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Egg and clutch sizes of western chicken turtles (*Deirochelys reticularia miaria*)

Donald T. McKnight[1,2,*], Ethan C. Hollender[1], Hunter J. Howell[3], John L. Carr[4], Kurt A. Buhlmann[5], Day B. Ligon[1]

[1] *Department of Biology, Missouri State University, Springfield, Missouri, USA. *Corresponding author.* Email: donald.mcknight@my.jcu.edu.au
[2] *College of Science and Engineering, James Cook University, Townsville, Queensland, Australia*
[3] *Department of Biology, University of Miami, Coral Gables, Florida, USA*
[4] *Department of Biology, University of Louisiana at Monroe, Louisiana, USA*
[5] *Savannah River Ecology Laboratory, University of Georgia, Aiken, South Carolina, USA*

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Abstract. Chicken turtles (*Deirochelys reticularia*) are generally characterized as having atypical reproductive characteristics relative to other sympatric emydids. However, the comparatively understudied western chicken turtle (*D. r. miaria*) has been shown to exhibit some reproductive characteristics that differ from the other subspecies. Therefore, we examined clutch and egg sizes from six *D. r. miaria* (13 clutches) in Oklahoma and compared the results to values that have been reported for the other two subspecies. Females nested up to three times per year, with clutches ranging from 8-13 eggs per clutch (mean = 10.9). The eggs were 19.4-25.3 mm wide (mean = 22.2 mm). These values are greater than the means reported for the other subspecies, but the differences were not statistically significant.

Keywords. Chelonia, Emydidae, subspecies, reproduction, geographic variation.

Chicken turtles (*Deirochelys reticularia*) occur in shallow swamps and ponds throughout the southeastern United States, and they exhibit several traits that are uncommon for North American turtles, the most noteworthy of which relate to their unusual reproductive patterns. The eastern chicken turtle (*D. r. reticularia*) is the most studied of the three recognized subspecies, and, unlike most North American turtles, females nest in the fall through early spring (Gibbons and Greene, 1990; Buhlmann et al., 2009). Eggs deposited in the ground in the fall enter embryonic diapause and resume development in spring after a period of chilling (Ewert et al., 2006). Additionally, when conditions become unfavourable (too cold) in the fall, females can retain eggs, overwinter with them, and lay them the following spring (Buhlmann et al., 1995, 2009). This behavior is uncommon in turtles but has also been reported for the Sonoran Desert tortoise (*Gopherus morafkai*) in Arizona, USA (Lovich et al., 2017). Florida chicken turtles (*D. r. chrysea*) extend this strategy by nesting throughout the winter and laying up to three clutches (rarely four) per year (Iverson, 1977; Jackson, 1988). In contrast, the western subspecies (*D. r. miaria*) nests in the late spring and summer (May-July), a pattern that is more consistent with other sympatric emydids (McKnight et al., 2015a; Carr and Tolson, 2018). However, this subspecies is the least-studied of the three, and although current research indicates that it differs from the other subspecies in diet, seasonal activity patterns, and some reproductive patterns (McKnight et al., 2015a, b), there is still a great deal that we do not know about its life history. One of these knowledge gaps is a dearth of information about their clutch and egg sizes. Currently, clutch and egg

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sizes for *D. r. miaria* are only reported from five clutches in Louisiana (Carr and Tolson, 2018), three clutches in Arkansas (Dinkelacker and Hilzinger, 2014) and one in Texas (David, 1975).

To expand our knowledge of this topic, we captured *D. r. miaria* in shallow beaver ponds and similar habitats in southeastern Oklahoma from 2013-2015 using a variety of hoop nets (detailed trapping methods available in McKnight et al. 2015c). We also attached radio transmitters (RI-2B 10 g or RI-2B 15 g, Holohil Systems Ltd., Corp., Ontario, Canada) to the carapaces of three females and recaptured them approximately every two weeks. Upon capture (via trapping or tracking) we used a portable ultrasound (Echo Camera SSD-500V, Hitachi Aloka Medical, Inc., Tokyo, Japan) to check for the presence of follicles or shelled eggs. If shelled eggs were observed, we immediately transported the turtles to a nearby veterinary clinic and used X-radiography to record the size and number of eggs (Gibbons and Greene, 1979). We included a coin or other object of known size to correct for magnification from the X-radiograph (Graham and Petokas, 1989). We counted the eggs and measured their width, but we did not measure their length, because length is only accurate when the eggs are oriented parallel with the plastron (Bertolero et al., 2007), a position that cannot be confirmed in a live animal.

Additionally, we included data from a female that was captured in southeastern Oklahoma on 16 June 2008 and transported to the Tulsa Zoo. Shelled eggs were observed via X-radiography on 9 July 2008, and 11 eggs were laid on 20 July 2008, following induction using oxytocin (Ewert and Legler, 1978; a single egg was laid and crushed on the previous day). These 11 eggs were measured after being laid (including the mass and length), rather than measuring the X-radiograph (they were included with the data from the other clutches for all summary statistics).

We measured and recorded clutch sizes for a total of 13 clutches from six females (two additional clutches were observed but could not be X-rayed for quantification or measurements). The clutch and egg sizes are shown in Fig. 1 and compared to other studies and subspecies in Table 1. The female that laid eggs at the Tulsa Zoo had a mean egg mass of 9.9 g (SD = 1.14; range = 8-12), mean egg length of 31.6 mm (SD = 1.43; range = 30.2-34.4), and mean egg width of 23.6 mm (SD = 0.88; range = 21.9-25.3; the width measurements are included in Figure 1 and Table 1).

For the three transmittered females that produced multiple clutches in a year, two produced at least two clutches for each of the two years we monitored them (we were not able to X-ray one of those clutches and it was not included in our summary statistics or the total number of clutches). The third individual produced at least one clutch in the first year, and at least three clutches in the second year (the third clutch was undergoing calcification when we X-rayed it, and the shells were not sufficiently visible to reliably count or measure the eggs; therefore, it was not included in our summary statistics or total number of clutches).

This is the first report of *D. r. miaria* producing up to three clutches in a single year. *Deirochelys r. chrysea* is known to nest up to four times per year (typically 2-3), but *D. r. reticularia* and *D. r. miaria* have only previously been reported nesting twice per annual cycle (Gibbons, 1969; Gibbons and Greene, 1978; Ewert et al., 2006; McKnight et al., 2015a).

Our mean clutch and egg sizes agreed closely with the results reported by Carr and Tolson (2018) and Dinkelacker and Hilzinger (2014) for *D. r. miaria* in Louisiana and Arkansas, respectively. All three studies found large mean egg and clutch sizes relative to most reports for the other subspecies. Indeed, the maximum egg widths in both our study and Carr and Tolson (2018) were the largest egg widths reported for any *D. reticularia* subspecies.

To statistically compare egg and clutch sizes between *D. r. miaria* and *D. r. reticularia*, we combined our data with the clutches reported in Carr and Tolson (2018) and compared them with data from *D. r. reticularia* that were captured in South Carolina between July 1995 and February 1996 (previously published in Buhlmann et al., 2009). This resulted in a total of 17 clutches from nine female *D. r. miaria* and 25 clutches from 16 female *D. r. reticularia* (one clutch from Carr and Tolson [2018] was not included because female plastron length was not recorded). We constructed mixed-effects models in R (v3.4.1; R Core

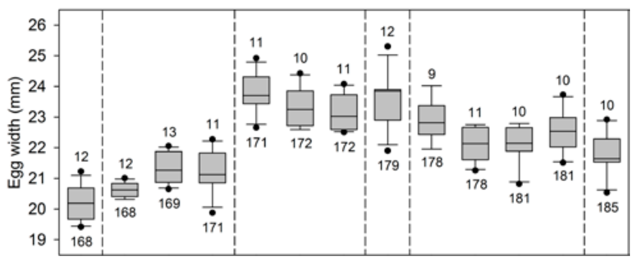

Fig. 1. *Deirochelys reticularia miaria* egg widths in Oklahoma. Each box is the data from a single clutch, and the dashed lines separate individual females. Numbers above the boxes indicate clutch size and numbers below the boxes indicate female plastron length at the time the clutch was observed. Whiskers indicate the 10th and 90th percentiles and all outliers are shown. The center box (clutch size = 12, plastron length = 179) shows the data for the female that laid eggs at the Tulsa Zoo. All other boxes are from X-ray data.

Table 1. Clutch and egg sizes for chicken turtle (*Deirochelys reticularia*) subspecies. Mean PL = mean plastron length of females. For results from the current study, mean egg width was calculated by averaging the means per clutch, whereas the range is the maximum and minimum of any measured eggs.

Subspecies	State	Mean clutch size	Clutch size range	Mean egg width (mm)	Egg width range (mm)	# of clutches	Mean PL (mm)	Source
D. r. miaria	OK	10.9	9–13	22.2	19.4–25.3	13	174.8	Current study
D. r. miaria	LA	10.6	8–13	22.9	20.7–24.7	5[a]	186.1	Carr & Tolson, 2018
D. r. miaria	AR	10.7	9–12	22.3 [b]	21.1– 23.5 [b]	3[b]	174.9	Dinkelacker & Hilzinger, 2014
D. r. miaria	TX	8	—	—	17–23	1	—	David, 1975
D. r. reticularia	SC	7.2	1–12	—	—	57	159	Gibbons et al., 1982
D. r. reticularia	SC	8	—	20.8	—	15	—	Congdon et al., 1983
D. r. reticularia	SC	8	—	20.8	—	13	160	Congdon & Gibbons, 1985
D. r. reticularia	SC	9.8	5–17	21.8	17.4–23.4	32	177.5[c]	Buhlmann et al., 2009
D. r. chrysea	FL	10.5[d]	8–12[d]	21.2	19.9–22.9	4[d]	—	Iverson, 1977
D. r. chrysea	FL	9.5	2–19	22.4	20.1–23.6	29	176.3	Jackson, 1988
D. r. chrysea	FL	10.9	6–18	—	—	16	—	Ewert et al., 2006

[a] Plastron length was only recorded for four females.
[b] Egg width was based on only two clutches.
[c] WebPlotDigitizer (Rohatgi 2017) was used to extract measurements from Figure 3 of Buhlmann et al. 2009.
[d] One female contained 5 eggs and "6 ovarian follicles of ovulatory size." Based on our experience with *D. r. miaria*, we treated the follicles as unshelled eggs and assumed a clutch size of 11 (otherwise, the mean is 9 and range is 5–12).

Team 2017) using the package lme4 (v1.1-15; Bates et al. 2015) with subspecies and plastron length as fixed effect factors (including an interaction). For egg width, we used a linear model via the lmer function, and for clutch size, we used a generalized linear model with a Poisson distribution via the glmer function. To avoid pseudoreplication, we included female ID as a random effect factor for the model comparing clutch sizes, and for the model comparing egg widths, we include female ID and clutch ID as random effect factors, with clutch ID nested in female ID. We used quantile-quantile plots and residual plots to check the models' assumptions, and we assessed the significance of both comparisons using the Anova function in the package car (v2.1-6; Fox and Weisberg, 2011) with type II sums of squares and the chi-square test statistic.

We did not find a significant difference in egg width between *D. r. miaria* and *D. r. reticularia* ($\chi^2 = 0.8715$, P = 0.3505), but egg width and plastron length were correlated ($\chi^2 = 8.1154$, P = 0.0044), which is consistent with previous research on *D. r. reticularia* (Gibbons et al., 1982; Buhlmann et al., 2009). The interaction term was not significant ($\chi^2 = 1.4155$, P = 0.2341). For the comparison of clutch size, there was not a significant difference between subspecies ($\chi^2 = 0.6655$, P = 0.4146), or a significant association with plastron length ($\chi^2 = 1.4070$, P = 0.2355), or a significant interaction ($\chi^2 = 0.2898$, P = 0.5904). These results do not suggest a difference in egg or clutch sizes between the subspecies after accounting for female body size, however, our sample size was small, resulting in limited statistical power. It would be useful for future researchers to expand this sample and conduct a more robust comparison.

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