THE REPRODUCTIVE BIOLOGY OF THE RIO GRANDE COOTER (*PSEUDEMYS GORZUGI)* IN TEXAS

by

Austin Michael Adrian Bohannon, B.S.

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Committee Members:

Michael R.J. Forstner, Chair

Dan H. Foley III

Sarah R. Fritts

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1. INTRODUCTION

Understanding reproductive biology is essential when attempting to understand the overall life history of any given species. Knowing the reproductive biology of a species influences management decisions, informing structured decisions for conservation status, conservation recovery plans, and assessment of population viability (Shine, 1980; Joanen & McNease, 1980). Understanding the aspects of the reproductive biology for non-marine chelonians is especially important due to 41.6% of this group being recognized as threatened (Stanford et al., 2018). Furthermore, given these animals are extremely sensitive to increases in adult mortality, understanding fecundity and recruitment rates are exceedingly important for this taxonomic group (Stanford et al., 2018).

Documenting the breeding season for a given species is critical to the conservation of that species. When considering the nesting season of turtles, understanding this season can help mitigate mortality of adult females due to vehicular collisions when turtles move to land to lay their eggs. Aresco (2012) found that female turtles routinely use the shoulder of roads for nesting and that populations of turtles in ponds near highways were significantly male biased due to female mortality from vehicle strikes. Some species of turtles such as *Chelydra serpentina* have been known to travel as far as 1,625 meters away from the water source to lay their eggs increasing the chance of mortality due to vehicular collision (Congdon et al., 1987). Roads in west Texas are routinely placed very close to *P. gorzugi* habitat. Two spring fed pools in Bracketville, TX and in Del Rio, TX that served as field sites for this study. Each of these water systems are located very close (< 30 m) to highway 90 and other less traveled roads. I would assume when female *P. gorzugi* move from their aquatic habitat onto land to nest, they would be at risk from vehicular collision.

The Rio Grande Cooter (*Pseudemys gorzugi*) has been petitioned for federal listing by the United States Fish and Wildlife Service owing to potential reductions in the extent of occurrence, population size, and enduring threats to population stability (FWS-R2-ES-2015-0061). Although originally considered a sub-species of the River Cooter (*Pseudemys concinna*), it has been subsequently elevated to full species status due to morphological differences (does not have "C" markings on plastron, rather has black and yellow concentric circles) and lack of gene flow between *P. gorzugi* and the nearest populations of *P. concinna* (Ward, 1984; Ernst, 1990; Siedel, 1994; Degenhardt et al., 1996). Threats to this species include modification of habitat due to the construction of dams and the extraction of water for agricultural, industrial, and municipal uses (Bailey et al. 2008; Pierce et al. 2016). Habitat modification is believed to have caused reductions in population size and geographic range (IUCN 2008). Additionally, collection of *P. gorzugi* for the pet trade may impact populations. Bailey et al. (2008) indicated they routinely observed wild caught *P. gorzugi* for sale online. Mali et al. (2014) report that *Pseudemys* and *Trachemys* genera dominate freshwater turtle exports from the United States, while additionally commenting on a lack of taxonomic resolution in those export data. At the currently available resolution, the quantity of *P. gorzugi* exports may be underestimated and their actual provenance unresolved. Moreover, within the United States the distribution of *P. gorzugi* is quite limited, as the species occurs only in the Devils, Pecos, and the Rio Grande rivers and their tributaries (Bailey et al. 2008).

Despite having been formally described 34 years ago, *P. gorzugi* remains among the most understudied turtle species in North America, having near the lowest number of published text and number of citations of all turtles in North America (Lovich & Ennen, 2013). Most published data detail one-time encounters such as distributional records (Bailey et al., 2005), melanism (Bailey et al., 2005), maximum size records (Stout et al., 2005) and other aspects of natural history (MacLaren et al., 2017; Sirsi et al., 2017). Detailed studies of *P. gorzugi* are necessary to inform the listing process and potential management options. Nonetheless, these are rare. For example, currently only 3 published records of clutch size for this species (Mali et al., 2014; Lovich et al., 2016; Letter et al., 2017).

The reproductive cycles of many North American turtles have been extensively studied over the past 75 years (Cagle, 1944; Gibbons & Coker, 1977; Christiansen & Burken, 1979; Gibbons et al., 1982; Aresco, 2004; Rose, 2011; Kuchling, 2012). These reproductive studies have progressed with the technological advancements in the medical field, more specifically with radiography and by enabling the use of ultrasound tomography on turtles (Kuchling, 2012). The newer generation ultrasound machines are much smaller and can be easily transported. Additionally, the probes on these machines have become sufficiently reduced in size enough to use in areas such as the inguinal pocket of turtles. These advancements have allowed researchers to study the growth and development of follicles and eggs in female turtles *in vivo* (Kuchling, 2012). There have been a few comprehensive reproductive studies of turtles related to *P. gorzugi* (*Trachemys scripta, Pseudemys concinna & texana*) that may serve to enable speculative extrapolation to the reproductive cycles of *P. gorzugi* (Cagle, 1944; Gibbons, 1977; Aresco, 2004).

Vitellogenesis and follicular growth has been recorded in *Trachemys scripta* and *Pseudemys floridana* in the fall before a halt in growth during the winter, then growth starts again once the turtle emerges the following spring (Ewert, 1991; Aresco, 2004; Kuchling, 2012). This follicular growth is then followed by either oviposition into the oviducts and the addition of an albumin layer, followed by gradual calcification of the outer layer of the egg, or follicular atresia (Kuchling, 2012). Female *T. scripta* are considered sexually mature when they produce follicles of 15 mm or greater (Cagle, 1944). *Pseudemys concinna* are considered mature when their follicles reach 10 mm, and follicles are ovulatory sized at 18 mm (Iverson, 2001). I would presume that *P. gorzugi* would be considered mature at a similar size due to the close phylogenetic relationship between these species. The process of albumin addition happens in approximately three days and can be identified by ultrasound (Kuchling, 2012). Once an egg is calcified it can be detected accurately by a radiograph. I would further predict a positive correlation between clutch size and plastron length of *P. gorzugi* to exist as in many other turtles such as *T. scripta*, *P. floridana*, and *P. concinna* (Gibbons et al., 1982; Aresco 2004). From this study, I plan to answer five main questions regarding the reproductive ecology of *P. gorzugi* 1) average clutch size, 2) length of breeding season, 3) size of females at sexual maturity, 4) number of clutches per year, and 5) time of year breeding/egg development occurs.

2. METHODS

Study Duration and Field Sites:

For this study I surveyed three different sites in west Texas (Fig. 1). The sites I sampled were: 1) Dolan Falls Preserve (The Nature Conservancy) and Finnegan Springs (Texas Parks and Wildlife Department) on the Devils River, Val Verde County (Fig. 2), 2) San Felipe Creek in the city of Del Rio, Val Verde County (Fig. 3), and 3) Las Moras Springs in Brackettville, Kinney County (Fig 4.). This project took place from April 7, 2018 to August 13, 2019. During the summer of 2018 (April–September) I sampled three field sites a minimum of once a month and on few occasions, I sampled a site twice. In April 2018 I sampled San Felipe creek twice (the first sample was to test equipment). On July 6th I sampled San Felipe Creek and Las Moras Springs only, then on July 24th I sampled all sites. July 6th sampling took place at San Felipe Creek and Las Moras Springs only due to not having access to Dolan Falls at that time.

After September 2018 I continued to sample Las Moras Springs on a monthly basis until March 2019. From March 2019 through August 2019 I sample Las Moras Springs twice a month. All data collected from Dolan Falls and Finnegan Springs were pooled into one dataset and hereafter called Dolan falls. This decision was informed by a recently published telemetry study concluding that turtles were not exclusive to one site and thus considered one population of interbreeding individuals (MacLaren et al., 2017).

I captured all turtles by hand, and when necessary, hoop traps and basking traps were used to supplement capture. To capture turtles by hand I used a diving mask and fins and extensively searched the area until no more turtles could be found. Hoop traps and basking traps were used from October 2018 to February 2019. During this time turtles became increasingly difficult to locate and capture by hand. I presume that turtles were difficult to find due to the water becoming increasingly murky, and the turtles sensing human presence and seeking shelter. Each turtle I captured was cohort marked by notching the marginal scutes on the shell with a mark for the year (“18” or “19”) (Cagle, 1950), and individually tagged using a subcutaneous Passive Integrated Transponder tag (Avid Identification Systems, Inc., Norco, CA). This identification also allowed generation of individual reproductive histories and track development of follicles/eggs within an individual over time. I weighed, measured, and collected a blood sample from each turtle on initial capture. Specific morphometric data I collected included carapace length, carapace width, plastron length, plastron width, and body depth. All measurements taken were straight line (not measuring the curvature of the shell). Blood samples were submitted into the MRJF tissue catalog at Texas State University for utilization in future projects.

Sonogram and X-Radiograph:

I used an Ibex Pro ultrasound machine (E.I. Medical Imaging) in the field on every female turtle captured that was large enough (inguinal pocket greater than 2cm x 3cm) for the probe to properly be inserted into the inguinal pocket. I found through trial and error that to achieve the best depth and clarity within *P. gorzugi* the machine should be set with the gain at 27 dB, the near setting at 40 dB, and the far setting at 46 dB.

Only turtles captured from San Felipe Creek and Las Moras sites were radiographed due to the close proximity to a veterinary facility in Del Rio, Texas. San Felipe Creek was approximately a six-minute drive at 3.8 km, and Las Moras Springs was approximately a 32-minute drive at 51 km. Turtles captured at Dolan Falls were not radiographed due to a long drive at approximately 2.5 hours, and 96 km with 46 km being rough rural dirt roads.

When a turtle indicated the presence of eggs via sonogram image, they were transported to Val Verde Veterinary Hospital to undergo radiography. Eggs could be accurately identified against follicles since eggs were much larger and had a visible calcified membrane around the yolk. Furthermore, with the use of the ultrasound, I could accurately identify which eggs could be detected by the radiograph. If the egg membrane was very faint and mostly transparent, the egg would not be detected via radiograph. When the egg membrane was more pronounced and less transparent, then eggs would be detectable on the radiograph image. Each turtle was positioned dorsally on its carapace and an image was taken through the plastron. As shown by Divers & Mader (2005) I found that 70 kVp and 100 mA settings provided the optimal image clarity for detecting shelled eggs.

I obtained information regarding follicle size and growth, length of breeding season, female turtle size at sexual maturity, and number of clutches per season from the ultrasound readings. The clarity of this portable ultrasound unit also gave the ability to measure individual eggs and follicles inside the turtle in the field. Because of the three-dimensional nature of follicles, it is not possible to accurately obtain a count of total follicles or eggs using this method.

Ultrasound further allowed for the detection of shelled eggs and thus individually select which turtles were subsequently taken to the veterinarian to undergo radiography. This minimized the amount of time turtles which did not have shelled eggs were outside of their natural habitat. Radiography was performed on turtles with shelled eggs as a method to estimate clutch size of *P. gorzugi.* This clutch size was then compared to plastron length to observe if any correlation between size of turtle and clutch size exists.

Data Analysis:

I used the statistical program R for all data analysis (R core team, 2019). I performed 6 main hypotheses tests (clutch size predicted by plastron length, follicle size predicted by Julian date for all sites combined and for each individual site, and egg presence predicted by Julian date). Data were combined from three years (2017–2019) and dates were assigned a Julian date numbering from 1 to 365, with 1 representing January 1st and 365 representing December 31st. This analysis was only performed with pooled data across all three sites. When testing clutch size vs plastron length, clutch size could not be normally distributed due to clutch size being discrete. Therefore, I used a Hosmer and Lemeshow goodness of fit test to determine if the data fit the Poisson distribution. This test confirmed that the data did in fact fit the Poisson distribution. A generalized linear model was then performed with class set to "Poisson". For this test I used plastron length as the independent variable and clutch size as the dependent variable.

Because follicle size is a continuous variable we could test for normality within these data. For each hypothesis, I first used a qqnorm plot and qqnorm line to visually assess look normality. After all plots appeared normal, I used a Shapiro-Wilk's test if this correlation was statistically significant. For each site individually and for all sites combined, follicle sizes for every site individually, and combined they all proved to be normally distributed. I then used a simple linear regression on each. When comparing follicle size to Julian date, date was used as the independent variable and follicle size was used as the response variable.

The data used for egg presence best fit the binomial distribution since measurements were considered 0 for the absence of eggs, and 1 for the presence of eggs.

I used a generalized linear model and a quadratic model with the family set to “binomial” to account for this distribution. Once again Julian date was used as the independent variable and the presence of eggs was used as the dependent variable. An analysis of variance test was performed on these two models to determine which model best fit these data. For this regression Julian date was used as the independent variable, and presence/absence was considered dependent.

3. RESULTS

From April 2018–August 2019, 99 individual turtles were captured 176 times among the three sites. Dolan Falls had the greatest number of individuals captured (45 turtles captured 58 times), followed by Las Moras Springs (41 turtles captured 102 times), and lastly by San Felipe Creek (13 turtles captured 16 times).

I observed follicles year-round; however, there is a decline in overall follicle size as the year progresses (Fig 8). Regressing date with follicle size indicated a daily decrease in follicle size of 0.018 mm, ($ß=0.0183 \pm 0.009$, $r^2=0.12$, & $p<0.01$).

Overall, there were 18 individual turtles bearing eggs. The smallest turtle captured bearing eggs had a carapace length of 239 mm, and the largest had a carapace length of 304 mm (avg. 279.9 ±16.54 mm). Of these 18 females, I obtained reliable radiographs from 10. The smallest clutch size observed totaled 7 eggs and the largest clutch size totaled 17 eggs (avg.=11.8, stdev= 2.89). I found that clutch size was not related to plastron length ($ß=0.0053 \pm 0.0058$, $p > 0.3$) (Fig.9). Females were bearing eggs every month beginning on April 7th and ending on August 13th. I predicted that female *P. gorzugi* bear eggs between March 15th and August 23rd $p < 0.01$ (Fig. 10).

Dolan Falls:

At Dolan Falls 45 individual female *P. gorzugi* were captured 58 times from April 20th, 2018 to September 30th, 2018. Of these 45 individuals, 11 turtles were captured twice each, and one turtle was captured three times. A total of 14 individuals that were captured had no detectable follicles (31.1% of individuals). Eight females were captured twice with detectable follicles (17.7% of individuals), allowing for measurement of follicle growth within these individuals between April and September. Nineteen turtles captured only once possessed follicles of various sizes (42.2% of individuals). Follicles ranged in size from 9 mm to 27 mm in diameter (mean = 16.7 mm). Three turtles captured once were found with eggs (6.6% of individuals). Eggs were found in the months April and June suggesting that the nesting duration may occur within this time.

There was a decline in overall follicle size as the year progresses (Fig 5). The slope indicates that as each day progresses the size of the follicles decrease by 0.047 mm ($ß= -0.047 \pm 0.018$, $r^2=0.46$, $p < 0.01$).

San Felipe Creek

At San Felipe Creek 14 individual female *P. gorzugi* were captured 18 times. Only three of the turtles were recaptured, two turtles were captured two times and one turtle was captured three times. A turtle (ID# 072323546) initially captured on June 8th, 2018 possessed 30 mm follicles, but when later recaptured on August 24th, 2018 showed no visible follicles or eggs, indicating the potential for a nesting event within this time. Turtle (ID# 073378072) was initially captured on May 8th, 2017 with 49 mm eggs. Eleven months later (April 8th, 2018) this same turtle was recaptured with 43 mm eggs, then recaptured again on June 8th, 2018 with 21 mm follicles. The last recaptured turtle (ID# 071546606) possessed follicles that grew from 12 mm to 16 mm over a 16–day period (April 7th–April 23rd, 2018). Female 071520099 captured on April 7th, 2018 had no visible follicles or eggs.

Over the summer of 2018 six of the female *P. gorzugi* possessed eggs (42.8%). Of the six total females bearing eggs only five were radiographed. One turtle was not radiographed because collection occurred on a "trial run" to test equipment on a day that the veterinarian was not open. Additionally, one of the turtles that was radiographed showed no presence of eggs, indicating that although the eggs were mature in size and detectable by the ultrasound, sufficient calcification had not yet taken place. Three females did show eggs on the radiograph revealing clutch sizes of 8,10, and 12 eggs Egg size ranged from 35 to 49 mm at the longest measurable point. Finally, five turtles each captured once had visible follicles (35.7%). Follicles ranged in diameter from 12–30 mm. Eggs at San Felipe Creek were observed every month starting on April 7th and ending on August 3rd, suggesting the nesting season for *P. gorzugi* at this site is likely from April–August. Although follicle size was normally distributed, a linear model indicated a non-significant positive slope for this site (ß= 0.01282 ± 0.0576, r^2= 0024, p= 0.66) (Fig. 6).

Las Moras Springs

At Las Moras 41 individual turtles were captured 102 times. The maximum number of times an individual turtle was captured was 12. All turtles were captured from May 2018 to August 2019. *Pseudemys gorzugi* were observed with follicles every month of the year, and eggs were detected each month starting April 7th and ending August 13th.There was a decline in overall follicle size as the year progressed (Fig 8). The slope indicates that as each day progresses the size of the follicles decrease by 0.018 mm (ß= -0.0183 ± 0.009, r^2=0.06, p=0.03).

Looking at the per capture data of the female (ID#1221) that I captured 12 times, I can track egg/follicle development within a single individual over time (Fig. 11). This female (ID#1221) was first captured on May 15th, 2018 and found with 25 mm follicles. She was then captured again on June 7th, 2018 with 46 mm eggs, and again on July 6th of 2018 with 45 mm eggs. During each of these captures she was found with calcified eggs detectable by radiograph. Only 21 days later 1221 was recaptured again showing 14 mm follicles. This indicates that egg deposition happened between July 6th, 2018 and July 27th, 2018. This turtle was then captured an additional 8 times after July 27th, 2018, from August 24th, 2018 to July 29th, 2019 never again having eggs present, but possessing follicles each and every capture. This turtle was radiographed two times, once on June 7th, 2018 and once on July 6th, 2018. On June 7th she showed a clutch size of 7 (Fig. 16), then when radiographed again on July 6th, she showed a clutch size of 10 (Fig. 17). This suggests not all eggs are shelled at the same time, and when a single radiograph is taken of a particular turtle the clutch size shown may not be the true number of eggs that are eventually deposited for a full clutch.

4. DISCUSSION

Results indicated that *P.gorzugi* carry eggs from April–August, and there is no correlation between clutch size and plastron length. I found females carrying eggs each month from April 7th to August 13th. The binomial model from these data suggests with 95% confidence that female *P. gorzugi* develop eggs from mid-March to late August, which greatly increases the time frame from what had been previously been proposed May–June (Degenhardt et al. 1996; Lovich et al. 2016; Letter et al. 2017). Albeit, these results are from Texas wherein the other publications emphasize New Mexico populations (Degenhardt et al., 1996; Lovich et al., 2016; Letter et al., 2017).

Results contradict previous thoughts of follicular development in other members of the genus *Pseudemys* where follicles develop in spring after turtles become active following brumation, and development halts in fall when turtles return to brumation (Iverson, 2001.). Instead, large oviductual follicles, follicles < 15 mm (Cagle, 1944; Iverson, 2001) were found every month of the year (January–December); however, the turtles studied herein were in Texas were all taken from generally isothermic spring fed systems (Dolan Falls 22ºC (Phillips et al., 2011), San Felipe Springs 24 ºC (López et al., 2005), Las Moras Springs 20ºC (Fortclarksprings.com, 2019)). Similar findings have been seen in isothermic populations of mud turtle (*Kinosternon subrubum*) in the eastern United States (Gibbons, 1983). Turtles in my study show that the average size of follicles decrease as the year progresses. This negative correlation is a logical consequence of follicles enlarging in the late winter and early spring (January–February) and then developing into eggs from late spring to late summer (March–September). Once eggs were deposited during this time, then the turtles started developing eggs for the next year or began producing a second clutch from smaller follicles.

It has been shown in other emydids that a positive linear correlation between clutch size and plastron length exists (Tinkle, 1961; Gibbons, 1968; Gibbons, 1970; Ernst, 1971;); however, I found no significant correlation between plastron length and clutch size in *P. gorzugi*. Although I had a relatively small sample size (n = 10), it is only six turtles fewer than previously tested by Suriyamongkol & Mali (in press) who found a positive correlation in New Mexico. Moreover, the turtle I observed with a clutch size of 17 is currently the largest clutch size known for *P. gorzugi*. The largest known clutch size before this study totaled 14 eggs (Suriyamongkol & Mali, in press). The average clutch size from my study also increased the previously suggested average clutch size from 9.3 eggs to 12 eggs per clutch (Suriyamongkol & Mali, in press).

One turtle increased her clutch size by 3 eggs over 29 days between captures. In this situation there were two possibilities of what could have happened. First, the female could have shelled and deposited a clutch size of 7 in early June then immediately started shelling a second clutch of 10 that I found in July. This is possible since I have observed turtles with eggs and large oviductual follicles at the same point in time. When looking at the radiographs side by side I can see that the clutch in June seems to have a thicker, more calcified shelling, whereas the clutch in July seems to have a thin layer of calcium deposited on the outside of the egg lending weak evidence towards double clutching. Although it is known that multiple clutches occur in other species of *Pseudemys* (Gibbons et al., 1982) this would document the first account of *P. gorzugi* laying multiple clutches per year.

Challenging the previous statement is the position of the eggs in the oviduct, although not exact, they are very similar. This leads to a few questions or even potential problems considering radiographs as reliable tools to assess the number of eggs in a clutch. If the two radiographs taken in June and July were images of the same clutch, then this could mean that many clutch size data that have been recorded in the past could present inaccurate clutch sizes. Also, this could mean that eggs that seem to have a thick calcium layer in the radiograph may not actually be more strongly calcified and were simply further into the process than those observed later the next month. I have not found another occurrence like this in the literature.

There were a few anecdotal observations made throughout the course of this study regarding the reproductive biology of *P. gorzugi.* First, I observed male courtship behavior beginning as early as in the year as January 12th. This would correspond to the correct timeline for fertilization of the eggs I observed in early April. It is known that turtles have an organ for storing sperm, and sperm to be retained for at least 423 days (Gist & Jones, 1989). I did not observe an exact date to when the courtship behavior ended but occurred into late August. Second, I found multiple *P. gorzugi* nests at San Felipe creek, but all had been predated before hatching. After setting up a trail camera on this nesting beach I found racoons (*Procyon lotor*) to be the main predator of these nests, with most nests being predated within 24 hours of deposition. No nests were found at either Dolan Falls or Las Moras springs. Lastly, I found a hatchling *P. gorzugi* at Las Moras Springs on November 11, 2018 (carapace length 34 mm, carapace width 34 mm, plastron length 33 mm, plastron width 25 mm, body depth 18 mm, weight 8 grams). This marginal scutes of this hatchling were still very soft and pliable indicating that it had hatched only a few days prior.

Understanding the reproductive cycles of a species is a vital step for informing the conservation management (Joanen & McNease, 1980; Shine 1980). With the information collected here conservation efforts around the known nesting times for this species can be implemented. This study provided valuable insight to the nesting duration of *P. gorzugi* as well as the first year-round reproductive study conducted on this species. To further understand the reproductive aspects such as double clutching and nest site selection of *P. gorzugi*, telemetry studies should be conducted in conjunction with reproductive studies. It would be valuable to track the production of follicles and eggs within each individual turtle on a consistent two-week interval over multiple years with the aid of telemetry to accurately define whether this species regularly deposits multiple clutches each year.

APPENDIX SECTION

Fig. 1 Three sample locations visited during 2018–2019. *Pseudemys gorzugi* were captured at all three sites during 2018, with only Las Moras Springs being sampled in 2019.

Fig. 2 Dolan Falls (The Nature Conservancy) on the Devils River, Texas.

Fig. 3 San Felipe Creek in Del Rio, Texas.

Fig. 4 Las Moras Springs (Fort Clark Springs) Bracketville, Texas.

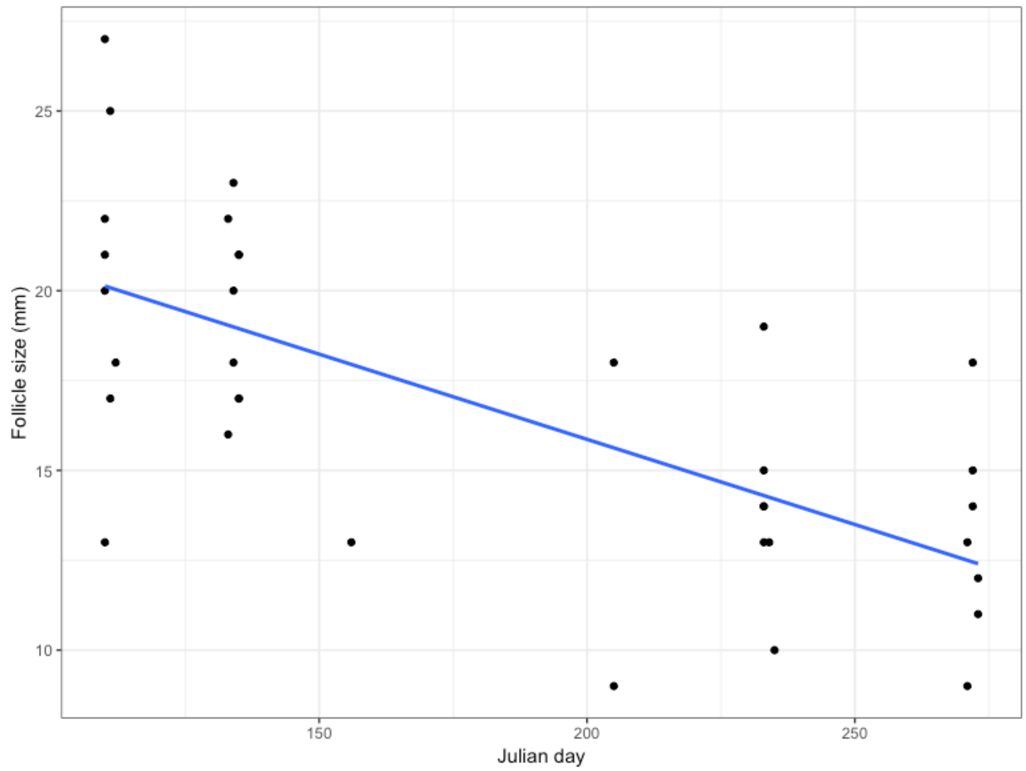

Fig. 5 Relationship of follicle size to Julian date in *Pseudemys gorzugi* at Dolan Falls on the Devils River (The Nature Conservancy) of Texas. The graph indicates a negative relationship in follicle size and day of year, slope = -0.047 ± 0.018, r^2=0.46, $p < 0.01$.

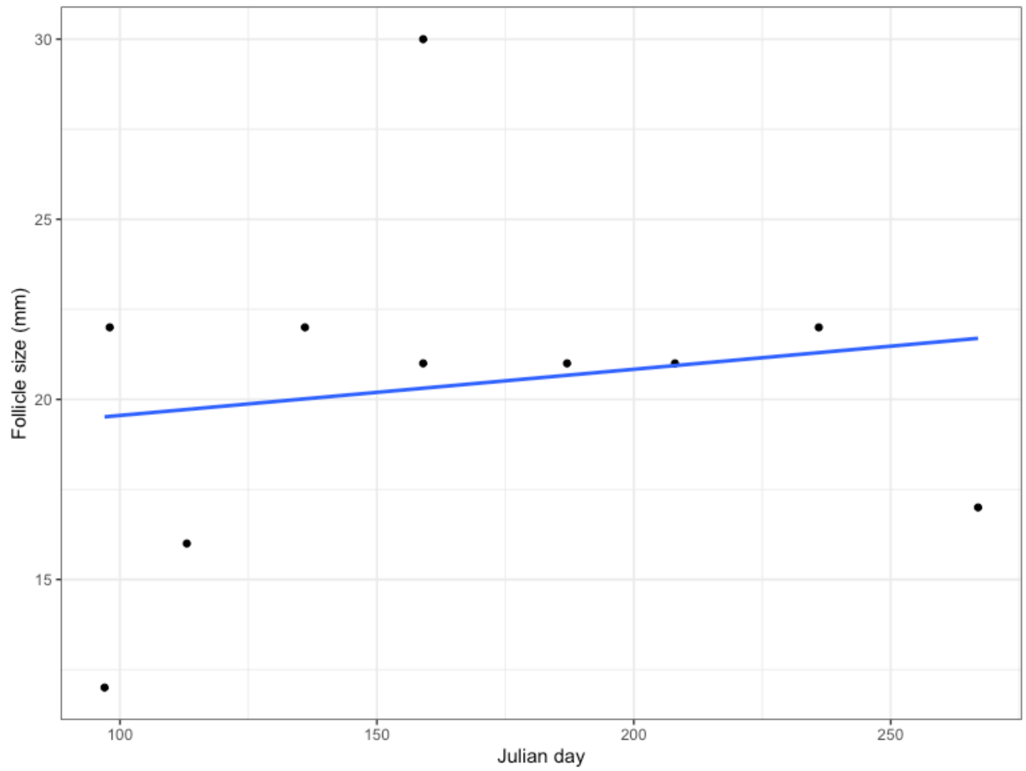

Fig. 6 Relationship of follicle size to Julian date in *Pseudemys gorzugi* at San Felipe Springs (Del Rio, Texas). The graph indicates a positive relationship in follicle size and day of year, slope = 0.012 ± 0.0576, r^2= 0.024, p= 0.66).

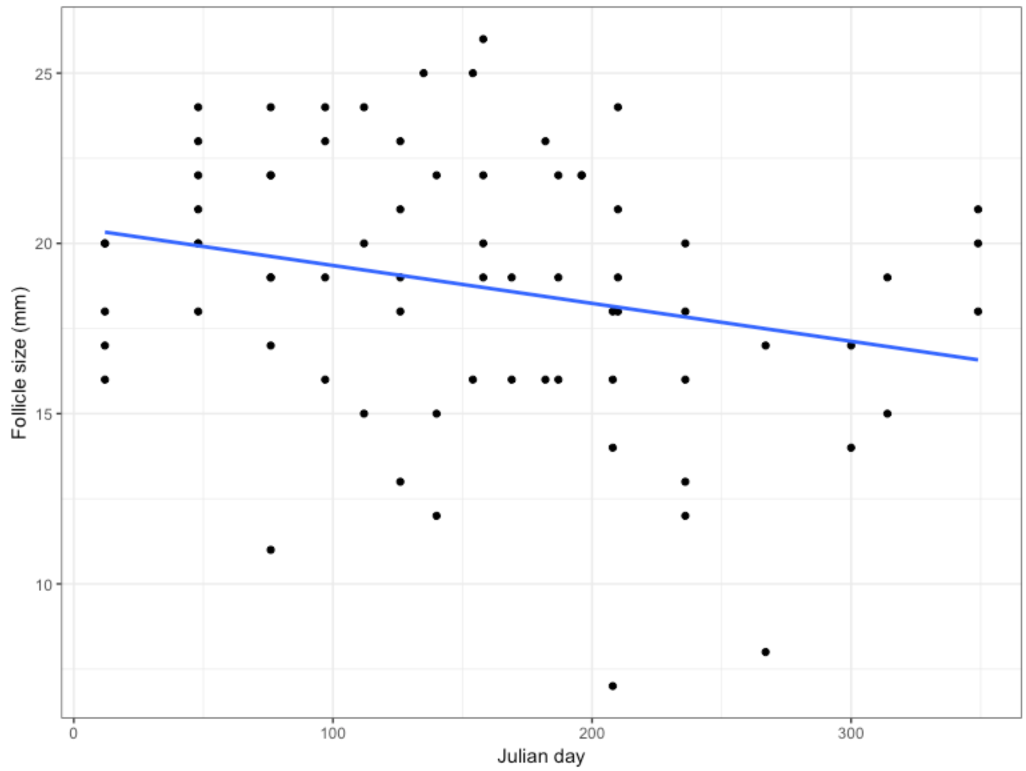

Fig. 7 Relationship of follicle size Julian date in *Pseudemys gorzugi* at Las Moras Springs in Bracketville, TX. The graph indicates a negative relationship in follicle size and day of year, slope = - 0.018 ± 0.009, r^2=0.06, p=0.03).

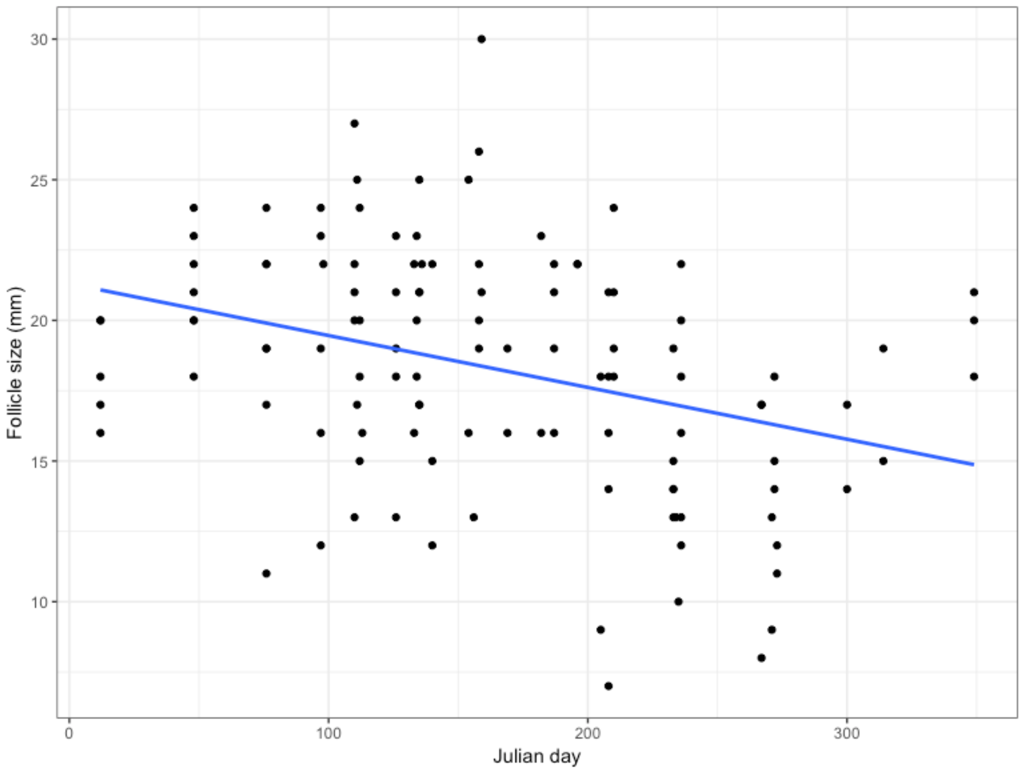

Fig. 8 Relationship of follicle size to Julian date in *Pseudemys gorzugi* over all study sites in Texas combined. The graph indicates a negative relationship in follicle size and day of year, slope = -0.018 ± 0.009, r^2=0.12, & p<0.01).

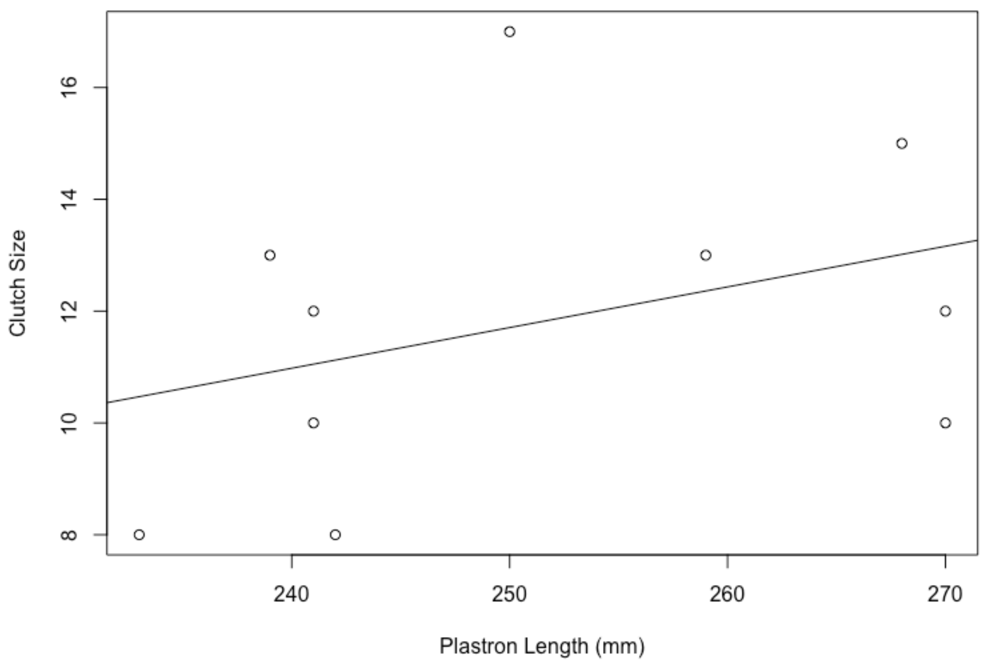

Fig. 9 Relationship between plastron length and clutch size. Linear model was conducted on 10 individual *Pseudemys gorzugi* with clutch sizes varying from 8 eggs to 17 eggs. The slope indicates a positive relationship but was non-significant ($ß=0.0053 \pm 0.0058$, $r^2=0.129$, $p > 0.3$)

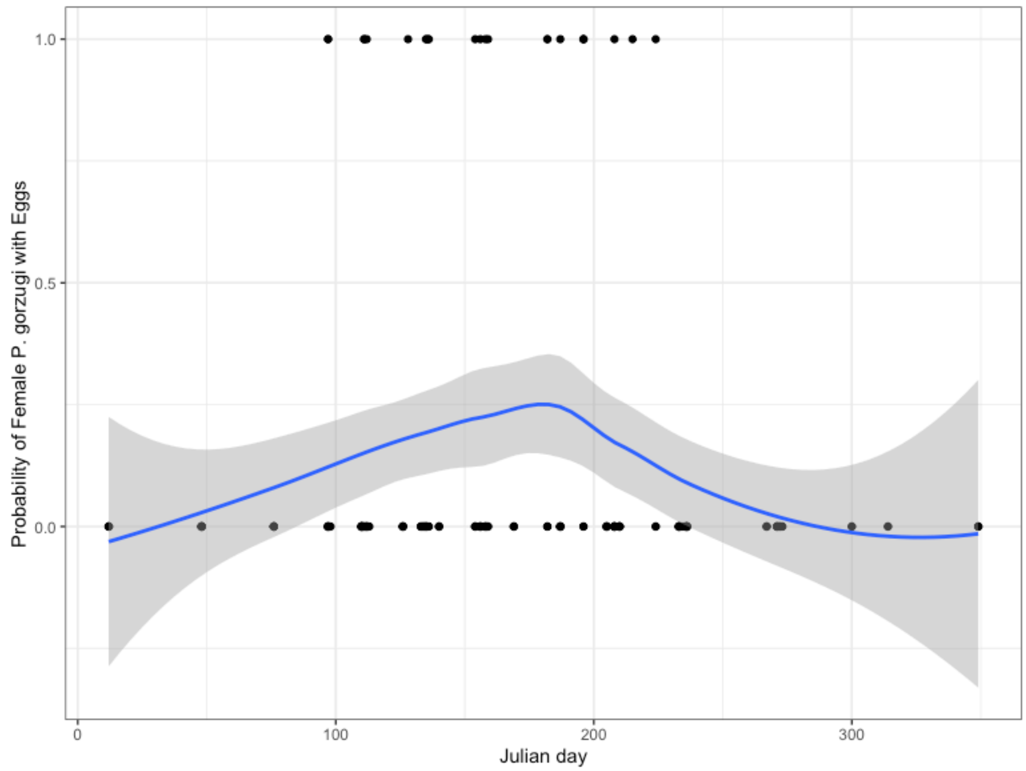

Fig. 10 Probability of female *Pseudemys gorzugi* carrying eggs. Julian date was plotted on the x axis with the probability of detecting eggs on the y axis. A total of 176 points were plotted with data combined over three years (2017–2019). 95% confidence interval indicates first predicted eggs appearing on March 15th and the last eggs found on August 23rd.

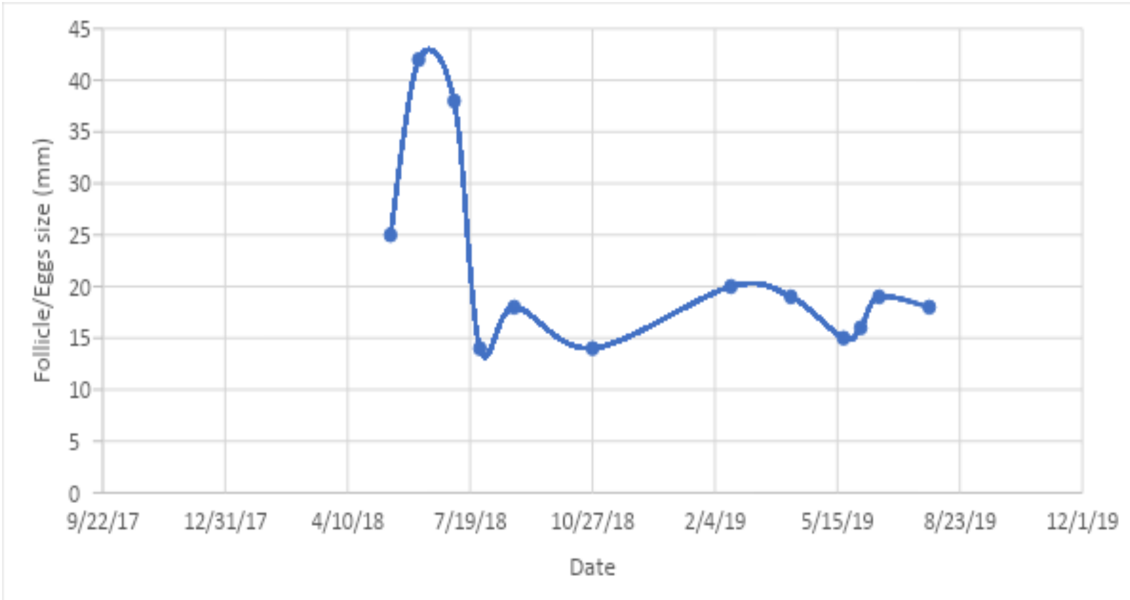

Fig. 11 Individual egg/follicle history for a single female *Pseudemys gorzugi.* This female was captured at Las Moras Springs near Brackettville, Texas from May 2018 to August 2019. The two points at 43 mm and 38 mm indicate the presence of shelled eggs. All other points represent follicles.

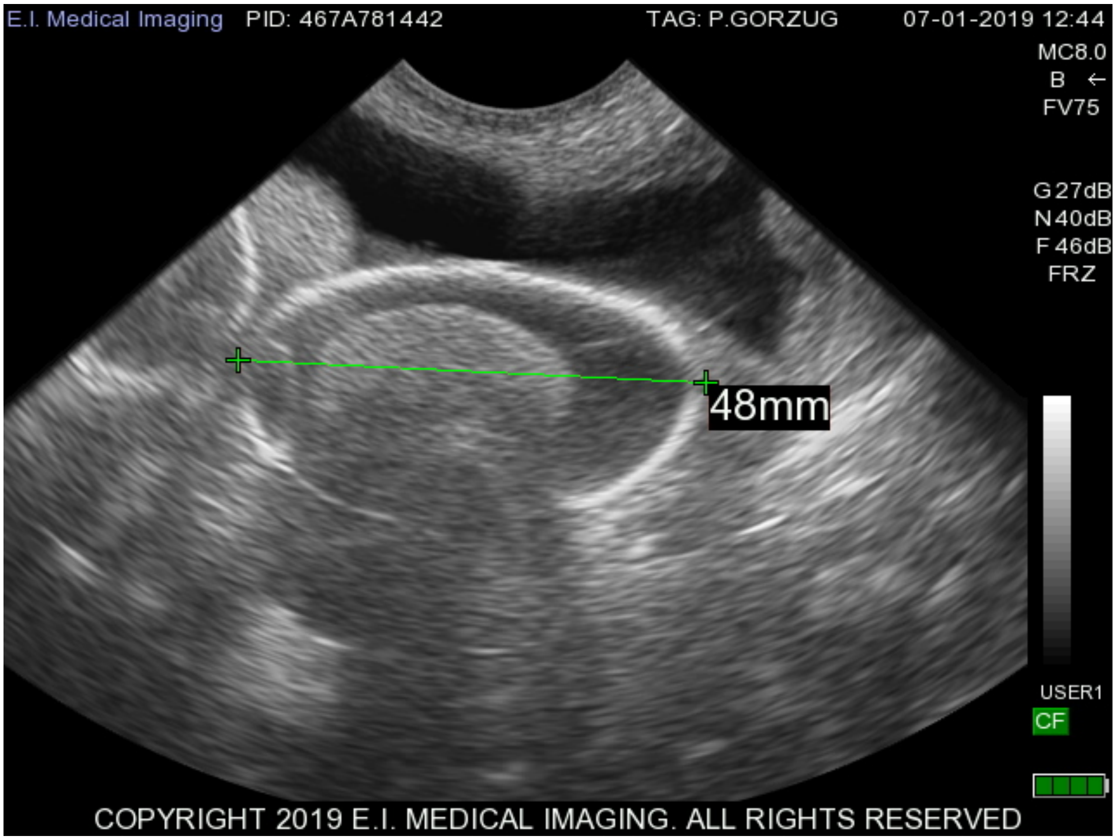

Fig. 12 Sonogram depicting a 48 mm shelled egg within a *Pseudemys gorzugi* (ID# 467A781442) on July 15th, 2019.

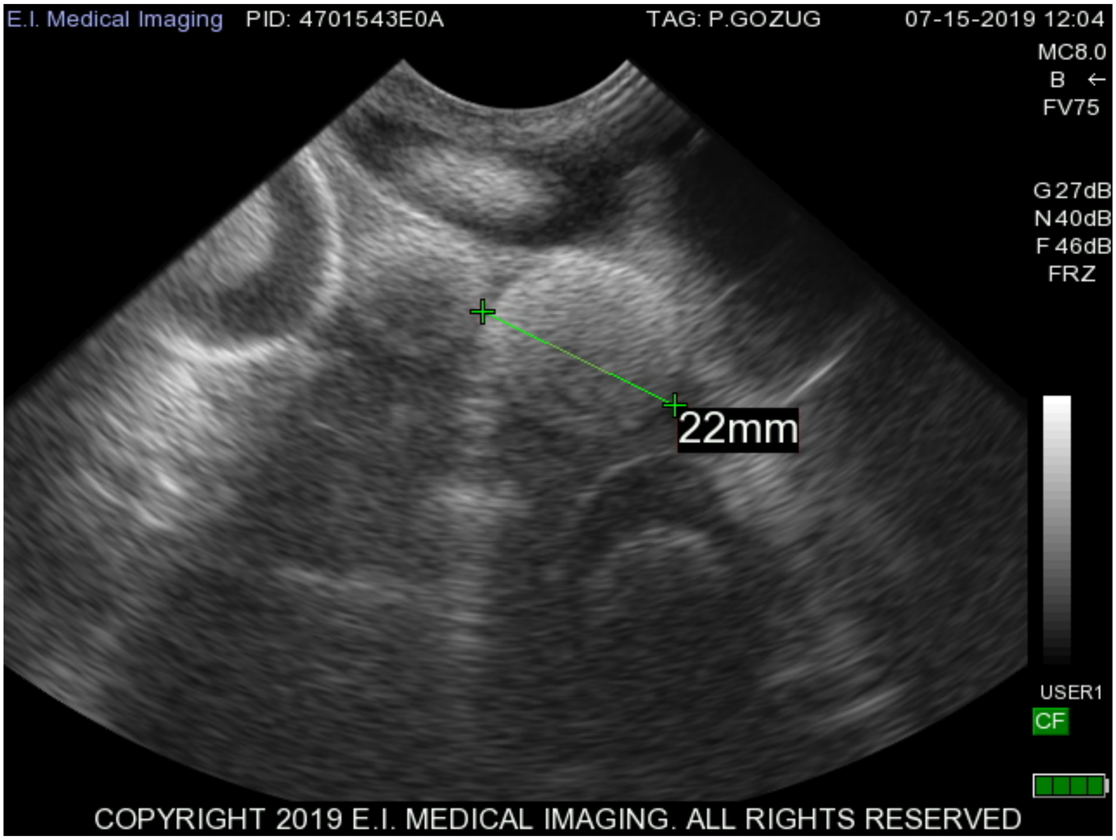

Fig. 13 Sonogram showing a 22 mm follicle among other follicles and shelled eggs within a *Pseudemys gorzugi* (ID# 4701543E0A) on July 15, 2019.

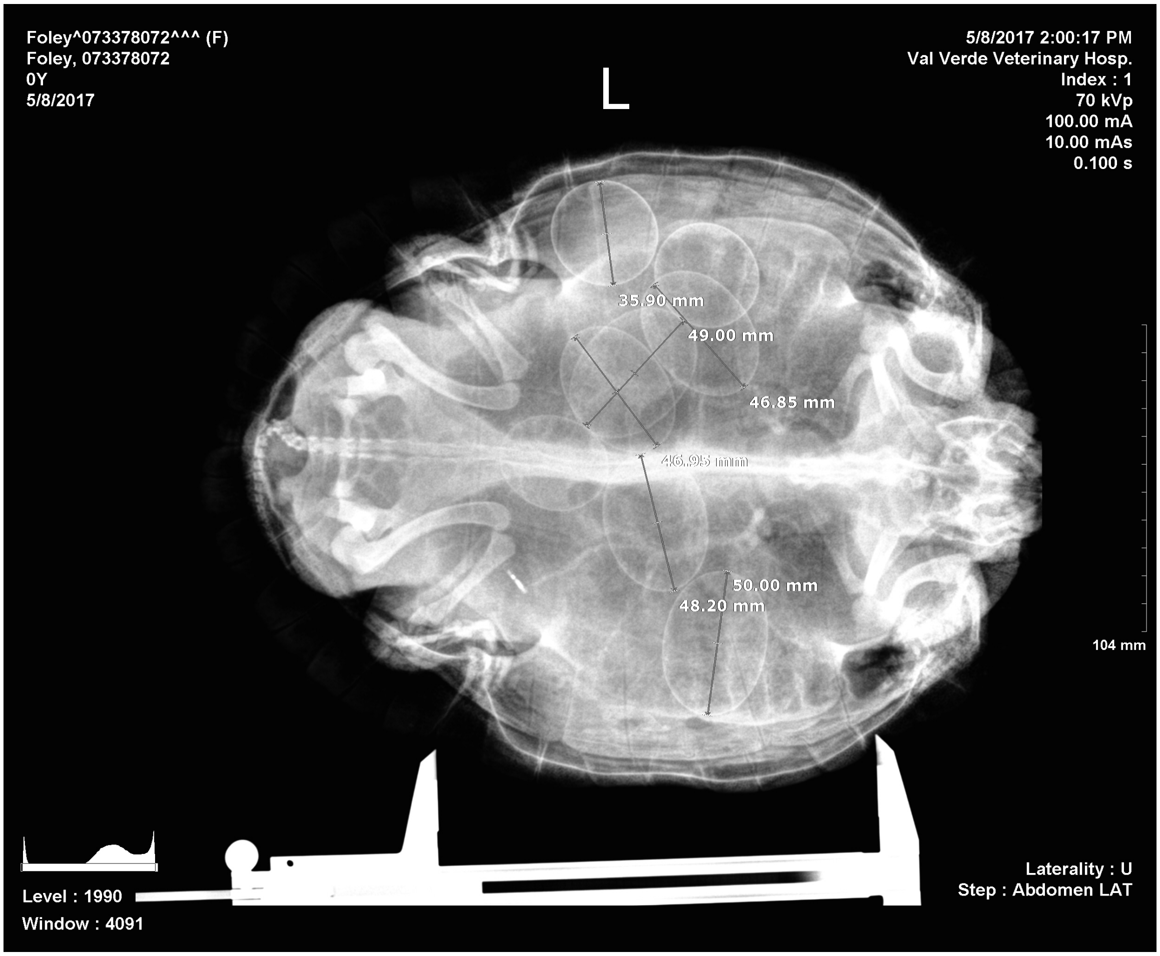

Fig. 14 Radiograph of *Pseudemys gorzugi* (ID# 073378072). Radiograph taken on May 8th, 2017 showing 8 eggs. Five measurements were taken of the egg's length (48.5 mm avg) and one eggs width (35.9 mm).

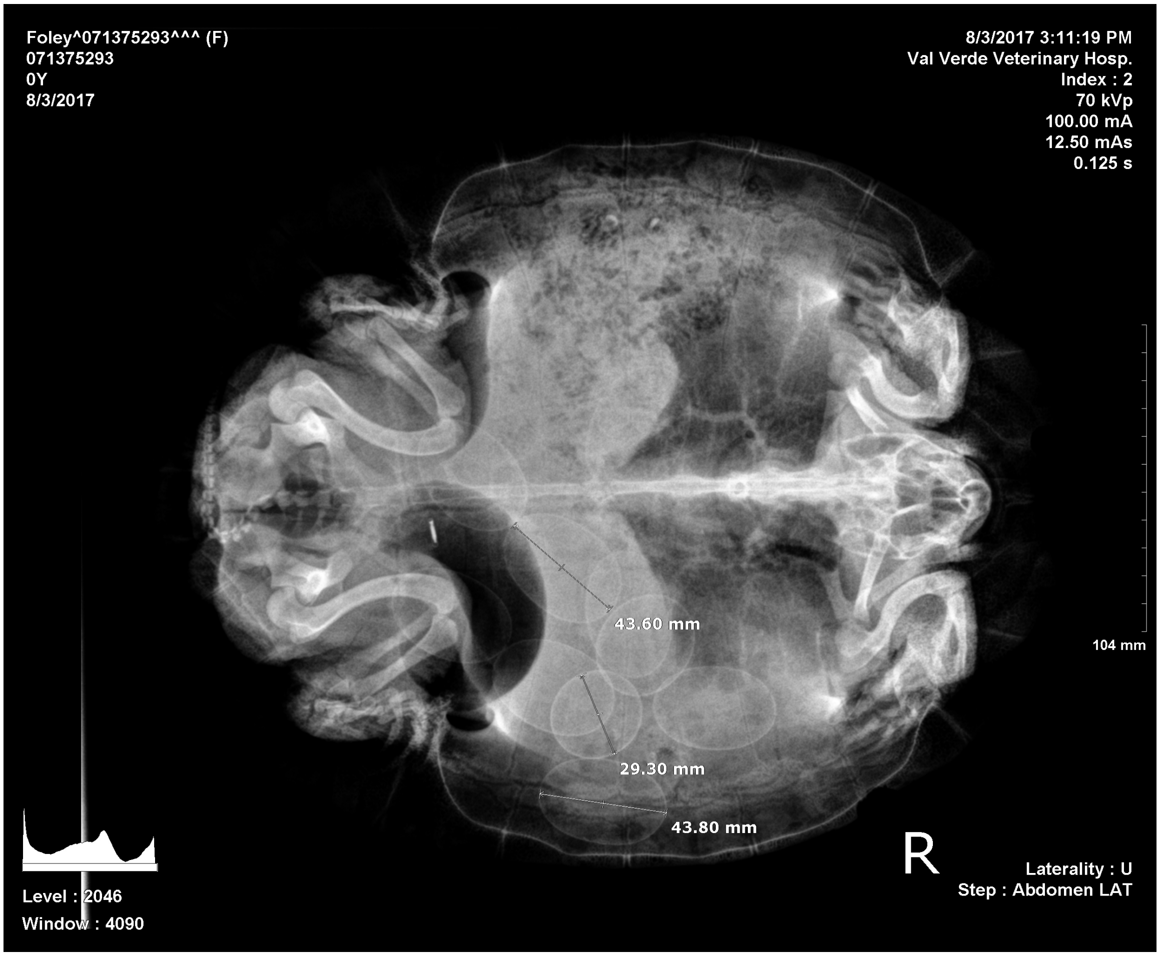

Fig. 15 Radiograph of *Pseudemys gorzugi* (ID# 071375293). Radiograph taken on August 3rd, 2017 showing 10 eggs within a single *Pseudemys gorzugi*. Measurements were taken of two egg's length (43.7 mm avg.) and one eggs width (29.3 mm).

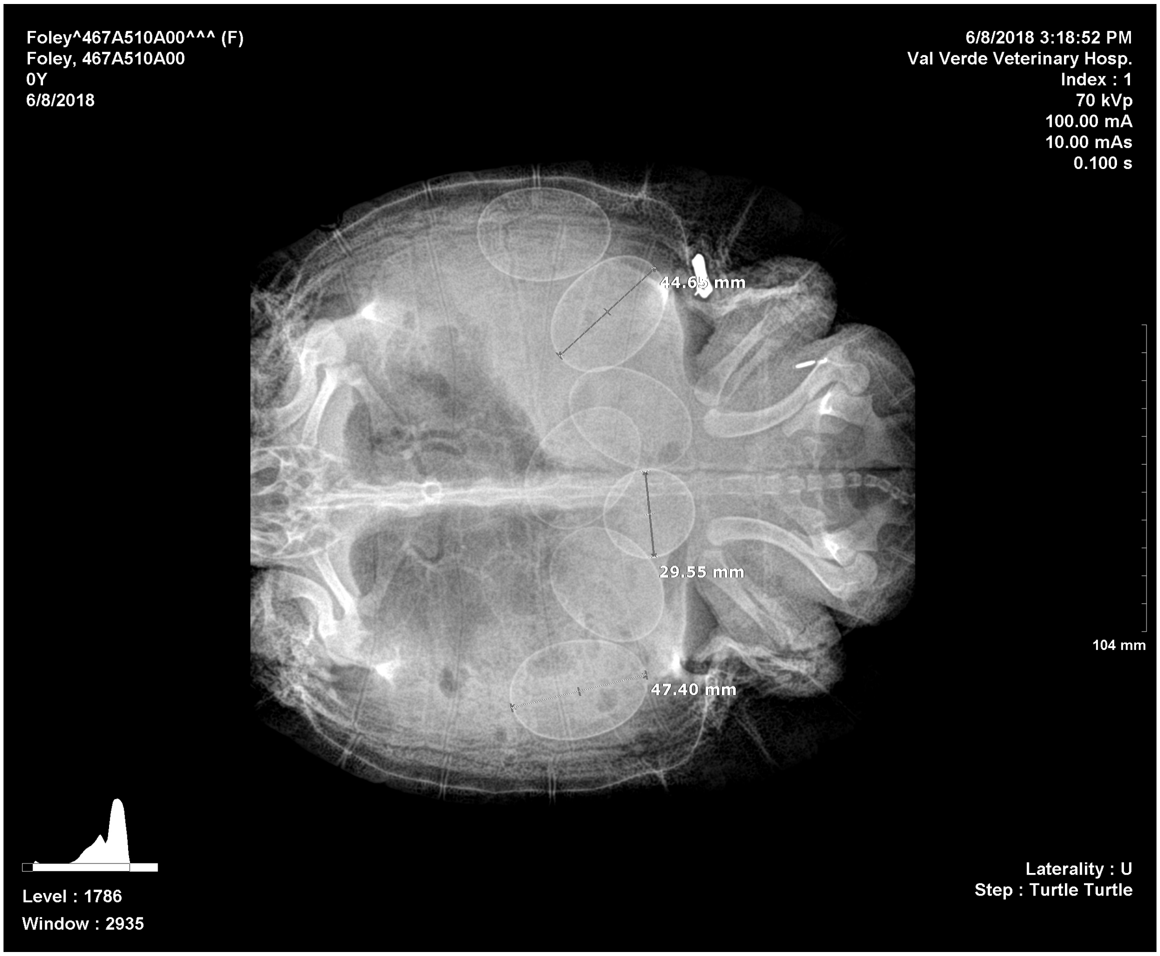

Fig. 16 Radiograph of *Pseudemys gorzugi* (ID# 1221). Radiograph taken on June 8th, 2018 showing 7 eggs within a single *Pseudemys gorzugi* female. Measurements were taken of two egg's length (46.03 mm avg.) and one eggs width (29.55 mm).

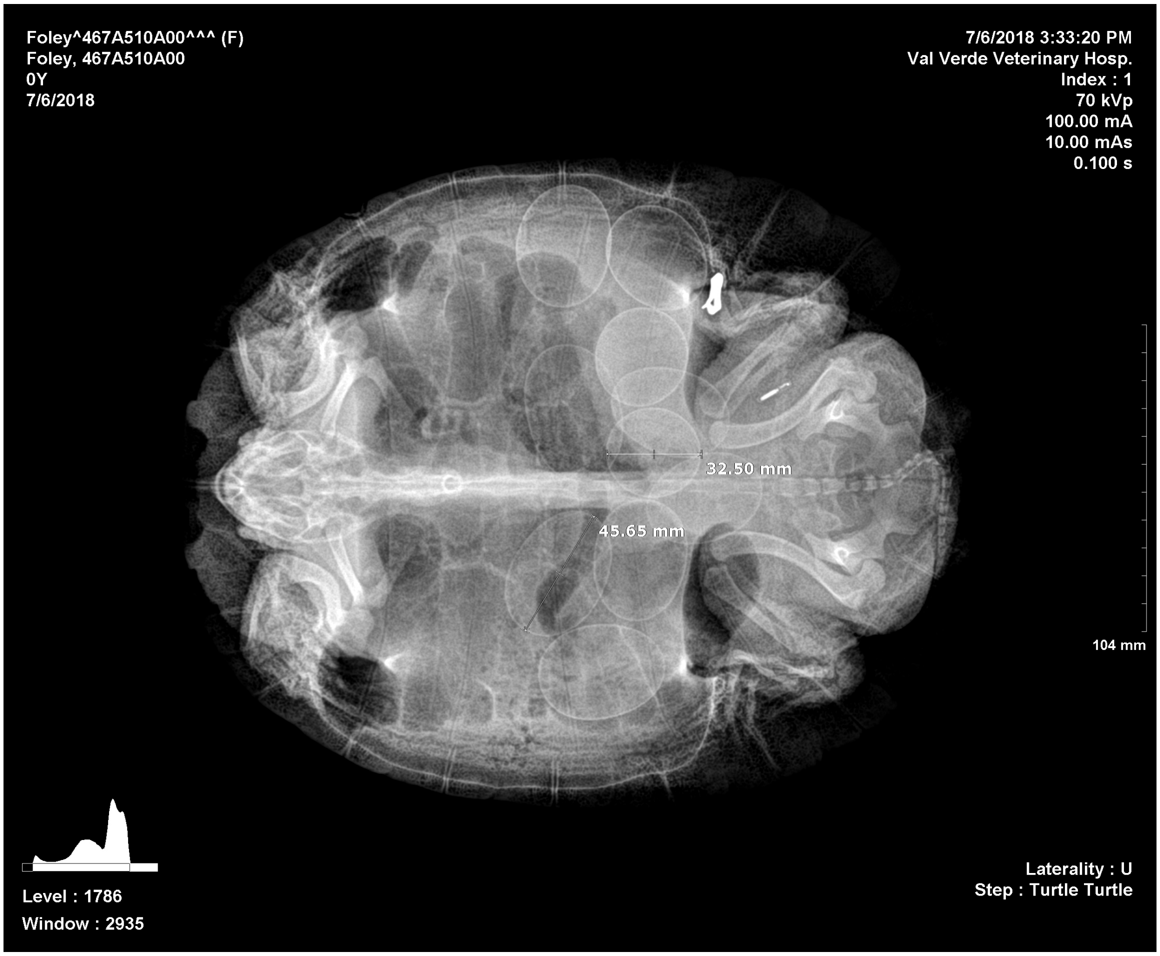

Fig. 17 Radiograph of *Pseudemys gorzugi* (ID# 1221). Radiograph taken on July 6th, 2018 showing 10 eggs within a single *Pseudemys gorzugi* female. Measurements were taken of one egg's length (45.66 mm) and one eggs width (32.5 mm).

Fig. 18 Radiograph of *Pseudemys gorzugi* (ID# 470151183F). Radiograph taken on June 8th, 2018 showing 12 eggs within a single *Pseudemys gorzugi* female. Measurements were taken of one egg's length (48.05 mm) and one eggs width (33.55 mm).

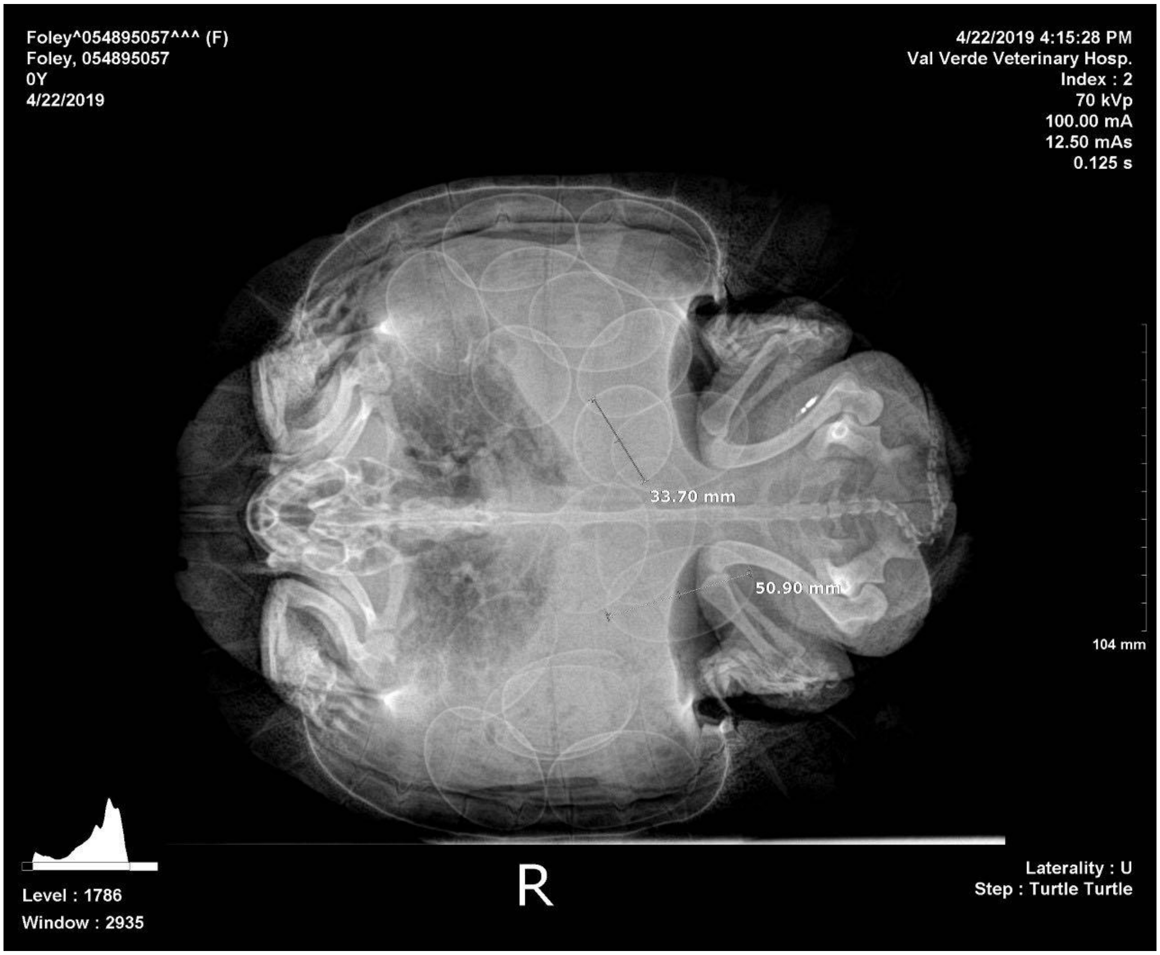

Fig. 19 Radiograph of *Pseudemys gorzugi* (ID# 054895057). Radiograph taken on April 22nd, 2019 showing 17 eggs within a single *Pseudemys gorzugi* female. Measurements were taken of one egg's length (50.9 mm) and one eggs width (33.7 mm).

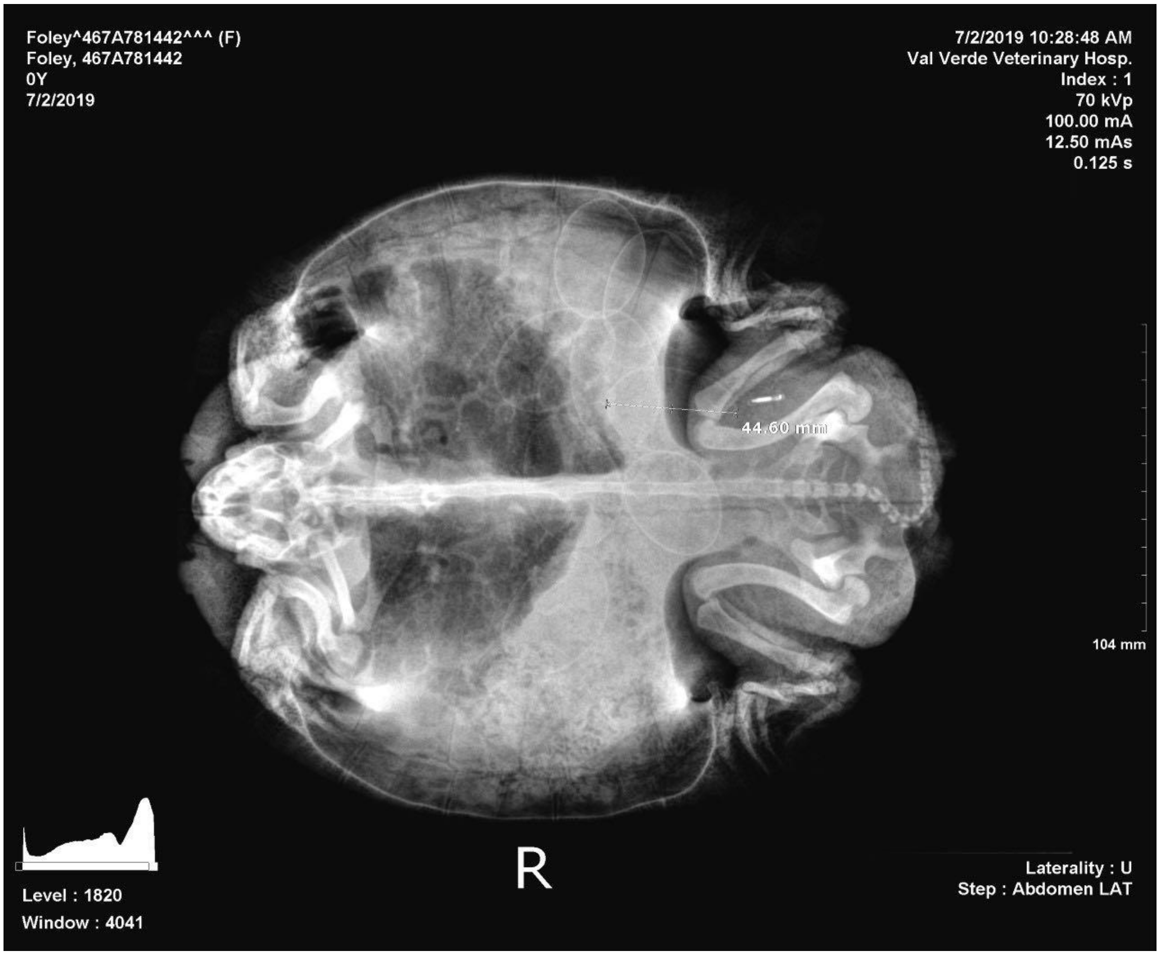

Fig. 20 Radiograph of *Pseudemys gorzugi* (ID#467A781442). Radiograph taken on July 2nd, 2019 showing 8 eggs within a single *Pseudemys gorzugi* female. Measurements were taken of one egg's length (44.6 mm). These eggs are very lightly shelled indicating that the ultimate clutch size could be greater than 8.

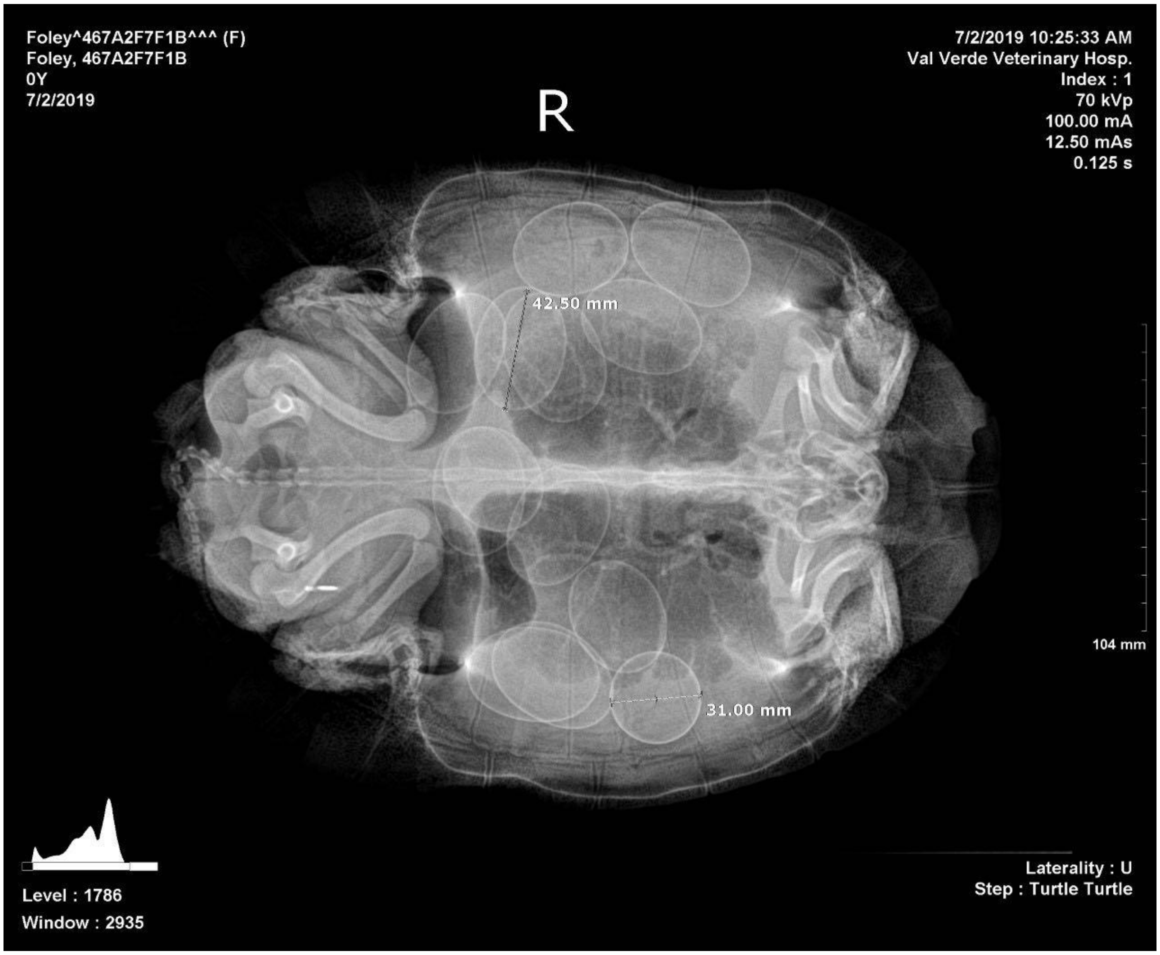

Fig. 21 Radiograph of *Pseudemys gorzugi* (ID# 467A2F7F1B). Radiograph taken on July 2nd, 2019 showing 13 eggs within a single *Pseudemys. gorzugi* female. Measurements were taken of one egg's length (42.5 mm) and one eggs width (31.0 mm).

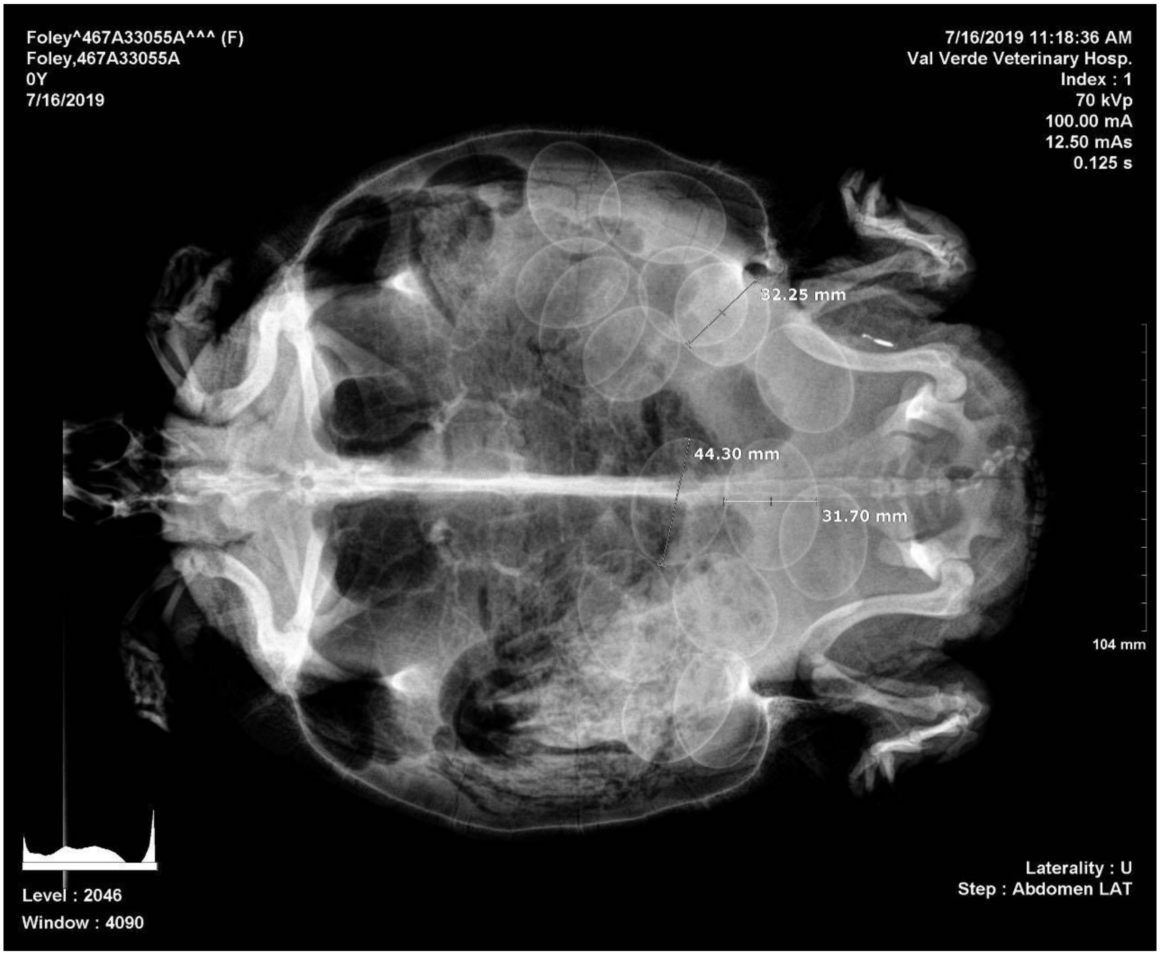

Fig. 22 Radiograph of *Pseudemys gorzugi* (ID# 467A33055A). Radiograph taken on July 2nd, 2019 showing 15 eggs within a single *Pseudemys. gorzugi* female. Measurements were taken of one egg's length (44.3 mm) and two eggs width (31.98 mm avg.).

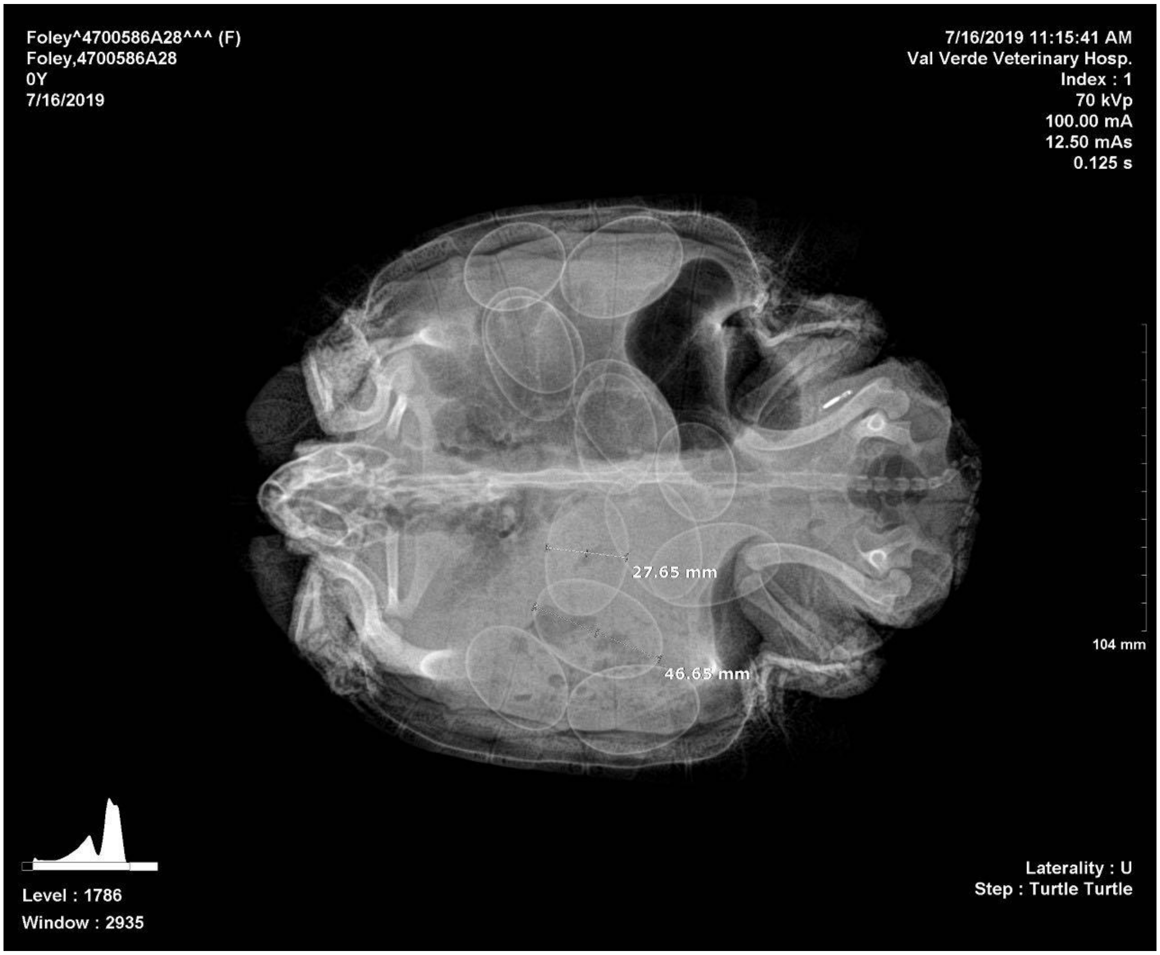

Fig. 23 Radiograph of *Pseudemys gorzugi* (ID# 4700586A28). Radiograph taken on July 2nd, 2019 showing 13 eggs within a single *Pseudemys gorzugi* female. Measurements were taken of one egg's length (46.65 mm) and one eggs width (27.65 mm).

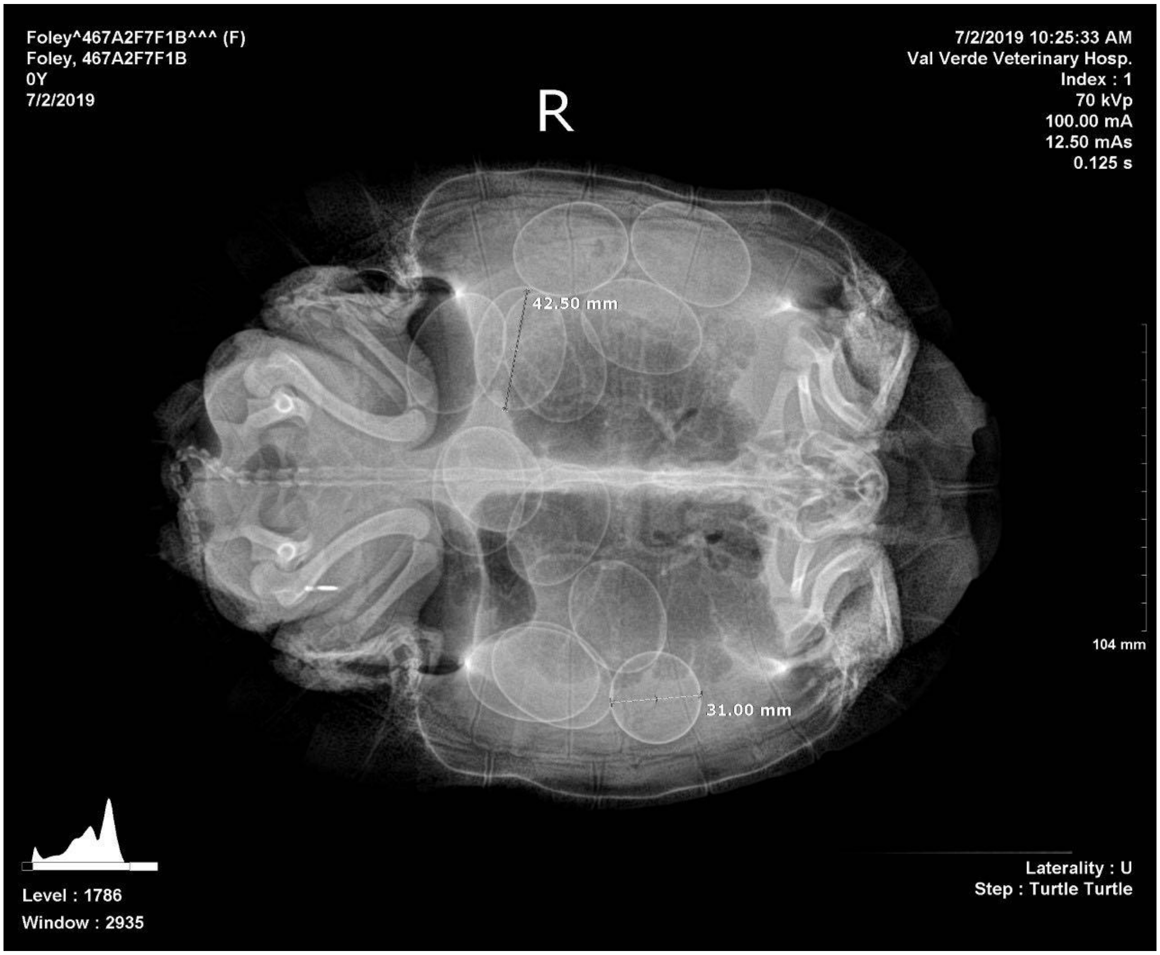

Fig. 24 Radiograph of *Pseudemys gorzugi* (ID# 467A2F7F1B). Radiograph taken on July 2nd, 2019 showing 13 eggs within a single *Pseudemys gorzugi* female. Measurements were taken of one egg's length (42.5 mm) and one eggs width (31 mm).

Fig. 25 Radiograph of *Pseudemys gorzugi* (ID# 054781873). Radiograph taken on August 13th, 2019 showing 13 eggs within a single *Pseudemys gorzugi* female. Measurements were taken of one egg's length (53.95 mm) and one eggs width (32.95 mm). This individual was found bearing eggs at the latest date known for this species.

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