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## OBSERVATIONS ON POPULATION ECOLOGY AND ABUNDANCE OF THE MICRO-ENDEMIC OAXACA MUD TURTLE (*KINOSTERNON OAXACAE*)

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**Abstract.**—The Oaxaca Mud Turtle (*Kinosternon oaxacae*) is one of the least known turtles in Mexico. It was described in 1980 and until now it is only known from 44 specimens and 15 localities. It is distributed from the Papagayo River near the state line with Guerrero to the western limits of Isthmus of Tehuantepec. It is under special protection by the Mexican Government, but it is not included on other conservation lists. The Mexican Turtle Center has monitored *K. oaxacae* for more than a decade. That work resulted in a database of 233 records, with annotations on its natural history and phenology. We conducted a survey from August to November 2013 at three localities in the vicinity of Mazunte. We captured 238 turtles but only recaptured 36 individuals. We found a sex ratio that was significantly biased to females (1:2.2). Males (plastron length [PL] = 119.3 ± 12.69 mm) were not significantly larger than females (PL = 113.9 ± 12.41 mm). We estimated population size at the San Roque locality as 287 individuals and at El Aguacate locality as 195 individuals. We only captured 47 individuals at the Escobilla locality. Population structures in the three localities were mainly composed of adults and juveniles, and we caught few hatchlings. Average density was 3,128 individuals/ha for the study areas. Populations of *Kinosternon oaxacae* in the vicinity of Mazunte seem healthy and reproductively active.

**Key Words.**—body size variation; conservation; population size; population structure; sex ratio

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### INTRODUCTION

Reliable demographic data are the best approach to understand population size, structure, survivorship, and recruitment (Begon et al. 1996; Caswell 2001). This kind of data has practical uses in conservation biology, population genetics, and life-history evolution (Stearns 1992; Primack 2012), which makes demography essential for the design of conservation and management plans for threatened species (Gibbs and Amato 2000; Rhodin et al. 2011; van Dijk et al. 2012). Most of the time demographic data come from capture-recapture studies; however, for long-lived organisms like turtles, such studies could be difficult to achieve although there are long term studies from northern latitudes (Crouse et al. 1987; Frazer 1991; Doak et al. 1994; Ruane et al. 2008; Enneson and Litzgus 2008).

Long-term population ecology studies on kinosternid turtles are available for Yellow Mud Turtles, *Kinosternum flavescens* (Iverson 1991), Eastern Mud Turtles, *K. subrubrum* (Frazer 1991), Sonora Mud Turtles, *K. sonoriense* (Hulse 1982; Stone 2001), and Common Musk Turtles, *Sternotherus odoratus* (Edmonds and Brooks 1996). The basic patterns suggest

that kinosternids are highly seasonal, with low juvenile survivorship and high adult survivorship, and the reproductive effort is low per reproductive event. That is why turtles are recognized as having a bet-hedger life-history strategy (Wilbur and Morin 1988; Janzen et al. 2000; Lovich et al. 2015).

Demographic data are rare for Mexican turtle species, even though Mexico has the second richest turtle fauna of any country (van Dijk et al. 2012; Legler and Vogt 2013). For the 61 known taxa of Mexican non-marine turtles (van Dijk et al. 2012; Legler and Vogt 2013; Flores-Villela and García-Vázquez 2014), the knowledge of basic population ecology is minimal. Few studies have explored freshwater turtle demography in Mexico (Iverson et al. 1991; Macip-Ríos et al. 2009, 2011; Legler and Vogt 2013), and even fewer have focused on kinosternids. Demography data of Oaxaca Mud Turtles (*Kinosternon oaxacae*) are completely unknown, with only some data available about its natural history and diet (Berry and Iverson 1980; Iverson 1986). This species is known from only 44 specimens from 15 localities (Legler and Vogt 2013). It is distributed from the Ometepec River basin near the state of Guerrero to the western edge of the Isthmus of Tehuantepec, where it

is replaced by *Kinosternon scorpioides cruentatum* (Iverson 1986; Carr 1993; Legler and Vogt 2013).

Personnel from the Mexican Turtle Center in Mazunte, Oaxaca, have done surveys and irregular monitoring of local populations of *K. oaxacae* from 1997 to 2010, and their archives include 233 observations. These records suggest that the Oaxaca Mud Turtle is active all year with a peak of captures from May to September. Because few data are available for this species, we conducted a preliminary study to obtain basic demographic information from three localities on the coast of Oaxaca. Our aims were to determine: (1) sex ratios of the three populations; (2) population sizes; (3) densities; (4) population structures; and (5) variation in sexual size dimorphism.

## MATERIALS AND METHODS

**Study site.**—We studied turtles at three localities in Oaxaca, along the Pacific Coast of México. We located sites at the low elevations of the Sierra Madre del Sur, between the Mexican Transvolcanic Belt and the Isthmus of Tehuantepec, at 94°30'W and 99°34'W and 17°36'N and 17°37'N (NAD27). The climate of the study site is seasonal with heavy rainfalls during summer (García 2004). Average annual temperature in the region is 24° C and annual precipitation average between 800 and 1000 mm (Rodarte 1997). The maximum distance between the three study sites was approximately 34 km, but two of the localities were only about 15 km apart. Because the three localities were in different drainage systems (see section below), we considered them as different populations isolated by distance and basins.

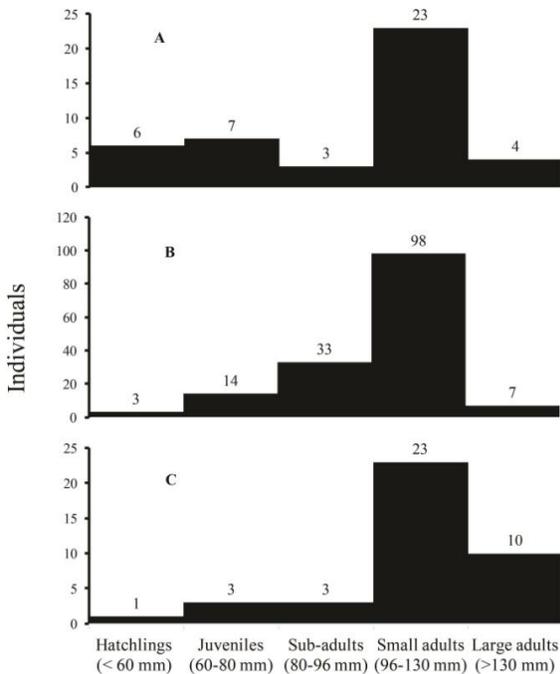
We conducted the study at El Aguacate (located at 96°25'38''W, 15°49'9''N, at about 40 m elevation), at Escobilla, located in Santa María Tonameca Municipality (96°49'15''W, 15°43'59''N at about 10 m elevation), and at San Roque, located in a valley between the foothills of Sierra Madre del Sur and a low hill system associated to the coast at the vicinity of San Pedro Pochutla (96°27'37''W, 15°47'16''N, at 180 m elevation). Rivers and streams drain from the Sierra Madre to the Pacific coast. The drainage of El Aguacate is El Aguacate stream, a tributary of the Copalita River. We took 10 water temperature measurements during the sampling season with a Fluke 53 II thermometer (Fluke MEA, Arjaan Tower-Dubai Media City, Dubai, United Arab Emirates). Water temperature from El Aguacate stream was 27.85 (SD) ± 1.27° C. The main drainage at Escobilla is the Tonameca River basin (SEMARNAT 2009), a river system that ends in a seasonal river mouth, which is open in the rainy season only with high tides. Several seasonal ponds are associated with this basin that fill by early July or early August depending on when rainy season begins and dries by late November. The

pooled temperature of the seasonal pools when we conducted the sampling averaged 29.01 ± 1.15° C. Finally, San Roque is placed in the Xonene River basin, a drainage system with seasonal pools filled with rainwater. The average water temperature in the ponds from six measurements during the sampling season was 29.65 ± 2.05° C. The dominant vegetation type in the study area was gallery forest, with secondary vegetation along the rivers and streams, while around the ponds were patches of crops (Coconut Palm, *Cocos nucifera*), grasslands, and remnants of tropical semi-deciduous forest.

**Sampling.**—At each locality, we used two types of capture technique: active search in the bodies of water with hand capture (at El Aguacate we also used the help of the local people) and fyke nets in temporary ponds and streams when water systems allowed the use of traps. We took captured turtles to the lab at the Mexican Turtle Center in Mazunte, Oaxaca. We measured typical morphological traits such as body mass (BM), maximum carapace length (CL), maximum carapace width (CW), maximum plastron length (PL), and maximum carapace height (CH). We used dial calipers (0.02 mm; Truper model 1281084, Truper S.A. de C.V. México, D.F. México) to make measurements and an Ohaus (Ohaus Corp., Parsippany, New Jersey, USA) triple beam balance (0.5 g) to take body mass. Each turtle was individually marked with notches on the marginal scutes following the system by Cagle (1939). We made a photographic record of each individual to document variability in shell shape and form.

We determined sex of adult turtles by the prevalence of secondary sexual characters in males: long and bulky tail, a developed spine at the end of the male tail, a prominent notch in the hind lobe of the plastron, and a concave plastron (Iverson 1999). Individuals that we could not categorize as adult males or adult females were considered as juveniles. We did not kill any turtles nor did any die accidentally during the study. After we took measurements, we injected some females with oxytocine to induce oviposition (for another study), but we returned all the captured turtles to the capture location within 7 d.

**Statistical analyses.**—We estimated population size using the Schumacher–Eschmeyer Model (Lemos-Espinal et al. 2005), which we calculated by hand. We did not use the Jolly-Seber Model from the MARK program (Lebreton et al. 1992; White and Burnham 1999) because we only had preliminary data and the referred protocol only works with large data sets. To test for morphological variation among populations, we first checked for data normality and homogeneity of variances with a Shapiro-Wilk test and Bartlett test, respectively (Sokal and Rohlf 2011). Because data were



**FIGURE 1.** Population structure of Oaxaca Mud Turtles (*Kinosternon oaxacae*) at three localities of the study area in Oaxaca, México: A = El Aguacate, B = San Roque, and C = Escobilla.

normal and homoscedastic, we used a two-way ANOVA to explore variation between localities using sex as a second factor in a mixed model with plastron length as body mass estimators. If variation was detected, we used Tukey's HSD as a *post hoc* test (Zar 1999).

We compared population density between localities using a Chi-square homogeneity test. We estimated population structure following previous work on the genus (Macip-Ríos et al. 2011) and qualitatively compared structure among localities. Our body size categories were: hatchlings with a PL < 60 mm; juveniles (immatures) with a PL between 60–80 mm; sub-adults with a PL 80–96 mm; small adults with a PL between 96–130 mm; and large adults with a PL > 130 mm. The minimum reproductive size reported for *K. integrum* is 96 mm PL (Macip-Ríos et al. 2009), which is quite similar in body size to *K. oaxacae* (Iverson 1986). We estimated sex ratio for pooled data and for each population. We included individuals smaller than 96 mm of PL to determine the sex ratio if individuals had evident signs of sexual dimorphism, such as long tails, wide heads, and aggressive behavior for males and small tails, thin heads, and domed carapaces for females (Iverson 1986). To test for the null hypothesis of 1:1 sex ratio, we used a Chi-square. We used JMP ver. 5.0.1 (SAS Institute, Cary, North Carolina, USA) for all analyses, with  $\alpha = 0.05$ .

## RESULTS

We captured 238 turtles from August to November 2013. We only recaptured 36 individuals (15%) in the same sampling season. We captured 155 turtles in San Roque, 43 in El Aguacate, and 40 in Escobilla. Estimated population sizes ( $\pm$  SE) at San Roque were 402 individuals ( $\pm$  123) and at El Aguacate 272 individuals ( $\pm$  73). We did not recapture any of the 40 individuals captured at Escobilla. The population structure was similar among the three localities, with a high number of adults, followed by immatures, very few large adults, and very few hatchlings, however, the studied populations showed evidence of recruitment, as evidenced by the presence of hatchlings and juveniles (Fig. 1). Local estimated density differed between San Roque with 3,894 individuals/ha of water and the other two localities, with Escobilla at 2,848 individuals/ha, and El Aguacate at 2,640 individuals/ha ( $\chi^2 = 288.83$   $P < 0.001$ ).

We observed a female-skewed sex ratio of 1:2.2 with 72 males and 160 females ( $\chi^2 = 33.37$ ,  $P < 0.001$ ) after combining the data sets of the three populations. San Roque and El Aguacate also had a female-skewed sex ratio (1:2.4;  $\chi^2 = 26.63$ ,  $P < 0.001$ ; 1:2;  $\chi^2 = 5.23$ ,  $P = 0.020$ , respectively); however, male-female sex ratios were even at Escobilla (1:1;  $\chi^2 = 1.77$ ,  $P = 0.18$ ). Body size (PL) showed interpopulational variation but not intersexual among adults (Table 1). Males were not larger than females ( $F_{5,165} = 1.05$ ,  $P = 0.300$ ); however, PL was variable between localities, with larger individuals from Escobilla than El Aguacate and San Roque ( $F_{5,165} = 7.55$ ,  $P < 0.001$ ). When we pooled all data we found that males were larger than females in PL ( $t = 2.60$ ,  $df = 114$ ,  $P = 0.010$ ).

## DISCUSSION

Population sizes were similar among the studied populations of Oaxaca Mud Turtle and exhibited female biased sex ratios and population composed mainly of sub adults and reproductive adults. This is a typical pattern found for related species such *Kinosternon integrum* in Central Mexico (Macip-Ríos et al. 2009) and *K. scorpioides* in San Andres Island (Forero-Medina et al. 2007). Our data document that *K. oaxacae* is highly abundant in the region, which is expected because the Mazunte region is near the center of its distribution area. Other studies (Macip-Ríos 2010; Centro Mexicano de la Tortuga archives) and previous surveys in the region (Iverson, 1986) also found a high abundance of the species.

Variation in body size among populations was consistent with other observations on body size variation among sexes and among populations for other

**TABLE 1.** Plastron length (mm) comparisons (standard deviation in parentheses) of Oaxaca Mud Turtles (*Kinosternon oaxacae*) from three localities in the vicinity of Mazunte, Oaxaca, Mexico, with *P* values of a mixed effect two-way ANOVA. Abbreviations are M = males, F = females, n = sample size.

San Roque		El Aguacate		Escobilla		<i>P</i> value		
M (n = 33)	F (n = 73)	M (n = 11)	F (n = 16)	M (n = 14)	F (n = 19)	Locality	Sex	Mixed
118.34 (12.98)	110.2 (10.40)	121.14 (11.93)	115.97 (10.94)	120.03 (13.26)	126.60 (12.41)	< 0.001	0.300	0.009

kinosternids (Frazer 1991; Iverson 1991; Iverson et al. 1991; Macip-Ríos et al. 2012). According to Legler and Vogt (2013), most of the kinosternids from Mexico showed larger males than females; however, turtles in lentic environments (Escobilla and San Roque) were larger than those from El Aguacate, which inhabit a lotic environment. Regarding alimentary habits and diet, the two habitat types could be driving body size, because shallow lentic environments tend to be warmer (Macip-Ríos et al. 2010) than streams like those from El Aguacate. Our water temperature measurements indicate a two-degree temperature difference between El Aguacate and the other two localities.

Sex ratios were skewed toward females, as in other populations of kinosternids: *K. subrubrum* (Frazer 1991), *K. scorpioides albogulare* (Forero-Medina et al. 2007), and *K. integrum* (Macip-Ríos et al. 2009); however, 1:1 sex ratios have been reported for *Sternotherus odoratus* (Mitchell 1988) and *K. flavescens* (Iverson 1991). Male-biased populations also have been reported for *S. odoratus* in Canada (Edmons and Brooks 1996) and Indiana (Smith and Iverson 2002). A sex ratio can be biased for several reasons: costlier males when males are larger than females (Burger and Gochfeld 1981), temperature-dependent sex determination (Bull and Vogt 1979), and simply because of sampling bias. Nevertheless, female-skewed sex ratios with a population structure dominated by adults and sub-adults could indicate a high reproductive potential of the population (Begon et al. 1996), although Macip-Ríos et al. (2011) reported female-skewed sex ratios in a non-growing population of *K. integrum*.

Recruitment was estimated by the presence of hatchling and fertile eggs from the three populations at the study site (Vázquez-Gómez et al., 2015). We documented similar proportions in population structure for the three populations and data suggest that the three populations are structured by reproductive individuals and potentially could be growing. Our density data agree with observations that turtles are an important biomass component of aquatic environments (Iverson 1982). In this study we also found the highest inferred density per area for a kinosternid species (Iverson 1986).

We conclude that *K. oaxacae* is a common and abundant species in Southern Oaxaca. Reproductive adults mainly compose the populations of the *K. oaxacae*

in the study area. However, more research is needed to achieve a complete demographic assessment of these localities. Analyses such as those made with MARK (Lebreton et al. 1992; White and Burnham 1999) in protocols like Macip-Ríos et al. (2011), Molina-Zoulanga et al. (2013), and Pérez-Mendoza et al. (2013) should be conducted when more data are available.

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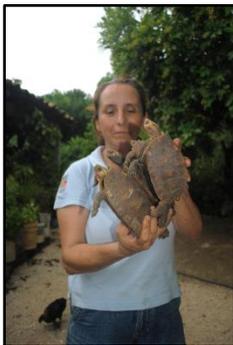
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## Herpetological Conservation and Biology



**ALMA VÁSQUEZ GÓMEZ** received her bachelor degree of biology with honors from the Benemérita Universidad Autónoma de Puebla, Puebla, México (2014). Alma is interested in the biology of turtles since her undergraduate studies. She is a graduate student from the Instituto de Ciencias del Mar, UNAM, México, and her research interest is focused on the effects of estradiol in the differentiation of ovary in the sea turtle *Lepidochelys olivacea*. Alma has been involved in turtle conservation in several ways. She has taken international turtle biology courses and has been a research assistant in the Sea Turtle Conservancy program in Tortuguero, Costa Rica. (Photographed by unknown).



**MARTHA HARFUSH** has been involved in turtle biology research and turtle conservation since at least 25 y ago. She earned her bachelor degree in Fisheries and Aquaculture Engendering from the Instituto Tecnológico de Boca del Río, Veracruz in 1990. Since then she participated in numerous projects on marine turtle conservation and biology at the Centro Mexicano de la Tortuga when she is based. Martha also works with freshwater turtles in Oaxaca, she run several projects on conservation of mud and Mexican wood turtles in the vicinity of Mazunte. She is a reference of Oaxaca turtle conservation and she collaborated with a lot of international researchers. (Photographed by Karina Contreras).



**RODRIGO MACIP RÍOS** received his Bachelor of Biology from the Universidad Autónoma de Puebla, Puebla, México (2003), his Master of Science (2005) and Doctor of Science (2010) degrees from the Universidad Nacional Autónoma de México (UNAM). His research focuses on reproductive ecology of kinosternid turtles, conservation biology of turtles, biological diversity studies, and herpetology. He is appointee Assistant Professor in the Universidad Nacional Autónoma de México at Morelia since 2015. He is Associate Editor of the Revista Mexicana de Biodiversidad. He has taught courses of evolution, evolutionary ecology, statistics, and science methods in undergraduate and graduate levels. He serves on the board of advisors for the American Turtle Observatory. (Photographed by Carlos A. Chavez Zichinelli).