Chelonian Conservation and Biology, 1995, 1(3):181–186

Reproductive Biology of the Chopontil, *Claudius angustatus* (Testudines: Kinosternidae), in Southern Veracruz, México

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ABSTRACT. – Adult male *Claudius angustatus* average slightly larger than females and attain sexual maturity at approximately 98 mm CL. Some females become sexually mature at 89 mm CL, although some individuals 20–25 mm larger are immature. Nesting and gravid females occur from November through February. Clutch size ranges from 1 to 6 (mode 2) ellipsoidal eggs ($\bar{x}$ = 17.5 x 30.5 mm, 6.0 g), and total clutch mass ranges from 5.2–27.2 g ($\bar{x}$ = 14.4 g). Clutch number and mass are positively correlated with carapace length. Spermiogenesis occurs throughout much of the year, peaking from November to January; spermatogenesis appears continuous for the population, although individuals may have discontinuous spermatogenesis with a resting period in the February to May interval.

KEY WORDS. – Reptilia; Testudines; Kinosternidae; *Claudius angustatus*; turtle; reproduction; oogenesis; spermatogenesis; sexual maturity; clutch size; nesting season; morphometrics; Mexico

Claudius angustatus, the chopontil or narrow-bridged musk turtle, occurs in a narrow band of land from south-central Veracruz, Mexico, eastward to Belize (Fig. 1). It lives principally in areas with seasonally flooded grasslands and forages in these grasslands during the flooding. After the floods, it reproduces and presumably estivates or greatly reduces its activity. This behavior during the drier months is speculative, like many other aspects of its biology.

Even though *Claudius angustatus* was described over a century ago (Cope, 1865), its life history is known only from a few anecdotal notes (e.g., Smith and Smith, 1979; Flores-Villela, 1980; Iverson and Berry, 1980; Alvarez del Toro, 1982). Few biologist have observed this species in the wild, and until recently, no more than 50 specimens were available in scientific collections (Smith and Smith, 1979). Thus, *Claudius* was believed to be a rare turtle. However, it was not! Biologists simply did not know where to find it.

Fishermen, presumably for centuries, have harvested *C. angustatus* and other freshwater turtles in coastal Veracruz and adjacent lowlands to the south. This local fishery allowed us to gather monthly samples of *Claudius* and other species of turtles from this area. The following study offers a summary of the data gathered on the reproductive biology of *C. angustatus*, the smallest extant staurotypine turtle and one of the smallest living kinosternids.

MATERIALS AND METHODS

Female and male *Claudius angustatus* were collected by local fishermen by hand from flooded grasslands along a tributary of the Papaloapan River, near Lerdo de Tejada, Veracruz (Fig. 2). *Claudius angustatus* is harvested from June through March but mainly during the wet season (June to October). Our monthly samples derive from collections made in the years 1982–1984, predominantly purchased from fishermen. To supplement the purchased samples and to make field observations, Flores-Villela and a field associate performed four 1–2 km transect censuses in early July 1985. Each transect traversed flooded fields in a series of nonoverlapping, widely spaced loops. The transects took 45–90 minutes and an attempt was made to capture all *Claudius* seen.

The composition of the monthly samples is variable; each sample can include turtles from one, two, or three years. The monthly samples for the analysis of size of gonadal structures and gametogenic stage consisted of females/males as follows: Jan 1/1; Feb 8/3; Mar 1/7; Apr and May 0/0; Jun 29/6; Jul 33/10; Aug 2/0; Sep 13/10; Oct 0/2; Nov 38/6; Dec 19/0; Total 144/45. The sample for determining oviducal clutch size contained 58 females. Specimens are deposited in collections of the Estacion de Biología Tropical "Los Tuxtlas" (UNAMLT), Instituto de Biología (IBH), and the Museo de Zoología, Facultad de Ciencias (MZFC), Universidad Nacional Autónoma de México.

Data on spermatogenesis derives from examination of histological sections (8 μm; Harris's haematoxylin and eosin, Berg's nuclear stain) from the middle of the left testis of each adult male. Each testis was classified using the Mayhew and Wright (1970) spermatogenic cycle stage-schedule. Seminiferous tubule diameter was recorded to the nearest micron on six tubules for each individual. Measurements (to 0.1 mm) and examination of all gross female and male reproductive structures were performed on recently preserved specimens with vernier calipers and with the naked eye or under a dissecting microscope. Testes and oviducal eggs were weighed to the nearest 0.001 g and 0.1 g, respectively. One to four size classes of ovarian follicles can occur in a female; we recorded only the diameters of the largest follicles and the total number of all follicles in the left ovary. Statistical analyses were performed using Systat 4.1.

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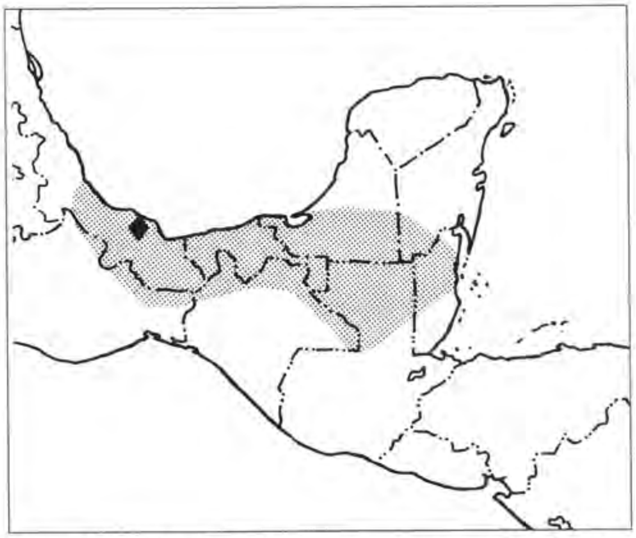

Figure 1. Distribution of *Claudius angustatus* and the location (diamond) of the study site in Veracruz, México. Distribution after Iverson (1992).

RESULTS

General Observations

Claudius angustatus is active only when the river floods the adjacent grassy marshes. Flooding normally occurs from June through October. As the marshes dry, turtles reproduce and then disappear. Presumably they burrow in the mud and remain inactive until the area floods again.

Sexual Differences. — Males were more abundant than females in four field censuses (28:11, males:females). Our reproductive samples were biased, however, toward females, because we preferentially purchased females from fishermen or in local markets.

Adult females and males differed in several morphological features, especially in overall body size and tail length. Males ($\bar{x}$ = 118.3 ± 14.5 mm CL, n = 118) averaged larger than females ($\bar{x}$ = 109.1 ± 11.0 mm CL, n = 132). This difference was evident in the size-frequency distribution of the two sexes (Fig. 3). The majority of the females (69%) were less than 115 mm carapace length (CL); in contrast, the majority of males (55%) were larger (> 115 mm CL). The largest female was 141 mm CL; three males exceeded this size and the largest was 147 mm CL.

Females

Sexual Maturity. — The attainment of sexual maturity was recognized by the presence of one or more of the following states: 1) presence of oviducal eggs (= gravid); 2) enlarged and maturing vitellogenic follicles (>10.0 mm diameter); 3) presence of corpora lutea. The smallest female with oviducal eggs was 94.0 mm CL. The smallest female with enlarged follicles was 88.9 mm CL and with corpora lutea 99.0 mm CL. A number of females (n = 41; all from June-September samples) did not meet the criteria for maturity, yet they were larger (range, 92.6–139.0 mm CL, $\bar{x}$ and SD = 110.1 ± 11.22 mm CL) than the smallest mature female. Three of these nonreproductive turtles (115.2, 126.0, 139.0 mm CL; all from September samples) had nonvitellogenic follicles (< 2.0 mm diameter) and no evidence of corpora lutea.

Oogenesis. — Females with maturing follicles (> 10 mm diam.) occurred in all monthly samples (Table 1). Females with corpora lutea were present in the January–March, June, November, and December samples, and those with oviducal eggs in the November–February samples. Three females caught and palpated in September 1982 were recorded as gravid; unfortunately, they were not retained for later dissection and confirmation of the presence of oviducal eggs. Samples were not available for April–May and October, so the gametic condition is unknown for these months.

All adult females in the November (n = 38) and December (n = 19) samples were gravid. The January sample included a single gravid female, and only one female from the February sample (n = 8) had oviducal eggs.

Corpora lutea occurred in all November, December, and January females. Most (7 of 8) February females also had corpora lutea; these seven females also had one or more large (> 14 mm diam.) follicles. The single March female and 16 of 29 June females had corpora lutea. Corpora lutea were not present in females from the July, August, and September samples.

Clutch Size. — Chopontils commonly had two or three hard-shelled eggs per clutch (Table 2). One, four, five, and six egg clutches were less common. The number of large corpora lutea in each female matched the number of oviducal eggs. Of the 47 females with corpora lutea, only 14 individuals showed symmetrical or equal production of eggs on the left and right sides. In 18 females, one ovary produced the entire clutch (1–4 eggs), the left ovary in eight females and the right ovary in ten females. In the remaining 15 females, each ovary produced eggs but production was asymmetrical, the left producing one or more eggs more than the right in two cases and the converse in 12 cases. Transuterine migration of ova was common, e.g., the number of corpora lutea

Figure 2. An adult *Claudius angustatus*. Photo by R.W. Barbour.

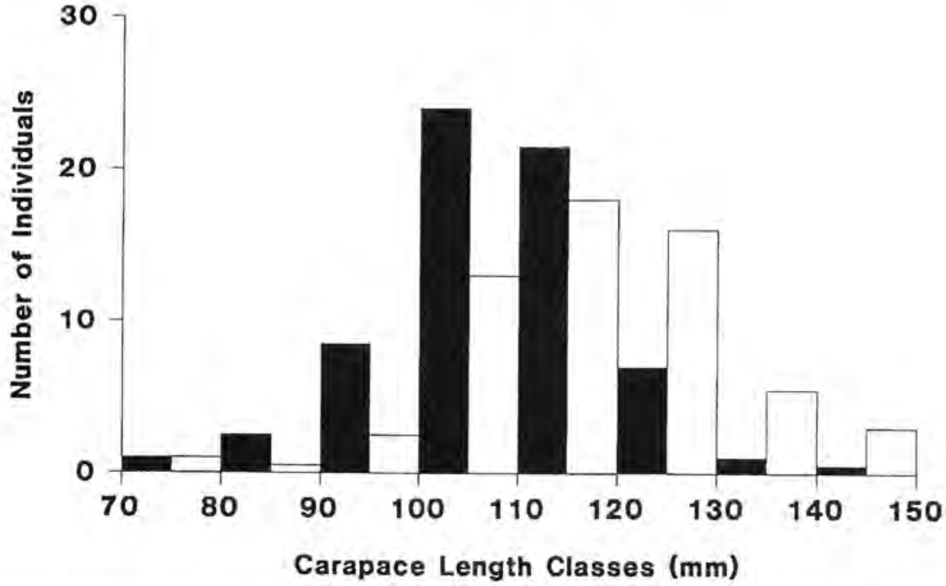

Figure 3. Size distribution of subadult and adult *Claudius angustatus* from the Veracruz study site. The sample (n = 250) encompasses specimens in 1982–1984. Class size is arbitrarily set at 10 mm CL. Females are depicted by solid bars, males by open bars.

in the right ovary equaled the number of eggs in the right oviduct in only 27% (n = 15) of the females.

Claudius eggs are ellipsoidal. Their maximum lengths varied from 26.4–33.8 mm ($\bar{x}$ = 30.5 ± 1.52, n = 130), and the maximum widths varied from 16.2–19.7 mm ($\bar{x}$ = 17.5 ± 0.70). Individual egg mass ranged from 4.3–7.9 g ($\bar{x}$ = 6.0 ± 0.70). There was a moderate and direct correlation of body size and clutch size (Fig. 4; egg number to CL, r = 0.62, P < 0.001; total clutch mass to CL, r = 0.65, P < 0.001, n = 54). The average egg weight of eggs in a clutch had no association with body size (r = 0.17, P = 0.221, n = 54).

Nesting. — Some natural nests (n = 7) were found during field work in July, although no actual nesting/egg-laying behavior was observed. Six of the nests had 3 eggs and one had 4 eggs. Females lay their eggs on or within vegetation (living or detritus) without digging a nest-pit in the soil.

Table 1. Monthly frequency of occurrence of six oogenic stages in adult (> 89 mm CL) female *Claudius* (n = 144). An individual is counted only once for the largest or oldest gametic stage. No samples exist for April, May, and October.

Month	n	Follicle Classes (diameters, mm)					Oviducal Eggs
		<1.0	1.0-4.9	5.0-9.9	10.0-14.9	>15.0	
Jan	1						1
Feb	8				3	4	1
Mar	1				1		
Apr	0						
May	0						
Jun	29		2	4	13	10	
Jul	33		1	25	6	1	
Aug	2			1	1		
Sep	13	1	2	7	3		
Oct	0						
Nov	38						38
Dec	19						19

Gravid females occurred in the November–January samples of all years, and this period presumably represents the major nesting season in southern Veracruz.

Males

Sexual Maturity. — Sexual maturity in males is demonstrated by presence of spermatozoa in the seminiferous tubules or the ducts of the epididymis. The smallest male meeting these conditions and also the smallest male examined histologically was 98.5 mm CL. A few larger males (6 of 43) had no spermatozoa and showed stage 2 or 3 spermatogenesis; these larger males lacking sperm derived from the months (March, June) of reduced spermatogenesis.

Spermatogenesis. — Active spermiogenesis occurred in some individuals from all monthly samples, except October. The histology of the two October males was difficult to decipher; stroma was absent between the seminiferous tubules, and cells within the tubules were fragmented. These tissue samples probably macerated prior to preservation. Three March males showed stage 2 spermatogenesis with primary spermatocytes as the most advanced sex cells; these males were the smallest (106, 107, 113 mm CL) in our samples. The other March males (112, 118, 119, 119 mm CL) showed a stage 6 condition with spermatids and spermatozoa bordering the lumina of the seminiferous tubules. Presumably the former group consisted of immature individuals because their tubule diameters were smaller ($\bar{x}$ = 126.2 vs. 193.0 μm, t = 3.06, P < 0.05) than those males with spermatozoa.

Testicular diameters and masses and seminiferous tubule diameters showed a cyclic pattern (Fig. 5). The general pattern of spermatogenesis matched that of testicular size with nearly continuous germ cell production, except for a brief resting period in February, then gradually intensifying

Table 2. Clutch size frequency as determined by the number of oviducal eggs and corpora lutea in *Claudius*. Corpora lutea were not recorded from all females from which oviducal eggs were measured.

Oviducal Eggs		Corpora Lutea	
Number per Clutch	Number of Females	Number per Clutch	Number of Females
1	6	1	8
2	27	2	20
3	19	3	13
4	4	4	3
5	2	5	2
		6	1
$\bar{x} \pm SD =$ 2.5 ± 0.090		2.4 ± 1.12	

through May and June. Spermiogenesis and spermatogenesis occurred at a high level from June through January.

DISCUSSION

The discovery of unburied clutches of eggs hidden in and among living and dead vegetation shows that the nesting behavior of *Claudius angustatus* lacks a preparatory digging phase and the resulting nest/egg cavity, similar to some other kinosternid turtles and a few other tropical turtles, such as *Rhinoclemmys* (Ernst and Barbour, 1989; Moll and Moll, 1990).

Our field censuses reveal that males outnumber females, 2.5:1. In contrast, egg clutches incubated in the laboratory yield a nearly 1:1 sex ratio (Vogt and Flores-Villela, 1992). Although this difference may be due to the census technique, most *C. angustatus* observed were captured, so there is no apparent bias in capture. Differences in behavior and activity levels between females and males could cause differential capture rates. It is also possible that males outnumber females in the adult population sampled.

Eggs and Nesting. — The nesting season in *Claudius angustatus* occurs from November to February, apparently with most nesting in November and early December, judging from the rapid decrease in the presence of oviducal eggs (Table 1). In 1982, we found three females with eggs in late September (as determined by palpation). This early oogonial maturation might have been stimulated by an unusual dry period (3 weeks in August) during the normally wet season. However, these specimens were not dissected or X-rayed to confirm the actual presence of oviducal eggs. The major nesting activity occurs in December and early January, as the major flooding of the streamside grasslands ends, although rain showers continue.

The duration of natural incubation remains unknown, although laboratory incubation at different temperatures yielded a range of 95–229 days ($\bar{x} = 194$ days, $n = 70$; Vogt and Flores-Villela, 1992; unpubl. data). Hausmann (1968) reported an incubation period of about 150 days (at 28°C) for eggs from captive animals. These two sets of observations suggest a long natural incubation period, comparable to that reported for other tropical kinosternids, e.g., 115–258 days for *Kinosternon scorpioides* or 122–293 days for *Staurotypus triporcatus* (Ewert, 1985: Table 6). The preceding taxa and other tropical kinosternids have an embryonic diapause (Ewert, 1985), and considering its long incubation period, *Claudius* probably does also.

Hausmann (1968) reported clutch sizes of 2–8 eggs. Table 3 shows that the reproductive parameters of *Claudius* match those of other comparable sized kinosternids.

Sexual Maturity. — Some females appear to attain sexual maturity at 89 mm CL and some males at 98 mm CL;

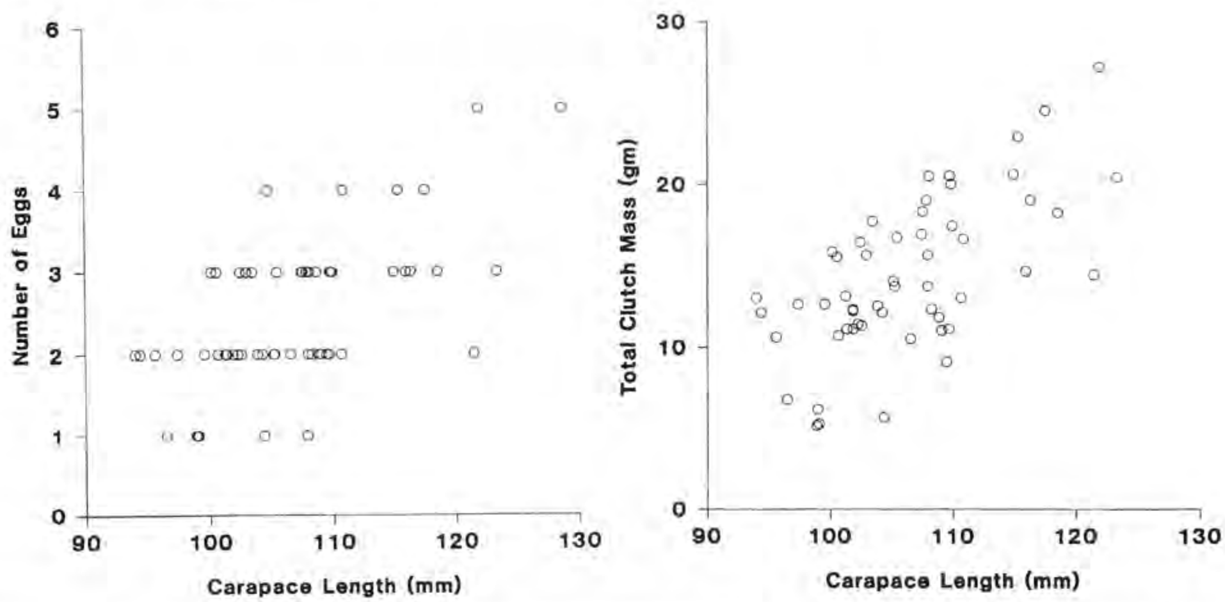

Figure 4. Relationship of clutch size to body size. The regression equations: number of eggs (y) to carapace length (x), $y = -5.59 + 0.08x$, $r = 0.62$, $P < 0.001$, $n = 58$; total clutch mass (y) to carapace length (x), $y = -32.49 + 0.44x$, $r = 0.65$, $P < 0.001$, $n = 54$).

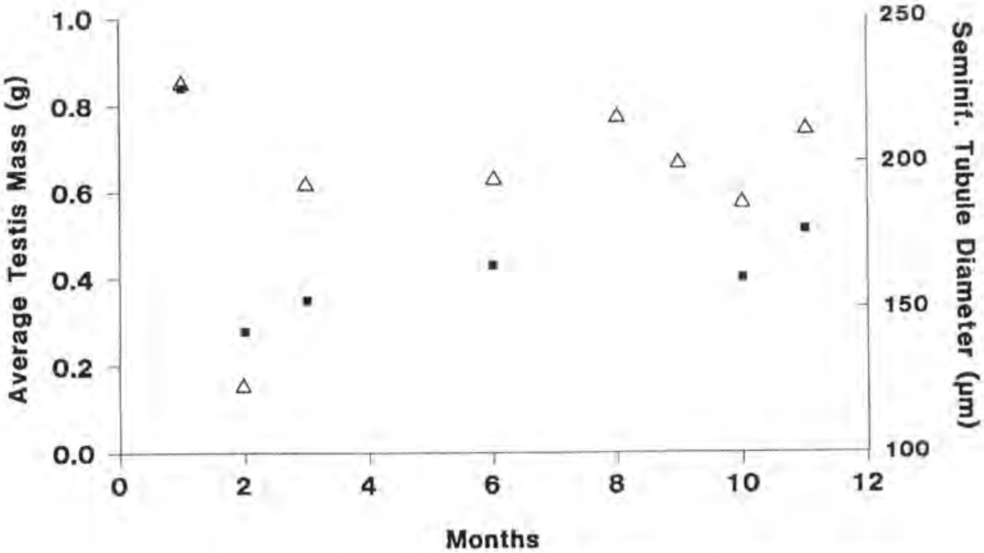

Figure 5. Spermatogenic activity reflected by the change in mean testicular mass (left axis, solid squares) and mean seminiferous tubule diameter (right axis, open triangles) during an annual cycle. Months, beginning with January, are designated numerically.

however, not all individuals of this size and larger were mature or at least reproductive. Female *Claudius* lacking mature follicles ranged from 93–139 mm CL. Whether these individuals were truly immature or their corpora lutea had disappeared (or were missed by the observer) and vitellogenesis had not yet begun is uncertain. All questionably immature females derived from summer (June–September) samples. Although it is unlikely that all individuals mature at the same size, a range of 20–30 mm (i.e., maturity attained between 90–115 mm CL) is larger than expected based on other studies (e.g., 10–15 mm CL range in *Sternotherus odoratus*; Mitchell, 1985). Three explanations are possible for nonreproductive females > 114 mm CL. 1) Females with small but vitellogenic follicles are mature and the corpora lutea had regressed or were still present but small and escaped observation. 2) Some females do not reproduce every year and remain in oogenic quiescence. 3) Adult-sized females with only previtellogenic follicles may be reproductively senescent. Two of the larger females (126, 139 mm CL) had only previtellogenic follicles and were from the September sample when all other adult females had large vitellogenic follicles; possibly, these females were senescent. Reproductive senescence is difficult to prove, and temporary gonadal involution can result from starvation, injury, or similar circumstances where energy resources must be redirected away from reproduction. Presumably males mature at < 98 mm CL, but our histological sample does not extend below that carapace length. Some larger males (to 114 mm CL) are immature, suggesting at least a 15 mm CL range over which maturity occurs in males.

Gametogenesis. — Combining these data with the presence of early and mid-vitellogenic follicles in July–September females suggests the following reproductive scenario. Follicular development begins in May and June, but growth is slow throughout the summer and early fall. Maturation and ovulation occur in late October and continue through early February, but in November most adult females are gravid. Many females will ovulate and deposit a second clutch in December–February. During March, April, and May, the ovaries rest and follicular growth is slow. The corpora lutea are slowly resorbed and disappear by August.

Table 3. Some reproductive parameters in selected species of warm-temperate to tropical kinosternid turtles (see Iverson et al., 1991, for data on other kinosternid species and populations).

Taxon	Size at Sexual Maturity (mm CL) female	male	Egg-Laying Season	Clutch Size (range/mode)	Number of Clutches/yr	Location	Source
Claudius							
angustatus	89	98	Nov-Feb	1-6 / 2	1-2	Veracruz	Present study
Kinosternon							
alamosae	100	–	Jul-Aug	3-5 / 4	1-?2	Sonora	Iverson, 1989
herrerai	116	–	–	2-4 / –	multiple	Tamaulipas	Carr and Mast, 1988
hirtipes	97	–	May-Sep	1-6 / 3	multiple	Chihuahua	Iverson et al., 1991
leucostomum	102	118	Continuous	1-2 / 1	multiple	Panama	Moll and Moll, 1990
Sternotherus							
odoratus	66	–	May-Jul	1-5 / 3	2-4	Alabama	McPherson and Marion, 1981

The preceding scenario predicts two egg clutches for many females. Double clutches are certainly possible in *Claudius* because most (> 90%) females in November and December have mature follicles ($\geq$ 15 mm diam.) and corpora lutea and/or oviducal eggs. These follicles are near ovulation size. Unfortunately, the absence of March–May samples does not allow us to determine the presence or absence of mature or atretic follicles immediately following the nesting period. Multiple clutching occurs in some kinosternids, e.g., Alabama *Sternotherus odoratus* with 2–4 clutches (McPherson and Marion, 1983), but not all, e.g., Iowa *Kinosternon flavescens* (Iverson, 1991) (also see Table 3).

Spermatogenesis is apparently continuous at the population level, since one or more individuals in each monthly sample (except in the small and peculiar October sample) displayed spermatogenesis and spermiogenesis. Individuals (adult-sized) show early stages of spermatogenesis in March and June. Perhaps males use a variety of spermatogenic behaviors during this post-nesting period: some continuing full spermatogenesis at a reduced rate; others producing primary spermatocytes that delay further meiotic maturation; and still others experiencing a full gametic resting phase. Our samples for this critical period are lacking or too small to provide an answer.

Acknowledgments

We thank the following individuals for their assistance with this study. Camilo and Agustin (fishermen of the Zubieta village near Lerdo de Tejada city), Miriam Benabib, Claudia Carnevale, Adrian Morales, Mardocheo Palma, and Richard Vogt assisted in gathering specimens and data in the field and laboratory. R. Vogt suggested, encouraged, and regularly offered advice during the field stage of this study. J.C. Mitchell and C.H. Ernst provided valuable suggestions for improving the manuscript.

OFV had a scholarship from Programa de Superación del Personal Académico, UNAM at the Instituto de Biología. During the final phase of this work OFV received support from the Dirección General de Asuntos del Personal Académico grant no. IN-201789 to J. Llorente and OFV. This support was greatly appreciated.

Resumen

Los machos adultos de *Claudius angustatus* alcanzan la madurez sexual aproximadamente a 98 mm de longitud del carapacho y son ligeramente de mayor talla que las hembras. Algunas hembras alcanzan la madurez sexual a los 89 mm de longitud del carapacho, aunque algunas entre 20 y 25 mm más grandes son inmaduras todavía. Las hembras grávidas y la anidación ocurren de noviembre a febrero. La puesta de huevos varía de 1 a 6 huevos elipsoidales ($\bar{x}$ = 17.5 x 30.5 mm, 6.0 g) con una masa total de la puesta de 5.2-27.2 g ($\bar{x}$ = 14.4 g). El número de huevos y la masa de la puesta está correlacionada positivamente con la longitud del carapacho. La espermiogénesis se presenta la mayor parte del año, siendo de noviembre a enero la época de mayor intensidad; la espermiogenésis parece ser continua en la población estudiada, aunque algunos individuos pueden presentar este fenómeno de forma discontinua con un periodo de descanzo entre febrero y mayo.

LITERATURE CITED

Alvarez del Toro, Miguel. 1982. Los Reptiles de Chiapas. Tercera Edición, corregida y aumentada. Tuxtla Gutiérrez, Chiapas: Publicaciones del Instituto de Historia Natural, 248 pp.

Carr, John L., and Mast, Roderic B. 1988. Natural history observations of *Kinosternon herrerai* (Testudines: Kinosternidae). Trianea 1:87-97.

Cope, Edward D. 1865. Third contribution to the herpetology of tropical America. Proc. Acad. Nat. Sci. Philad. 17:185-198.

Ernst, Carl H., and Barbour, Roger W. 1989. Turtles of the World. Washington, D.C.: Smithsonian Institution Press, 313 pp.

Ewert, Michael A. 1985. Embryology of turtles. In: Gans, C., Billett, F., and Maderson, P.F.A. (Eds.). Biology of the Reptilia. Vol. 14, Development A. New York: John Wiley and Sons, pp. 75-267.

Flores-Villela, Oscar A. 1980. Reptiles de importancia economica. Thesis, Faculdad de Ciencias, UNAM, México.

Hausmann, Peter. 1968. *Claudius angustatus*. Int. Turtle Tort. Soc. J. 2(3):14-15.

Iverson, John B. 1989. Natural history of the Alamos mud turtle, *Kinosternon alamosae* (Kinosternidae). Southw. Nat. 34(1):134-142.

Iverson, John B. 1991. Life history and demography of the yellow mud turtle, *Kinosternon flavescens*. Herpetologica 47(4):373-395.

Iverson, John B. 1992. A Revised Checklist with Distribution Maps of the Turtles of the World. Richmond, Indiana: Privately printed.

Iverson, John B., Barthelmess, Erika L., Smith, Geoffrey R., and de Rivera, Catherine E. 1991. Growth and reproduction in the mud turtle *Kinosternon hirtipes* in Chihuahua, México. J. Herpet. 25(1):64-72.

Iverson, John B., and Berry, James F. 1980. *Claudius* Cope narrow-bridged musk turtle. *Claudius angustatus* Cope narrow-bridged musk turtle. Cat. Amer. Amphib. Rept. 236.1-236.2.

Mayhew, Wilbur W., and Wright, Shirley J. 1970. Seasonal changes in testicular histology of three species of the lizard genus *Uma*. J. Morphol. 130:163-186.

McPherson, Roger J., and Marion, Ken R. 1981. The reproductive biology of female *Sternotherus odoratus* in an Alabama population. J. Herpetol. 15(4):389-396.

Mitchell, Joseph C. 1985. Female reproductive cycle and life history attributes in a Virginia population of stinkpot turtles, *Sternotherus odoratus*. Copeia 1985(4):941-949.

Moll, Don, and Moll, Edward O. 1990. The slider turtle in the Neotropics: Adaptation of a temperate species to a tropical environment. In: Gibbons, J.W. (Ed.). Life History and Ecology of the Slider Turtle. Washington, D.C.: Smithsonian Institution Press, pp. 152-161.

Smith, Hobart M., and Smith, Rozella B. 1979. Synopsis of the Herpetofauna of Mexico. Vol. VI. Guide to Mexican Turtles Bibliographic Addendum III. North Bennington, Vermont: John Johnson, 1044 pp.

Vogt, Richard C., and Flores-Villela, Oscar A. 1992. Effects of incubation temperature on sex determination in a community of neotropical freshwater turtles in southern Mexico. Herpetologica 48(3):265-270.

Accepted: 15 Oct 1994