REPRODUCTIVE DEMOGRAPHY OF TWO CLOSELY RELATED EMYDINE TURTLES IN A SPRING FED SYSTEM

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ABSTRACT—Freshwater turtles are appropriate organisms for studying maternal investment in offspring because, unlike most long-lived vertebrates, turtles show high fecundities and most provide no parental care. We studied reproductive patterns of two emydine freshwater turtle species, the Texas river cooter (*Pseudemys texana*) and the red-eared slider (*Trachemys scripta elegans*) at Spring Lake, Hays County, Texas during the 2009 nesting season. Forty-six percent of all nesting Texas river cooters and 25% of all nesting red-eared sliders nested twice, with some Texas river cooters nesting more than twice. Mean egg mass, egg length, and egg width decreased in subsequent clutches in Texas river cooters. However, there was an insufficient sample size of subsequent clutches ($n = 6$) to draw conclusions for red-eared sliders. Red-eared sliders did not show a positive relationship between clutch size and body size. We found that in both species there was a positive relationship between egg width and egg mass in relation to maternal body size. However, only Texas river cooters showed a positive relationship between clutch size and maternal size, while only red-eared sliders showed a positive relationship between egg length and maternal size. By comparing reproductive parameters of these two coexisting populations, we concluded that the members of these two species allocate resources differently for reproduction.

RESUMEN—Las tortugas de agua dulce son organismos apropiados para el estudio de la inversión energética materna en las crías porque, a diferencia de la mayoría de vertebrados de larga vida, las tortugas muestran altos niveles de fecundidad y no proveen cuidado de los padres. Se estudiaron los patrones reproductivos de dos especies de tortugas de agua dulce de la familia Emydidae, la tortuga galápago de Texas (*Pseudemys texana*) y tortuga de orejas rojas (*Trachemys scripta elegans*) en Spring Lake, condado de Hays, Texas, durante la temporada de anidación en el 2009. El 46% de las tortugas galápago de Texas reproduciéndose y el 25% de las tortugas de orejas rojas reproduciéndose anidaron dos veces, con algunas tortugas galápago de Texas anidando más de dos veces. El promedio de la masa, longitud y ancho de los huevos en las tortugas galápago de Texas disminuyó sucesivamente en cada puesta. Sin embargo las muestras de las puestas subsecuentes para las tortugas de orejas rojas ($n = 6$) no fueron suficientes para inferir conclusiones posteriores. No se mostró una relación positiva entre el tamaño de la puesta y el tamaño de la hembra para las tortugas de orejas rojas. Encontramos que en ambas especies existió una relación positiva entre el ancho y la masa del huevo en relación con el tamaño de la madre. Sólo en las tortugas galápago de Texas hubo una relación positiva entre el tamaño de la puesta y el tamaño de la hembra, mientras que sólo en las tortugas de orejas rojas hubo una relación positiva entre la longitud del huevo y el tamaño de la hembra. Mediante la comparación de parámetros reproductivos de estas dos poblaciones coexistentes, concluimos que los miembros de estas dos especies asignan los recursos para la reproducción de maneras diferentes.

Turtles are appropriate vertebrates for investigating maternal investment in offspring because they are long-lived, have high fecundities and, with the exceptions of few species (see Agha et al., 2013), do not exhibit parental care. Female energy investment in egg laying can be directly measured by number of eggs produced, egg size, egg mass (Congdon and Gibbons, 1987; Rowe, 1995), and number of clutches produced during a single nesting season (Litzgus and Mousseau, 2003; Lee, 2007). Strategies related to parental investment are varied among turtle species.

All clutch variables (clutch size, egg size, and egg mass) increase in size with maternal body size in red-eared sliders (*Trachemys scripta elegans*), snapping turtles (*Chelydra serpentina*), painted turtles (*Chrysemys picta*), and Amazonian giant river turtles (*Podocnemis expansa*) (Rowe, 1994; Iverson et al., 1997; Tucker et al., 1998; Valenzuela, 2001). However, this trend is not universal. Chicken turtles (*Deirochelys reticularia*) and southern spotted turtles (*Clemmys guttata*) increase egg size but not clutch size with an increase in maternal body size (Congdon et al., 1983; Litzgus and Mousseau, 2006). Western box turtles
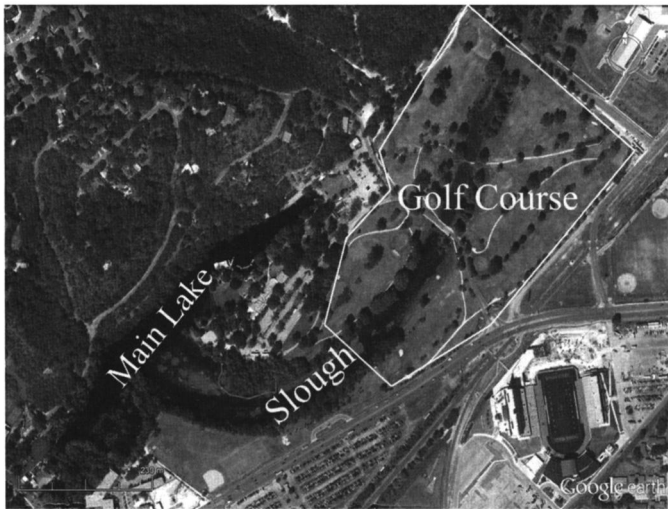
Fig. 1—Aerial view of Spring Lake (Hays County, Texas), the slough, and the golf course surrounding the slough. During the 2009 nesting season, we collected female Texas river cooters (*Pseudemys texana*), red-eared sliders (*Trachemys scripta elegans*), and their eggs primarily from the golf course.

(*Terrapene luteola*) increase clutch size rather than egg size (Nieuwolt-Dacanay, 1997). In addition to species-specific variation in reproductive output, variations between populations of the same species have been reported. For example, some populations of red-eared sliders are known to only increase egg size but not the clutch size with an increase of female body size (Mitchell and Pague, 1990).

Laying more than one clutch during a single nesting season is a common event in several species of freshwater turtles (Lee, 2007). Laying multiple clutches is believed to decrease the risk of losing the reproductive output of an entire season by separating clutches in time and space (Harless and Morlock, 1979; Lee, 2007). Similar to other clutch variables, the clutch frequency within the nesting season may vary among individuals within and among populations (Tinkle et al., 1981; Gibbons et al., 1982; Iverson, 1992; Iverson and Smith, 1993). Several studies examined differences in clutch variables between subsequent clutches. In yellow-bellied sliders (*Trachemys scripta scripta*), Florida cooters (*Pseudemys floridana*), and common mud turtles (*Kinosternon subrubrum*), last clutches have fewer eggs than clutches laid earlier (Gibbons et al., 1982). However, clutch size does not always decrease with clutch number. In southern spotted turtles, the number of eggs in second clutches is greater than that of the first (Litzgus and Mousseau, 2003), while for painted turtles first and second clutches are similar in size (Gibbons, 1968).

Reproductive variation is sometimes linked to geographic distribution for the populations of the same species. Clutch size and clutch frequency vary with latitude, with clutch size increasing at higher latitudes and frequency increasing with decreasing latitude (Litz-

gus and Mousseau, 2003; Lee, 2007). High-latitude populations have shorter nesting seasons and may lay only one large clutch, whereas low-latitude populations have fewer time constraints and may lay multiple clutches in a single year (Gibbons, 1983; Litzgus and Mousseau, 2006). Clutch frequency also varies annually in individuals as well as in populations and, therefore, may be the key variable which controls overall annual reproductive output (Gibbons et al., 1982).

Understanding relative reproductive output is fundamental to understanding the demography of a population, and species- and population-specific studies must be conducted to obtain insight into reproductive behavior and resource allocation. Comparative data are crucial to understanding and development of life history theories and models. Detailed local data sets are beneficial for comparisons to allopatric populations of the same species (and closely related species) inhabiting similar and different environments.

In this study, we evaluated the relationships between female size, clutch frequency, clutch size, and mean egg size per clutch in two emydine turtles, red-eared sliders and Texas river cooters (*Pseudemys texana*) inhabiting Spring Lake, Hays County, Texas. Additionally, we evaluated whether clutch variables changed with secondary laying bouts within the same season. Previous observations (FLR, pers. obser.) indicated that red-eared sliders laid fewer and smaller eggs than did Texas river cooters, which are larger than red-eared sliders. While the red-eared slider is among the most-studied species in North America (Gibbons, 1990), with known variation in reproductive output among different populations, little is known about the reproductive demography of the Texas river cooter. Thus far, researchers have looked at egg shape and volume (Rose et al., 1996), clutch frequency (Rose, 2011), and clutch-egg size (Lindeman, 2007), but in the primary literature there are no studies on reproductive output in relation to body size. At our study site we had an opportunity to make direct comparisons between two closely related species with similar life histories without concern about geographical variation because both populations occupied the same area and habitat. We therefore expected similar patterns in the reproductive output for both species.

MATERIALS AND METHODS—*Study Area*—Our study site was Spring Lake (29°53′N, 97°55′W) on the campus of Texas State University, Hays County, Texas. The San Marcos Springs (>200 springs emanating from the Edwards Aquifer at the Balcones Fault) are the source of the San Marcos River, which was initially dammed in 1849 (Fields et al., 2003), leading to the formation of Spring Lake, an 8-ha reservoir (Fig. 1). The site is composed of the main lake and a static slough that is confluent with the lake at its midpoint (Rose, 2011). A golf course and recreational fields border the slough. We collected nesting females and their eggs for analysis from the golf course.

Data Collection—We visited the study site daily during the nesting season (Rose, 2011), from 1 April to 1 August 2009, searching for nesting turtles and collecting eggs for measurements. We observed nesting females from 0730 to 2100h daylight savings time. Most turtles were previously marked for ongoing research projects. Nesting turtles were additionally marked with yellow spray paint on the carapace for uncomplicated visual recognition during subsequent nesting attempts (Rose, 2011).

After nesting we measured plastron length, carapace length, and carapace width to the nearest 1.0 mm using tree calipers (Haglof, Madison, Mississippi). We determined female mass to the nearest 10 g using spring scales (Pesola, Baar, Switzerland). Eggs were removed from the nest and transported to a laboratory for measuring and incubating. We counted the number of eggs per clutch, determined egg length and width to the nearest 0.1 mm using a digital caliper (Digimatic®, Mitutoyo, Aurora, Illinois) and determined egg mass using a digital balance scale (Ohaus Pro, Parsippany, New Jersey).

Statistical Analyses—We used female plastron length as our measure of body size in all statistical analyses. We used simple linear regression to define the relationship between plastron length and clutch size, mean egg width per clutch, and mean egg length per clutch for each species. In addition, we conducted analysis of covariance (Type III) to determine if clutch parameters differed between the two species, taking into account differences in female size. This allowed us to directly test whether or not the two species followed the same patterns in terms of allocating energy toward reproduction. For females that nested more than once, we used the measurements of the first clutch. Specifically, we used females that laid their first clutch prior to 8 May 2009.

We used paired t-tests to address the differences between the first and second clutches (mean egg size and mean number of eggs) and logistic regression to determine the relationship between female body size and clutch frequency. We inferred significance at $\alpha = 0.05$. All analyses were performed using the program R (R Development Core Team, 2008).

RESULTS—We collected eggs of 82 Texas river cooters and 24 red-eared sliders from 1 April to 7 May 2009. Of the 38 (46%) female Texas river cooters found nesting more than once, 30 nested twice, five nested three times, two nested four times, and one nested five times. Six red-eared sliders nested twice (25%), and none nested more than twice. The first nesting female of both species was found in the first week of April with the peak nesting activity occurring in May. The internesting period ranged from 18–66 days for Texas river cooters and 22–63 days for red-eared sliders. Means of measurements taken showed that Texas river cooters were larger than red-eared sliders, and the values for all clutch variables were larger in Texas river cooters (Fig. 2).

We found significant relationships between plastron length and most of the clutch variables in both species (Table 1; Fig. 2). There was no trend between body size and clutch size in red-eared sliders ($F_{1,21} = 0.30$, $P = 0.59$); however, both egg width and egg length increased ($F_{1,21} = 15.35$, $P < 0.01$ and $F_{1,21} = 6.76$, $P = 0.02$,

respectively) with body size. Larger Texas river cooters produced larger clutch sizes ($F_{1,74} = 11.60$, $P < 0.01$), as well as wider eggs ($F_{1,74} = 14.67$, $P < 0.01$), but not longer eggs ($F_{1,74} = 1.11$, $P = 0.27$). None of the statistically significant regressions had strong relationships (all R^2 values <0.47). There were different relationships of plastron length with clutch size ($F_1 = 3.51$, $P = 0.06$) and mean egg length ($F_1 = 3.63$, $P = 0.06$) between species, with marginal statistical support (Table 2; Fig. 2). There was no relationship difference between species regarding plastron length with mean egg width and mean egg mass.

There was no relationship between plastron length and clutch frequency for Texas river cooters ($\chi^2_{1,65} = 1.21$, $P = 0.27$). For comparison of variables for first and second clutches in Texas river cooters, three variables decreased significantly with the second clutch; mean egg length ($t_{27} = 6.42$, $P < 0.01$), mean egg width ($t_{27} = 4.64$, $P < 0.01$), and mean egg mass ($t_{27} = 4.83$, $P < 0.01$). There was no difference in clutch size between the first and the second clutches ($t_{27} = 0.28$, $P = 0.55$). We did not have an adequate sample size ($n = 6$) to conduct comparisons between the first and the second clutches for red-eared sliders.

DISCUSSION—We analyzed reproductive outputs of two closely related emydine turtles occupying the same habitat. Previous studies on sliders and cooters reported positive relationships between female body size and all clutch parameters such as clutch size and egg size (Gibbons and Greene, 1990; Tucker et al., 1998; Aresco, 2004). Red-eared sliders in our study did not produce larger clutches with increased body size, but both egg width and egg length increased (Fig. 2). Larger Texas river cooters increased clutch size, as well as egg width, but not egg length. Because both species showed a strong relationship of body size to egg width and the relationship did not differ between species, this might indicate that both species show some constraint on the diameter of the pelvic aperture. In addition, Texas river cooters laid multiple clutches more often than did red-eared sliders. In Texas river cooters, later clutches were generally smaller than first clutches, which is consistent with previous studies on emydine turtles (Rollinson and Brooks, 2008; McGuire et al., 2011). Although red-eared sliders did not lay multiple clutches as often as did Texas river cooters, they did lay multiple clutches more often than previously reported by Frazer et al. (1990; 9.9%) and Tucker (2001; 8.1%).

Our results indicate that the two study species show differences in allocating resources for reproduction, specifically in relation to clutch size and egg length (Table 2). On the other hand Aresco (2004), who studied reproductive outputs of red-eared sliders and Florida cooters living in the same habitat, found that both species increased egg size (width and length) and clutch size with
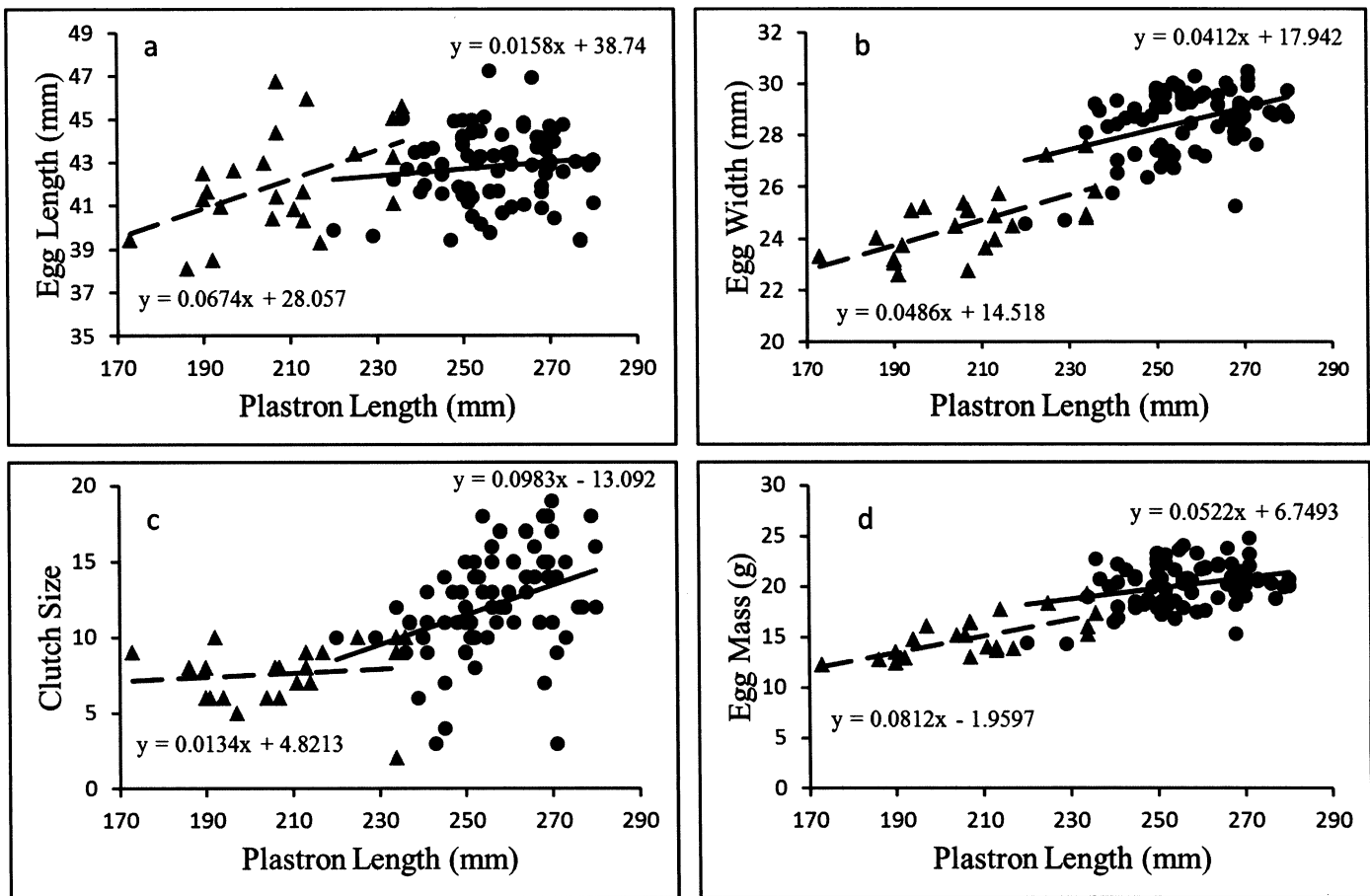
Fig. 2—Mean egg length (a), mean egg width (b), clutch size (c), and mean egg mass (d) as a function of plastron length in two species of turtles, Texas river cooter *Pseudemys texana* (—•—) and red-eared slider *Trachemys scripta elegans* (- -▲- -), demonstrating similar slopes for mean egg width and mean egg mass and different slopes for mean egg length and clutch size between the two species.

maternal body size. Intra- and interspecies interactions could influence reproductive outputs of coexisting populations. For example, competition for resources may affect growth rates as well as reproductive traits. Juveniles of both species are omnivorous but, as adults, Texas river cooters are primarily herbivorous while red-eared sliders remain omnivorous (Fields et al., 2003; Lindeman, 2007; Piña, 2012). Aresco (2010) reported that under low-resource conditions due to competition,

red-eared sliders grew more slowly with conspecifics than did Florida cooters, which grew at the same rate with both red-eared sliders and conspecifics. The study of Aresco (2010) was conducted in a closely controlled environment and, therefore, it is uncertain if similar dynamics are occurring at Spring Lake. However, it is worth exploring a relationship between environmental quality and intra- and interspecies competition and how these interactions

TABLE 1—The results of simple linear regressions determining relationships between female size (plastron length) and clutch variables in two species of emydine turtles, red-eared sliders (*Trachemys scripta elegans*) and Texas river cooters (*Pseudemys texana*), inhabiting Spring Lake, Hays County, Texas. While most clutch variables increased with the increase in maternal body size, Texas river cooters did not show a positive relationship between egg length and maternal size, while red-eared sliders did not show a positive relationship between egg clutch size and maternal size.

	Trachemys scripta elegans					*Pseudemys texana*				
Variable	F	df	SE	P	R^2	F	df	SE	P	R^2
Clutch size	0.30	1,21	1.98	0.59	0.01	11.60	1,74	3.17	<0.01	0.12
Egg length	6.76	1,21	2.09	0.02	0.21	1.11	1,74	1.66	0.27	0.01
Egg width	15.35	1,21	1.00	<0.01	0.39	14.67	1,74	1.18	<0.01	0.15
Egg mass	20.1	1,21	1.46	<0.01	0.46	7.25	1,74	2.13	<0.01	0.08

TABLE 2—The results of analysis of covariance (Type III) determining if clutch variables differed between the two species of freshwater turtles (red-eared sliders and Texas river cooters, *Trachemys scripta elegans* and *Pseudemys texana*, respectively), taking into account differences in female size (interaction effects were not significant). Species had different relationships of plastron length with clutch size and mean egg length, with marginal statistical support. Species did not differ in their relationship of plastron length with mean egg width and mean egg mass.

Parameters	Clutch size			Egg length			Egg width			Egg mass		
	F	df	P	F	df	P	F	df	P	F	df	P
Plastron length	0.13	1	0.77	9.54	1	<0.01	11.73	1	<0.01	0.70	1	<0.01
Species	3.04	1	0.08	3.03	1	0.08	0.74	1	0.34	1.56	1	0.22
Plastron:species	3.51	1	0.06	3.63	1	0.06	0.17	1	0.68	0.88	1	0.35

affect reproductive output as measured by clutch sizes and intraseasonal clutch frequencies.

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