproduction in other similar and related species (Iverson, 1986; Iverson, 2008; Macip-Rios et al., 2009; Iverson, 2010; Iverson et al., 2013). Hand captures were made by an average of five persons per collect event, averaging a 520 hours/person. Traps sum 112 hours/trap for Escobilla and 96 hours/trap for San Roque.

Females were brought to the lab at the Centro Mexicano de la Tortuga (CMT) were they were measured, weighed, and marked with an individual code by shell notching (Cagle, 1939). No radiograph was used but all females were injected with a dose of 1.5 ml/kg of oxytocin to induce oviposition (Gibbons and Greene, 1979). Injected turtles were placed in individual enclosures with a sand substrate for oviposition. Deposited eggs were measured (length and width) with a dial caliper (0.02 mm) and weighed with a triple beam Ohaus Junior TJ611 balance (0.1 g).

Reproductive effort was estimated by the relative clutch mass (RCM) formula modified by Cuellar (1984): clutch mass/gravid females body mass. We compared clutch size, egg size and relative clutch mass among localities by a non-parametric (rank based) Kruskal-Wallis test (Zar, 1999). We also used a non-parametric Spearman ρ (Rho) to identify correlations between body size (carapace length) and reproductive parameters. All statistical tests were ran with JMP ver. 5.0.1 (SAS Institute, 2002) with an $\alpha = 0.05$. We also searched in the archives of the CMT for field journals and data sheets with information and data gathered by the CMT personnel from discontinuous surveys from the years 2009 and 2010. We used these data to support our direct observations in 2013. Turtles were collected under the permit SGPA/DGVS/04572/13 issued by the Ministry of Natural Resources of the Mexican Federal Government (SEMARNAT). No ethics permits are required in Mexico when organisms are used for scientific research, however no turtle were killed or harm during the study, and all individuals were handled and managed under international ethics on animal issues.

A total of 57 females were injected with oxycitocin, but only 21 females oviposited in the lab; 11 females were from San Roque, eight from Escobilla, and two from El Aguacate. Females with oviductal eggs were detected from late September to early November. The smallest female with eggs in the three localities was 118.8 mm CL. Clutch size (CS) ranged 1-4 eggs, with a mean of 1.95 (± 1.0) (mean ± SD) eggs; egg length (EL) averaged 30.61 (± 1.89) mm, egg width (EW) 17.11 (± 0.69) mm, and egg mass 7.64 (± 0.57) g. Relative clutch mass was 0.046 (± 0.023).

The comparison of reproductive traits among localities did not show significant variation in clutch size ($H_2 = 0.29$, $P = 0.86$); either EL ($H_2 = 3.81$, $P = 0.14$), EW ($H_2 = 0.87$, $P = 0.64$), EM ($H_2 = 3.73$ $P = 0.15$), and RCM ($H_2 = 0.94$, $P = 0.62$). Means and variation by localities are presented in Table 1. Body size (CL) was not correlated with

Table 2. Non parametric correlations of reproductive traits. ρ is Spearman Rho; CL is carapace length; RCM is relative clutch mass.

X	Y	ρ	P
Body Size (CL)	Egg Length	0.28	0.21
Body Size (CL)	Egg Width	-0.14	0.52
Body Size (CL)	Egg Mass	0.35	0.11
Body Size (CL)	Clutch Size	0.12	0.58
Clutch Size	RCM	0.92	<0.0001

any reproductive trait. Only RCM was correlated with clutch size (Table 2).

Archive data showed that eggs were laid in captivity from early May to early September, with the following average measurements for 21 captive eggs: EL 27.29 (± 2.81) mm, range 22-30 mm; EW 17.15 (± 1.79) mm, range 15-22 mm; EM 4.28(± 1.48) g, range 2.6-7.1g.

Based on our combined data (field survey and archive data), nesting season probably extends from early May to early November, significantly longer than Iverson's (1986) hypothesis of mid July to an unknown date. Actually, reproductive season of *K. oaxacae* is quite similar to that of the closely related *Kinosternon integrum,* for which reproductive season extends from May to early November (Iverson, 1999; Macip-Ríos et al., 2009), and *K. scorpioides*, which reproduce from May through November across its range (Iverson, 2010). The Oaxaca Mud turtle shows a similar reproductive season to those turtle species in the seasonal (dry) tropics of the Pacific Coast of México, concentrating all of their reproductive activity in the rainy season (Macip-Ríos, 2010), which in the study area extends from May to October (Trejo, 2004), even though in the study area water is available all year round in rivers, streams, ponds, and small lagoons.

The smallest female with oviductal eggs (118.8 mm of CL), a proxy of minimum body size at first reproduction, was also quite similar to that of *K. integrum* (Macip-Rios et al., 2009; 2011; 2013) and *K. scorpioides* (Iverson, 2008; Iverson, 2010). Apparently turtles of the *scorpioides* group mature around 114-120 mm CL, which is large compared with others kinosternids from higher latitudes (Macip-Ríos, 2010).

Clutch size was possibly underestimated because we did not use X-Ray photographs. Instead we estimated clutch size by the number of eggs oviposited after oxytocine was injected, a proceeding repeated for three days if no egg was oviposited, assuming all eggs were laid. This method produced clutches of one egg to four eggs. Iverson (1986) suggested a clutch size might range from two to five eggs for the Oaxaca Mud Turtle. Our estimate was similar, but we suspect that a higher clutch size could be attained, because congeners with similar body size and morphology (*K. scorpioides* and *K. integrum*) are capable of producing clutches from 2 to 10 eggs (Iverson, 1999; Macip-Ríos et al., 2009; Iverson, 2010). Macip-Ríos (2010) collected two gravid *K. oaxacae* (141 and 136 mm CL) in July 2008 from Chacalapa, Oaxaca (a locality 5.8 km northwest from San Roque), and found clutches (estimated by X-Ray photographs) of three eggs in both individuals.

Clutch size may actually be reduced in *K. oaxacae* compared with its relatives of the same genus (Macip-Ríos et al., 2013). *K. oaxacae* showed smaller variation in egg width (coefficient of variation = 4.07) compared with egg length (coefficient of variation, CV = 6.18), and egg mass (CV = 7.52). Egg size was similar to that other related kinosternids of about the same size, which depends in part on female body size and its relation with clutch size. RCM was similar to the average reported within related species (Iverson et al., 1991); however, it was lower than the calculated value for the two Chacalapa females (0.065 and 0.064) reported by Macip-Ríos (2010).

Comparisons between localities and correlations between reproductive traits did not show any pattern. The strong correlation between clutch size and RCM may reflect an underestimation of clutch size. A direct correlation between clutch size and RCM represents an isometric investment of body mass by each egg of the clutch, a previously unknown pattern which may be an artifact of the sample size, since it is absent in other species of mud turtles (Lovich et al., 2012; Macip-Ríos et al., 2013).

Finally, this is the first study that documents a minimum reproductive size, egg size (length, width, and mass), and reproductive output in *Kinosternon oaxacae*. This paper also extends the known nesting season from early May to early November. It also demonstrates that the reproductive phenology and major traits are quite similar to those of *K. integrum*, but more data are needed to confirm the reproductive traits of this locally endemic species in Oaxaca.

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