



Reproductive Phenology, Inter-clutch Intervals, and Among-Year Clutch Frequencies of Two Freshwater Turtles Inhabiting an Urban Spring System

Francis L. Rose¹ and Ivana Mali²

¹Department of Biology, Texas State University, San Marcos, Texas 78666, USA (rosefrancis743@gmail.com)

²North Carolina State University, Fisheries, Wildlife, and Conservation Biology Program, Raleigh, North Carolina 27695, USA

Abstract.—We combined three separate sequential annual data sets (2008–2010) to document general phenology of nesting seasons, reproductive frequencies, and inter-clutch intervals (ICIs) of female *Pseudemys texana* (Texas River Cooter) and *Trachemys scripta* (Red-eared Sliders) occupying a spring system in Hays County, Texas. We detected gravid females by frequently monitoring nesting areas over three entire nesting seasons. Of the 108 *Pseudemys texana* that nested in the first year, 54 (50%) also nested in two subsequent years. Of the 65 *Trachemys scripta* that nested in the first year, only 13 (20%) nested in all three years. Percent of females that nested in only the first year was 10% and 19% for *P. texana* and *T. scripta*, respectively. Individuals that nested only once in a season, did so over all days in a season. Production of multiple clutches within a season was observed for both species. Within-year ICIs of females were approximately 30 days for both species and only about 30% of both species nested.

Chelonians are one of the longest-lived reptilian groups and also one of the most prolific. Evaluating and understanding the varied reproductive patterns of many species of turtles can be daunting and time consuming. Nonetheless, such understanding is important for developing sound management plans and for understanding species’ reproductive potentials, which is necessary for projecting population growth and stability. Comparisons of reproductive patterns of females within the same environments can also be helpful in understanding differences in life-history trait values. Clutch frequency is often the major determinant of variation in annual reproductive output among females and is the most difficult trait to determine in field studies of freshwater turtles (Gibbons et al. 1982). Whereas documenting within-year reproductive frequencies is difficult, obtaining empirically sufficient data on among-year variation in reproductive frequency remains unreported, even though it is necessary for determining the fecundities used in the calculations of life tables and construction of population and life-history models (Stearns 1992; Roff 1992, 2002).

Our current understanding is that some individuals of numerous freshwater turtle species produce multiple clutches in a year (e.g., Litzgus and Mousseau 2003; Wilkinson and Gibbons 2005; Buhlmann et al. 2009). In many species, clutch frequency varies within and among populations

(Gibbons et al. 1982; Iverson 1992; Iverson and Smith 1993; McGuire et al. 2011). Such variation is often linked to latitude in temperate areas, whereas clutch frequency increases with decreasing latitude (Litzgus and Mousseau 2003; Lee 2007). This pattern is associated with the shorter and milder winters in lower latitudes that enable longer productivity and more time for harvesting and processing resources that can be available for reproduction (Congdon et al. 1987; Doody et al. 2003).

The number of annual sequential reproductive events for individuals, however, is not well known due to the paucity of data for within- and among-year reproductive frequency over continuous multiple years. The proportion of adult females in a population that do not reproduce in any given year is extremely difficult to determine in field studies; however, some estimates have been made for the most-studied species (Frazer et al. 1990). Some species such as Madagascan Big-headed Turtles (*Erymnochelys madagascariensis*) and Pig-nosed Turtles (*Carettochelys insculpta*) exhibited biennial reproductive cycles, producing single or two clutches every other year, respectively (Kutchling 1993; Doody et al. 2003). To obtain meaningful data on annual reproductive output, accurate identifications of initiation and termination dates of nesting seasons have to be known from observations made during each day of entire nesting seasons over multiple years; a

unique mark or PIT-tag should individually identify females, and egg deposition of individuals should be confirmed or estimated accurately; and the number of females in a population must be sufficient for statistical analysis.

A community of freshwater turtles became available for study when Texas State University purchased and added Spring Lake to the main campus. Intense study began on the turtle community in 1996 (Rose 2011; Rose et al. 2020) and continues. Observations confirmed the presence of a high density of four species of turtles: *Pseudemys texana* (Texas River Cooter), *Trachemys scripta* (Red-eared Slider), *Chelydra serpentina* (Common Snapping Turtle), and *Sternotherus odoratus* (Common Musk Turtle), and to date, over 6,000 individuals have been marked. The study site has several features that favored research on turtle reproductive phenology. The area was protected after it was incorporated into the main university campus. Stable thermal spring flow ensured annual turtle activity, which allowed females to sequester nutrition throughout the year. A golf course surrounded substantial segments of shorelines where turtles egressed to nest (Rose 2011; Mali et al. 2014; Rose et al. 2020).

We combined data from three intensive turtle nesting studies in 2008 (Rose 2011); 2009 (Mali et al. 2014), and 2010 (FLR, unpubl. data) of *P. texana* and *T. scripta*, the two most abundant turtles readily found nesting. The total number of each species recorded while nesting allowed us to evaluate and describe the general reproductive phenology of *P. texana* and *T. scripta* throughout the three nesting seasons, provided evidence of timing of single and multiple nesting events, and made possible the determination of inter-clutch intervals (ICIs) within a nesting season. The data also allowed us to study inter-annual reproductive frequencies of both species of turtles and assess in how many of the three years individual females reproduced.

Methods

Turtles were studied at Spring Lake (Coley 2016), headwaters of the San Marcos River, Hays County, Texas. More than two hundred springs emanate from the Edwards Aquifer in the 600-m spring-run, which was dammed in the 1840s to produce Spring Lake (Rose 2011). An 880-m lentic slough is confluent with the main lake at about its halfway point. Water temperature of the spring-run was 22 ± 2 °C (Groeger et al. 1997), but water temperature of the slough varied seasonally (Rose 2011). During the three studies, a manicured golf course and recreational fields bordered the slough. An entry road crossing the slough physically divided the habitat into four nesting areas. Turtles exited the water along the entire length of the slough to access the golf course. See the study site map and descriptions of the site in Rose et al. (2020).

Pseudemys texana is an emydid riverine turtle inhabiting five river drainages in Texas (Lindeman 2007; Dixon 2013). In general, riverine turtles inhabit large flowing bodies of water that are open-ended (Moll and Moll 2000). Turtles associated with this habitat are difficult to study because of the lack of physical environmental boundaries. In addition, such habitats are subjected to floods, perhaps altering population and community compositions. Spring Lake is an exceptional locality because it serves as an impoundment of the headwaters of a river and is delineated by shorelines, a headwall, and a dam. In contrast, *T. scripta* is one of the most widely distributed and studied aquatic turtle species in North America (Gibbons 1990), whereas *P. texana* is among the least studied (Lovich and Ennen 2013). As of 30 May 2020, 2,082 *P. texana* and 1,199 *T. scripta* were marked at Spring Lake. The comparison of reproductive activities of two species inhabiting the same environment at the same time gave us a unique opportunity to evaluate interspecific variances.

Observations from previous years at the study area confirmed that *P. texana* and *T. scripta* nested diurnally and exhibited fidelity within and among years to specific nesting areas (Rose et al. 2020). Members of both species moved onto the golf course and initiated nest chamber construction in open areas, with mean distances from water ranging from 88 to 100 m (Washington 2008; Rose 2011; Mali et al. 2014). We searched for turtles with the aid of a utility cart when they came out onto the golf course to nest. Grounds personnel, golfers, park visitors, and students involved with various research projects notified us of turtles on land. From the first observations of nesting turtles until 14 July, the average last documented date of nesting, we patrolled quadrants at least every 2 h from 0700 until 2100 h. Two-hour survey intervals allowed us to efficiently intercept females, knowing that it takes a turtle about 2.5–3 h to exit the water, construct a nest cavity, deposit eggs, cover them, and return to the water. Post-nesting females were transported to the biology field lab, where they were measured and weighed. If a PIT-tag (American Veterinary Identification Devices, Norco, California) was not present, one was inserted subcutaneously in the turtle's right forearm. Females were released along the shore nearest to their nest.

The start dates for the three years of the study periods were 23 April 2008, 8 April 2009, and 1 April 2010 and are based on observing a minimum of two females of either species constructing nests in one day. Starting dates were standardized to compare daily nesting activity over the three nesting seasons. Although the start dates varied yearly, the nesting seasons of both species were simultaneous. To compare within-year ICIs between the first two clutches and second and third clutch, we performed a *t*-test using Prism 8GPS-1639614-EKRU-E4697. Data are presented as means \pm standard deviation (min-max).

Results

Five hundred and four *P. texana* (Fig. 1) reproduced over all three seasons. Of these, 162 (32.1%) reproduced twice in a single season, 29 (5.8%) three times, and two (0.4%) four times. Females that reproduced only once (311; 61.7%) did so at any time from early to late in a nesting season.

Two hundred and sixty-five female *T. scripta* (Fig. 1) reproduced in three seasons. Of these, 63 (23.8%) reproduced twice in a single season and 14 (5.3%) reproduced three times. Females that reproduced only once (188; 70.9%) did so at any time within the season but 78.5% did so within the first 59 days. No *T. scripta* produced four clutches during a single season, but several individuals did so in previous years.

The mean number of days between first and second clutches of *P. texana* was 28 ± 14 (17–81) and between second and third clutches it was 30 ± 9 (18–55). The difference between mean intervals was not significant ($P = 0.32$, $t = 0.98$, $df = 186$). The mean number of days between the first and second clutch of *T. scripta* was 34 ± 13 (16–72) and between the second and third clutches 29 ± 8 (17–43). The difference between mean intervals was not significant ($P = 0.21$, $t = 1.2$, $df = 82$).

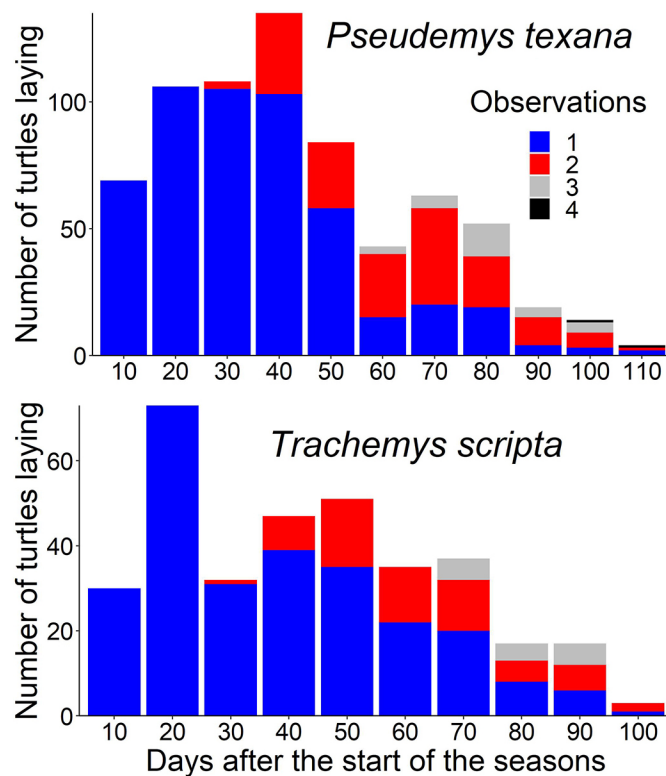


Figure 1. Reproductive sequences of 504 *Pseudemys texana* and 265 *Trachemys scripta* at Spring Lake, Hays County, Texas, from 2008 to 2010, grouped by clutch number (i.e., observations). For example, Observation 4 indicates turtles that laid their fourth clutch within a season. The initiations of the three laying seasons were adjusted to standardize comparisons.

Table 1. Number and percent of *Pseudemys texana* (108) and *Trachemys scripta* (65) producing from 0–4 clutches annually at Spring Lake, Hays County, Texas, from 2008 to 2010. To account for possible mortality, we included only turtles that were known to be alive in 2010 or later.

Years	<i>Pseudemys texana</i>		<i>Trachemys scripta</i>	
	N	Percent	N	Percent
2008	108		65	
2008 only	11	10	12	19
2008/2009/2010	54	50	13	20
2008/2009	34	32	30	46
2008/2010	9	8	10	15

Inter-clutch intervals among years.—To adjust for mortality, we assessed only females that nested in 2008 and were accounted for in the system (i.e., known to be alive) in 2010 or later. Of 108 *P. texana* that nested in 2008, 50% nested in both subsequent years whereas only 11 (10%) did not nest in either of the subsequent years (Table 1). The rest (39%) nested either in 2009 or 2010. Of 65 *T. scripta* that nested in 2008, 13 (20%) nested in both subsequent years whereas 12 (19%) did not nest in either subsequent year. The rest (61%) nested either in 2009 or 2010 (Table 1). A substantial number of females did not reproduce within a given season. Of the total *P. texana* and *T. scripta* that laid eggs over the three years, the mean numbers of gravid females per year were 168 and 80, respectively.

Discussion

We documented annual multiple clutches for female *P. texana* and *T. scripta* using three sequential years of observation. Although multiple within-season clutches were produced by females of both species, no clear pattern between the season's clutch production was evident in either species, and approximately 30% of females skipped reproduction for one or more years, proportions that were surprisingly higher than expected for long-lived and multiple-clutch producing species. However, the proportions of adult females that skipped reproduction reported here are similar to reports of Tinkle et al. (1981) and McGuire et al. (2011) for *Chrysemys picta* (2–3 clutches per year), Congdon (1989) for *Chelydra serpentina* (single clutch per year), and Frazer et al. (1990) for *T. scripta* (2 clutches per year).

Our knowledge of multiple annual clutches by freshwater turtles progressed from suggestions (Cagle 1954; Powell 1967; Moll 1979), to necropsies (Gibbons 1968), observations (Snow 1980), and X-radiography to the confirmation and clarification of its importance regarding the construction of life tables (Tinkle et al. 1981). Sequentially, studies and observations of multiple clutches per year were documented

for many wild and maintained turtles (Lee 2007). Tinkle et al. (1981), however, pointed out that most Painted Turtles in their study did not nest every year although studies of two other species documented laying every two years (Kutchling 1993; Doody et al. 2003).

Our anecdotal observations over many years suggested that within-season first clutches were laid early in the nesting season along with first clutches of females that laid multiple clutches. That view was altered upon observing the large number of first-time layers in the 40–100 day categories in our three-year data sets for *P. texana* and *T. scripta* (i.e., among females, first clutch nests were constructed during entire nesting seasons). Distributing annual egg production in multiple nests at different times and places may increase the probability of hatchling survival (Lee 2007; Refsnider and Janzen 2010). At Spring Lake, however, increased nest density increases predation by Raccoons (*Procyon lotor*) and imported Red Fire Ants (*Solenopsis invicta*). In addition, remaining to be determined is whether high numbers of clutches (i.e., 3 or 4) in a season decreases reproductive output in the following season(s). Annual and decadal variation in environmental conditions and predator types and densities render documentation of the relative success of each nest and resulting hatchling recruitment difficult.

Accurate ICIs obtained by frequent monitoring of nesting areas can usually detect missed nesting events because that would essentially double an ICI (e.g., a first to third clutch). Jackson and Walker (1997) speculated that Suwannee Cooters (*Pseudemys suwanniensis*) might lay up to nine clutches per year (ICI 35–50 or 55–75 days/mean ICI), a calculation that assumes no among-female differences in suites of developing follicles and resource acquisition histories. Inter-clutch intervals at Spring Lake for the second clutches of *P. texana* and *T. scripta* were 33 days (17–81) and 34 days (16–74), respectively. Mean intervals between second and third clutches were 29 and 30 days for *P. texana* and *T. scripta*, respectively. Clutch intervals of both species were similar between all clutches. In contrast, Tucker (2001) reported overall ICIs of 6–41 days for *T. scripta* in Illinois.

Although some females at Spring Lake nested in three consecutive years, the percentage was fewer than expected (50% for *P. texana* and 20% for *T. scripta*), but even fewer nested in years one and three (7.1% for *P. texana* and 12.2% for *T. scripta*). That females producing three or four clutches in a year may have insufficient time to sequester sufficient energy for the maturation of another clutch of average size in the following nesting season is a reasonable expectation. However, our current three-year data set did not reveal any patterns (i.e., some multi-clutch females nested the following season and some did not). The costs of reproductive activities, distance and duration of nesting migrations, and durations of nest construction do not represent a large part of a female's total energy

budget but can expose females to environmental extremes and encounters with predators that might be negative.

Assuming a nesting season of 100 days at our study site, sufficient time is available for females to produce a maximum of five clutches. A maximum of four clutches were rarely observed in either species even though turtles in Spring Lake are active and harvest resources throughout the year. At higher latitudes, winter inactivity reduces the time available to harvest resources, as a result clutch frequency is probably lower, and ICIs may be shorter (Litzgus and Mousseau 2003).

In summary, this study significantly contributes to our understanding of multi-seasonal reproductive patterns of emydid freshwater turtles. We demonstrated that *P. texana* and *T. scripta* showed differences and similarities in aspects of their reproductive phenology that suggest levels of investment in reproduction (e.g., multiple clutches) in the prior year and the time necessary to mature, ovulate, and shell eggs between clutches are important in setting the maximum number of clutches per year. The magnitude of the variation in females that reproduced and did not reproduce should be well documented when incorporating a single or a range of fecundities into population and life history models. For models to be helpful to conservation biologists and planners, empirical data spanning more than one year to bracket realistic variation in population models designed for future population trends is essential.

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