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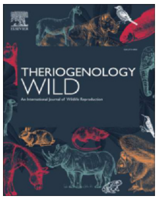

Ovarian cycle, reproductive performance and breeding seasonality of Amazonian yellow-footed tortoises (*Chelonoidis denticulatus*) in the wild

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ABSTRACT

Studies of the reproductive functional morphology of chelonians are important to understand the reproductive physiology, anatomy, and endocrinology of these species. However, data on the reproduction of this group have been difficult to obtain because of their cryptic habits and as a result the consequent inefficiency of conventional field methods in detecting individuals in the wild. In this study, we obtained reproductive organs from 347 adult female yellow-footed tortoises (*Chelonoidis denticulatus*), one of the most hunted and traded land vertebrates in South America, donated by local sellers in a wild meat market in the Peruvian Amazon. We described the species' reproductive cycle and the influence of climatic factors on reproductive performance. Of the sampled females, 116 (33.4%) were gravid and 231 (66.6%) were non-gravid, including 215 vitellogenic (62.0%) and 16 non-vitellogenic (4.6%). The mean number of ovarian follicles per female declined from 28.5 ($<$ 10 mm diameter), to 7.1 follicles (20–40 mm) to 3.2 follicles $>$ 40 mm. Gravid females had in average 3.7 shelled eggs in the oviduct, with a mean egg diameter of 53.7 mm. The estimated annual reproductive potential in gravid females was 5.1 eggs per clutch, ranging from one to 22 eggs. There was a strong positive relationship between the diameter of shelled eggs and the straight carapace length of gravid females. The final phase of the folicular growth, the most demanding energetic process in chelonian reproduction, correlated with annual rainfall peak, while oviposition was estimated to occur in the dry season. The yellow-footed tortoise should be considered an opportunistic seasonal breeder, though capable of reproducing throughout the year. Reproductive yields are linked to climatic events that influence food availability in the environment. To improve the sustainable use of this species, adult females should be harvested primarily during reproductive quiescence, from the end of the laying period in the late dry season to the mid-rainy season when large follicles appear, to avoid taking gravid females. Our results are useful not just to better understand the reproductive biology of terrestrial chelonians but can inform the conservation management of harvested species.

1. Introduction

Chelonians are oviparous shelled reptiles that have been hunted for centuries. This group of animals has been used as a source of high-quality food, oil, and income from the sale of whole individuals for the domestic and international pet market, meat and other subproducts [1,2]. Due to high rates of extraction, chelonians are currently classified as one of the most threatened taxonomic groups in the world, where populations of many species are suffering decreases or local extinctions [3].

Studies of the reproductive biology of any species are essential to better understand the demographics and dynamics of wild populations, and to describe the responses of populations to various levels of human disturbance [4-6]. In chelonians, the female reproductive cycle can be divided into four phases: vitellogenesis (follicular growth), ovulation and intrauterine period, nesting period and latent period [7]. Chelonians differ in their vitellogenesis process, presenting either short periods of follicular growth just before oviposition (prenuptial vitellogenesis), or extended periods after oviposition before entering quiescence

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(postnuptial vitellogenesis) [8]. This variation in ovarian activity results in two distinct reproductive patterns: (1) continuous with variable intensity levels of reproductive activity over the year; or (2) discontinuous with periods of extended gonadal activity alternating with periods of quiescence [8].

The yellow-footed tortoise (*Chelonoidis denticulatus*) is amongst the most hunted and traded vertebrates in South America, particularly in the Amazon Basin; some populations here are being heavily depleted as a result of overexploitation [9,10]. Most of the information on the reproductive performance of this species have been derived from captive studies or from anecdotical records. Results from these studies indicate that the species reaches sexual maturity between 12 and 15 years old, at 250 mm of carapace length [11-13]. Nesting information is scarce and unclear; while some authors reported that eggs are buried in the ground, others claim that gravid females lay eggs exposed on the ground surface or just cover them with leaf litter [11,12]. The yellow-footed tortoise can lay an average of five (range 3–15) spherical eggs, 48 mm average diameter, with rigid but slightly flexible shells [11,12,14]. The reported incubation period may vary from 128 to 152 days [14].

Understanding the reproductive biology of chelonians is important for designing conservation strategies. However, the reproductive biology of terrestrial chelonians is often difficult to study in the tropics due to the cryptic habits of the species and the difficulty in acquiring biological information from specimens in the wild [15]. The yellow-footed tortoise is extensively traded in Amazonian markets [16] thus, by involving vendors in participatory data collection it is possible to gather sufficient data on the species' reproductive biology and use this information to inform community-based management schemes to encourage the sustainable use of the species. In this study, we employ participatory data collection by local wild meat vendors in the Belén Market in Iquitos, one of the most important wild meat markets in the Peruvian Amazon [17,18], to describe the reproductive cycle of the yellow-footed tortoise from the Napo River basin.

2. Material and methods

2.1. Biological sampling

Although wildlife trade is prohibited by Peruvian Law No. 29763 of 2011, the sale of tortoise meat occurs openly. We obtained information on the reproductive cycle of wild yellow-footed tortoises sold in the Belén Market in Iquitos from regular wild meat sellers. Because of the long-term trusting relationship between researchers and tortoise sellers from decades of market monitoring [17,18], we trained one tortoise seller to take standardized photographs of fresh genital organs (ovaries and oviducts) and measure carapaces of butchered tortoises from November 2014 to October 2016 (Fig. 1A).

All sampled tortoises were captured in the Napo River basin, shipped to the market and subsequently sacrificed. We recorded the date each tortoise was butchered, but we had no date of capture; however, the tortoise seller estimated it took between 1 and 2 weeks from capture to butchering of the animals. All photographs were taken with an autofocus digital camera (Camkory®, Model ES-DC311L-AF-BL +32G) at an approximate distance of 40 cm from the subject material (ovaries). The seller would open the ovaries as much as possible so to be able to view the main ovarian bodies (visibility of the minor ovarian bodies was occasionally obscured). The seller would include a coded paper when photographing the ovaries to be able to identify each tortoise picture and match the carapace with its respective genitalia. To scale all photographs and allow precise measurement of the size of follicles and eggs, as well as carapace length, the seller also included a coin with a known diameter (25.5 mm) at the same distance from ovaries.

2.2. Reproductive data

We analysed one photograph per female using the software ImageJ [National Institutes of Health, http://rsb.info.nih.gov/ij/), using the coin measure as a calibration scale (Analyze → Set Scale function). Reproductive parameters were recorded for right and left ovaries. These included: a) number of follicles, shelled eggs (eggs in the oviduct with developing or already formed calcified outer covering), corpora lutea (CL) and corpora atretica (CA), b) diameter of all follicles and shelled eggs, and c) diameter of the largest follicle (hereafter "maximum follicular diameter"). The diameter of each follicle and shelled egg was calculated as the mean length of the two maximum perpendicular axes. Ovarian follicles were classified into three classes according to the vitellogenesis process: early (< 10 mm), intermediate (10–30 mm) and preovulatory follicles (> 30 mm) [19]. The CL was characterized as a cystic fibrotic cuplike structure with a haemorrhagic or dark area (Fig. 1B); while the atretic CL was completely fibrotic and whitish (Fig. 1C).

Due to the length of the genital organs, the oviducts were photographed obliquely (inclination estimated at 15º), so as not to generate a difference in the calculation of the diameter of the shelled eggs. Following the expected magnification error of approximately 1% per centimeter of distance from the perpendicular axis due to projection geometry well described for rotational panoramic radiographs [20,21], we calculated egg diameters by multiplying the image diameter by the associated deviation from perpendicular registration (e. g., image x 1.05 for eggs photographed 5 cm from the perpendicular center of the photograph).

We classified females into gravid and non-gravid individuals. Gravid females are characterized by the presence of shelled eggs. Non-gravid females are characterized by the absence of shelled eggs and were classified into non-vitellogenic (absence of intermediate and preovulatory follicles), and vitellogenic (presence of intermediate and preovulatory follicles) females.

We calculated the gravidity rate as the number of gravid females per total adult females. The potential reproductive production in gravid females was calculated as the sum of preovulatory follicles (> 40 mm) and corpora lutea [22]. We also measured the straight length of the carapace (hereafter "carapace length").

2.3. Climatological data

We obtained data on the daily rainfall and river water level for the studied period from the Bellavista Hydrological Station (76º33'00"W, 07º03'00" S), located in the Mazan district on the Napo River, provided by the Peruvian National Meteorology and Hydrology Service [23].

2.4. Data analysis

Two-way ANOVAs were used to assess the differences in the reproductive parameters between females with distinct reproductive status (gravid vs non-gravid) and between reproductive seasons (high vs low reproductive season). We considered months with $\geq 50\%$ of gravid females as the high reproductive season (May-October), and the months with $< 50\%$ of gravid females as the low reproductive season (September-April).

Generalized Linear Models (GLM) were used to assess the relationship of the carapace length with the number of follicles (Reverse Gumbel distribution, link function identity), the number of large follicles and shelled eggs (Poisson distribution, link function log), and the probability of presence of preovulatory follicles and shelled eggs, i.e., females were assigned value 1 when preovulatory follicles/shelled eggs were present and 0 when not present (Binomial distribution, link function logit). We also used a GLM to test the association between the carapace length with the number of shelled eggs and the mean diameter

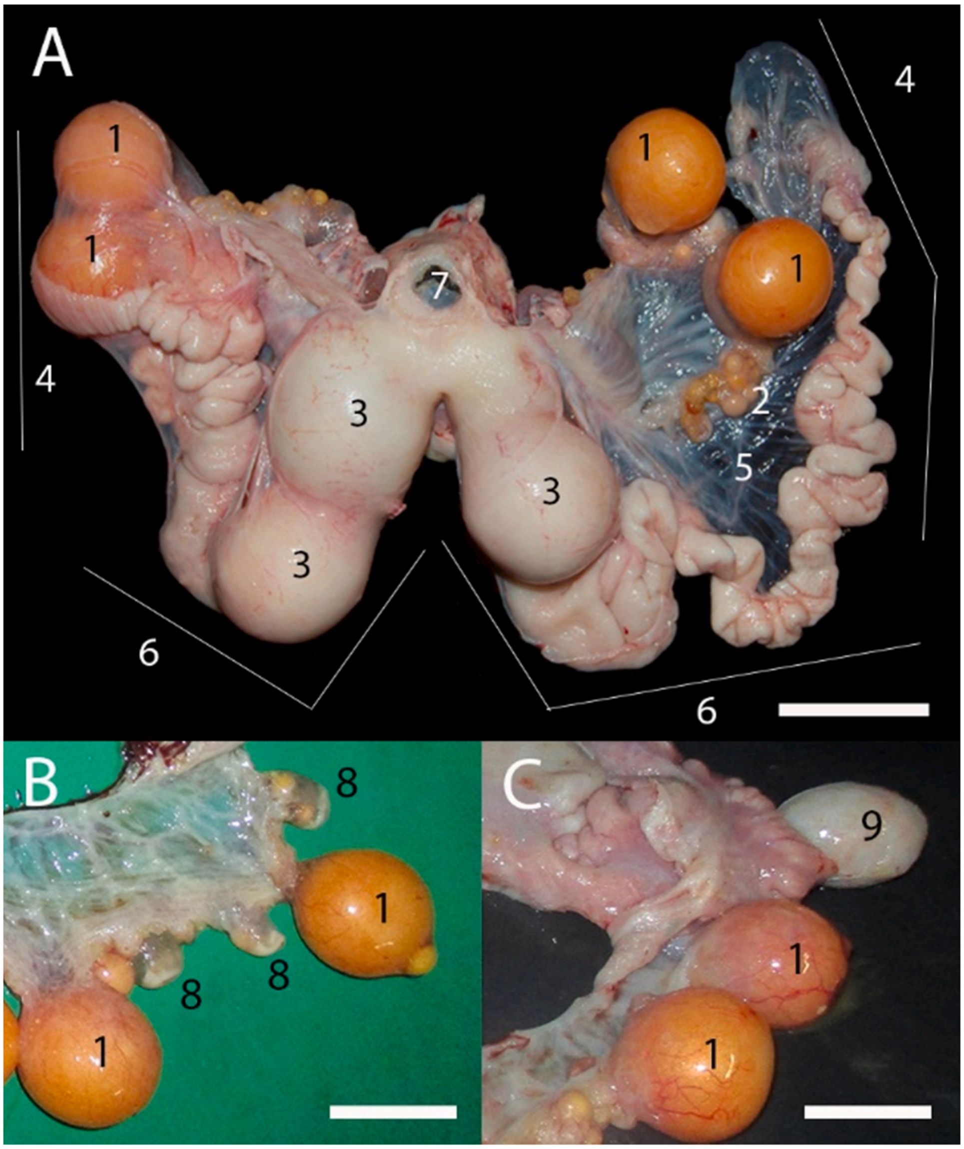

Fig. 1. Dorsal view of the genital organs of a gravid yellow-footed tortoise female (*Chelonoidis denticulatus*). (A) The micrograph shows the ovaries with large pre-ovulatory follicles (1), corpus luteum (2), shelled eggs (3), the infundibulum (4), the mesotubarum (5), the oviduct (6) and the cloaca (7); scale bar = 5 cm. (B) Image of CL (8); scale bar = 2.5 cm. (C) Atretic CL (9); scale bar = 2.5 cm.

of shelled eggs in gravid females (Box-Cox Normal distribution – link function identity). We selected the families of distribution based on the Q-Q plots (quantile-quantile plot) of the residuals.

We applied cross-correlation analyses to assess possible breeding seasonality in the species by identifying time lags and relationships between the presence of ovarian structures with rainfall and river water level. To do so, we used fortnights (half-month) as our time scale. For each fortnight, we calculated the mean number of follicles per class, shelled eggs, CL and CA, mean follicle diameter, percentage of gravid females, and average rainfall and river water level. We plotted time-series graphs of the annual variation of these parameters using values from each sampled tortoise to estimate the standard error in each fortnight period. We used the functions ccf for the cross-correlations, gamlss for the GLMs and ggplot for producing the graphs. All analyses were conducted in R (version 3.6.3). Differences with a probability value of 0.05 or less were considered statistically significant.

3. Results

3.1. Reproductive parameters

A total of 347 mature yellow-footed tortoise females were obtained in this study. Of these, 116 (33.4%) were gravid and 231 (66.6%) were non-gravid, including 215 vitellogenic (62.0%) and 16 non-vitellogenic (4.6%) non-gravid females. The mean number of ovarian follicles per female was 40.34 (SD 18.7), and mean number of shelled eggs 1.22 (SD 1.97) per female.

The mean follicle diameter in all females was 11.63 mm (SD 7.90; n = 14020), the average maximum follicle diameter was 35.43 mm (SD 8.84; n = 347) with the absolute maximum follicle diameter 58.24 mm. The average maximum follicle diameter was 33.62 mm (SD 8.79; n = 231) in non-gravid and 39.04 mm (SD 7.82; n = 116) in gravid females. The number of ovarian follicles per female decreased with

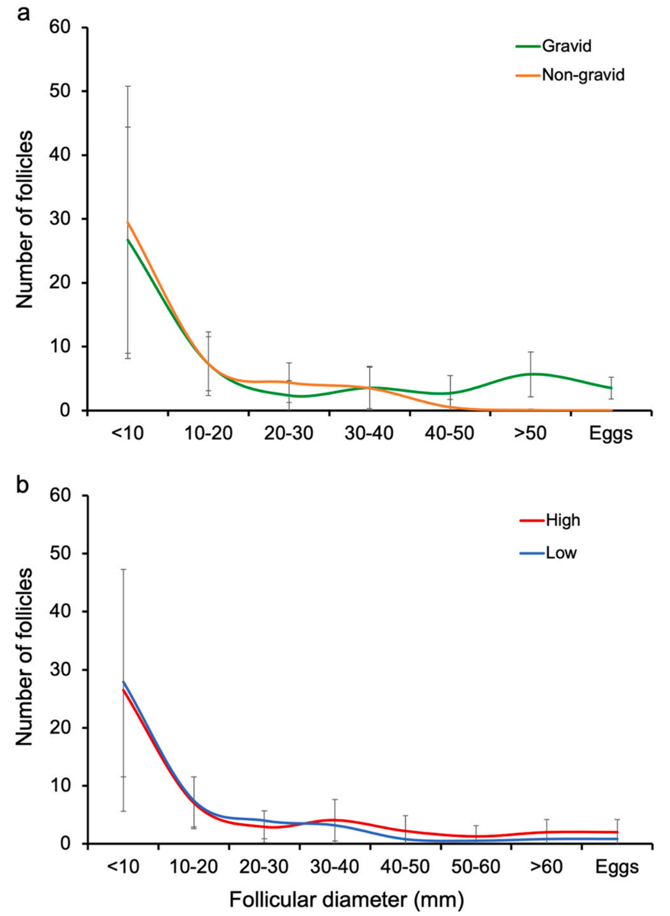

Fig. 2. Size distribution of ovarian follicles and eggs in female yellow-footed tortoises (*Chelonoidis denticulatus*): A) in gravid (n = 116) and non-gravid (n = 231) females; and B) in the high (May-October; n = 115) and low (November-April; n = 232) reproductive season. Whiskers represent the 95% CI.

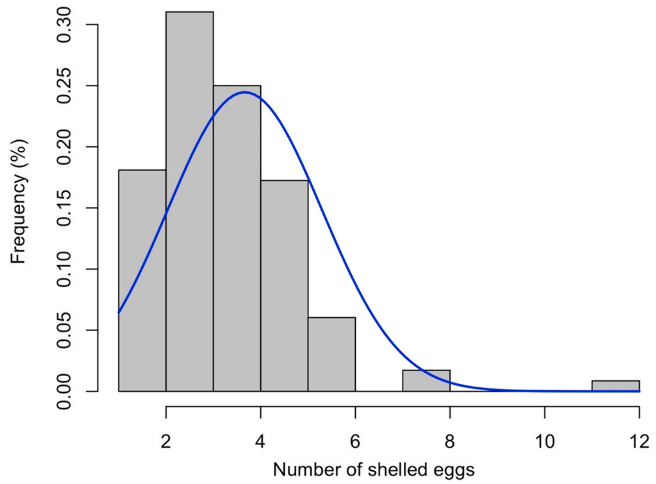

Fig. 3. Frequency of gravid yellow-footed tortoise (*Chelonoidis denticulatus*) females (n = 116) according to the number of shelled eggs.

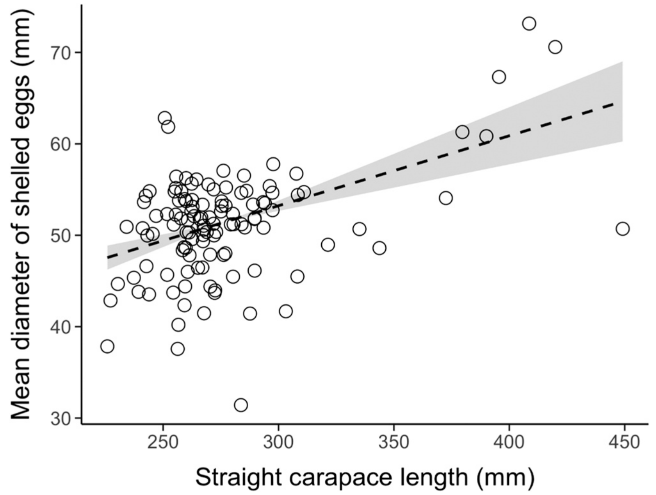

Fig. 4. Relationship between the mean diameter of shelled eggs and the straight carapace length in gravid yellow-footed tortoises (*Chelonoidis denticulatus*; n = 116).

follicle diameter from 28.5 (SD 20.2) ovarian follicles < 10 mm in diameter, to 7.3 (SD 4.5) follicles 10–20 mm, 7.1 (SD 4.5) follicles 20–40 mm, and finally to 3.2 (SD 4.9) follicles > 40 mm.

Gravid and non-gravid females had the same number of ovarian follicles < 40 mm; but gravid females had more large follicles than non-gravid females (estimate = 4.95, t = 2.342, p = 0.0198; Fig. 2), resulting in a strong tendency for gravid females to have a higher average follicle diameter (estimate = 0.996, t = 1.895, p = 0.0589), and a higher maximum follicle diameter (estimate = 3.809, t = 3.521, p = 0.000488).

Gravid females had 3.66 (SD 1.63, range 1–12) shelled eggs in the oviduct (Fig. 3). The estimated reproductive potential in gravid females was 5.09 eggs (SD 3.78, range 1–22) per nesting. Considering the average diameter in each clutch per female, the average diameter of the egg was 53.68 mm (SD 6.30, N = 116), ranging from 32.99 to 76.82 mm (Supplementary Table 1). The difference between the two perpendicular diameters was 95.5%. There was no association between the number of shelled eggs carried by gravid females and the egg diameter (estimate = −0.35, t = −1.49, p = 0.14). Compared to non-gravid females, gravid females had more corpora lutea (estimate = 1.1608, t = 3.329, p = 0.000964), but no difference in the number of CA (estimate = 0.1451, t = 0.214, p = 0.4981). We did not observe any correspondence between the number of eggs and CL (P = 0.813).

The mean carapace length of all females was 281 mm (SD 51). There was a strong positive relationship between the diameter of shelled eggs (estimate = 0.077, t = 6.38, p < 0.0001) and the largest follicle (estimate = 287.2, t = 25.16, p < 0.0001), and the carapace length of gravid females (Fig. 4). Small (carapace length ≤275 mm, medium (between 275 and 350 cm) and large females (≥350 mm) had an average egg diameter of 52.47 (SD 5.24, N = 71), 53.74 (SD 5.37, N = 38) and 65.69 (SD 8.75, N = 7), respectively. There was no relationship between the number of shelled eggs and the carapace length in gravid females (estimate = −0.00003, t-value = −0.023, p = 0.98). Carapace length did not differ in females according with the gravidity status (estimate = 6.109, t = 1.045, p = 0.297), nor with their vitellogenic status (estimate = 11.928, t = 0.809, p = 0.419).

3.2. Annual reproductive performance

The timing of the follicular growth was as follows (Supplementary Table 2, Fig. 5): a) between August and December, peak of number of early follicles; b) between January and July, higher number of intermediate and preovulatory follicles; c) between April and August, highest mean follicular diameter; and d) between May and October, higher number of shelled eggs and percentage of gravid females. There was no seasonal pattern in the mean number of CL and CA over the year (Fig. 6).

The number of preovulatory follicles at time 0 (no time lag) was negatively associated with the number of early (r = −0.447, p = 0.029) and intermediate follicles (r = −0.414 p = 0.044). The

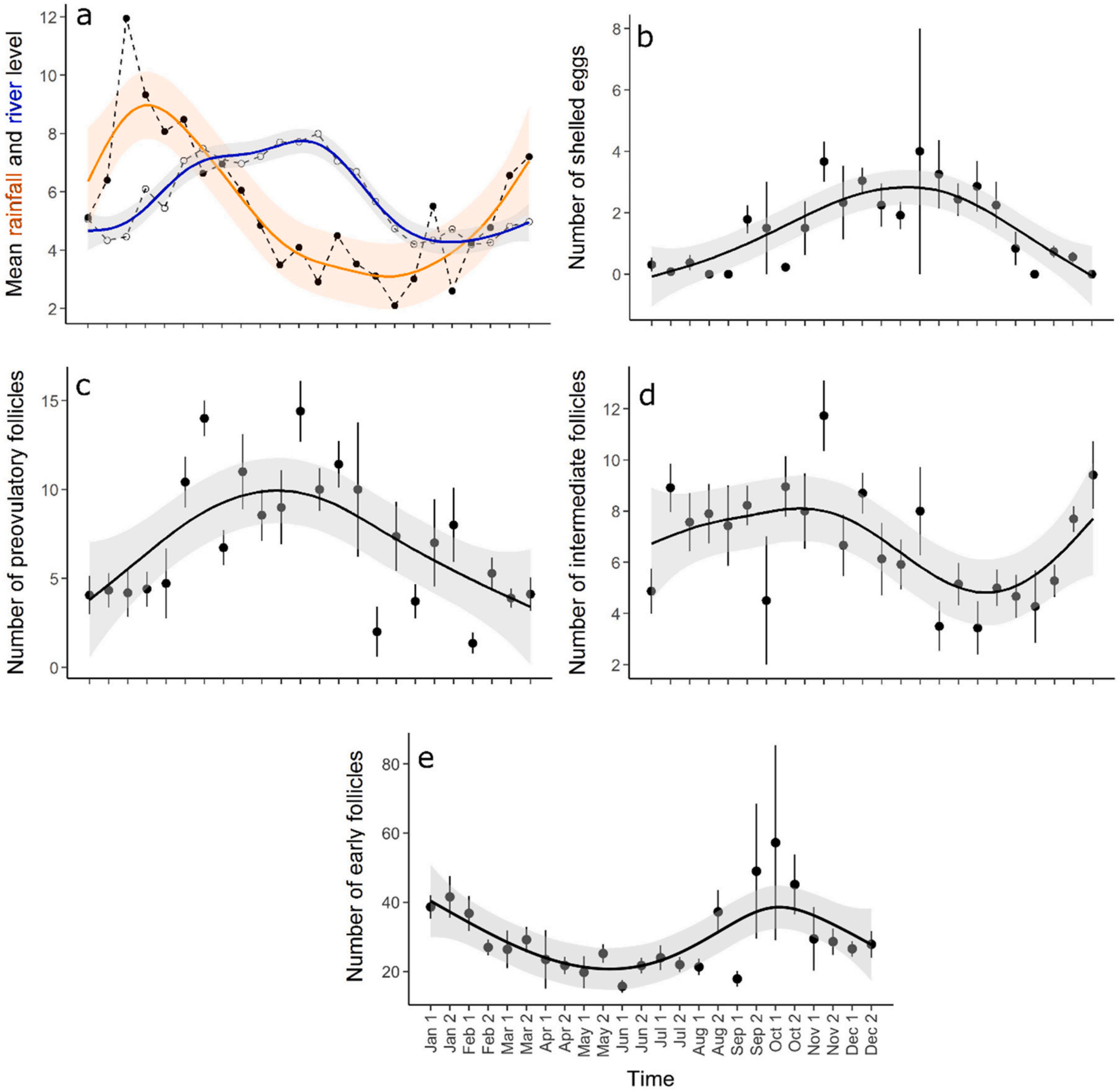

Fig. 5. Annual variations of climate measures, and reproductive parameters (mean, 95% CI) of the yellow-footed tortoise (*Chelonoidis denticulatus*) female (n = 347) collected from the Napo River basin: a) rainfall (orange) and water level (blue) in the Napo River; b) number of shelled eggs; c) number of preovulatory follicles (diameter > 30 mm); d) number of intermediate follicles (10–30 mm); and e) number of early follicles (< 10 mm). We used fortnights (half-month) as our time scale. River water levels had a lagging rainfall by + 2 months (r = 0.665, p = 0.0004). The number of preovulatory follicles showed a positive relationship with the river water level at time 0 (no time lag) (r = 0.770, p = 0.00001), and with rainfall + 2 month time-lag (r = 0.541, p = 0.006). Number of shelled eggs was positively associated with the river water level + 1.5 month time-lag (r = 0.750, p = 0.00002), and with rainfall + 3.5 month time-lag (r = 0.516, p = 0.01; gravid females: r = 0.421, p = 0.04).

number of preovulatory follicles showed a strong positive relationship with the number of shelled eggs with a time lag of + 1.5 months (r = 0.687, p = 0.0002) (Fig. 5). There was no temporal relationship between the number of any follicle class or shelled eggs with the number of CL or CA (r < 0.39, p > 0.05).

We identified two seasons with different reproductive performances (Supplementary Table 2): a) between May and September, a high reproductive performance with a gravidity rate > 50%, and b) between October and April, a low reproductive performance with a gravidity rate < 50%. The carapace length did not differ in females according with the reproductive season (estimate = 3.331, t = 0.548, p = 0.584). All non-vitellogenic females were observed in the low reproductive season.

Females in the high reproductive season had a higher average follicle diameter (estimate = 1.7830, t = 2.907, p = 0.00388; and estimate = 3.543, t = 3.154, p = 0.001754, respectively), higher maximum follicle diameter (estimate = 3.543, t = 3.154, p = 0.001754), and higher number of eggs (estimate = 2.001, t = 9.681, p < 2e-16). There was no significant effect of the reproductive season on the number of ovarian follicles (estimate = 2.700, t = 1.222, p = 0.222), egg diameter (estimate = 0.8262, t = 0.726, p = 0.469), number of CL (estimate = 0.4326, t = 1.179, p = 0.239), or number of CA (estimate = 0.1451, t = 0.678, p = 0.4981).

3.3. Reproductive seasonality

Compared to other months, rainfall levels were higher between January and April, while river water levels are higher between March and July, lagging rainfall by + 2 months (Fig. 5). Both environmental parameters were cues for the number of preovulatory follicles and shelled eggs, and the percentage of gravid females. The number of preovulatory follicles showed a positive relationship with river water level at time 0 (no time lag), and with rainfall + 2 month time-lag. Number of shelled eggs and percentage of gravid females were

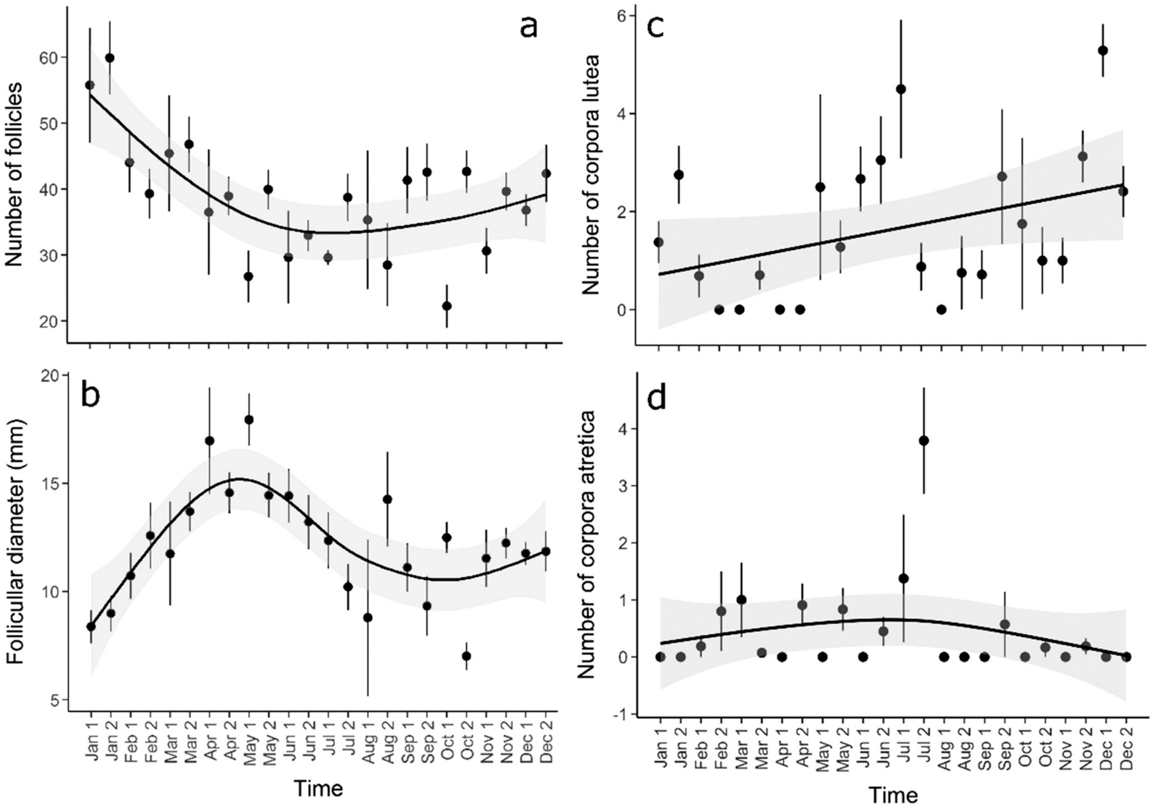

Fig. 6. Seasonal distribution of ovarian structures in the yellow-footed tortoise (*Chelonoidis denticulatus*; n = 347): a) number of ovarian follicles; b) average follicular diameter; c) number of corpora lutea; and d) number of corpora atretica. We used fortnights (half-month) as our time scale.

positively associated with river water level + 1.5 month time-lag and with rainfall + 3.5 month time-lag (Figs. 5 and 7).

4. Discussion

Reproductive information is critical for the development of management strategies for the sustainable use of any species [24]. However, although turtles are among the most threated vertebrates in South America [9], their reproductive biology remains poorly studied [15]. This study on the functional morphology of the female genital organs describes the reproductive cycle and reproductive performance of one of the most harvested chelonian in the Amazon, the yellow-footed tortoise.

4.1. Opportunist seasonal breeder

Main reproductive events are usually timed to occur during the most propitious periods for the survival of the parents and offspring. Our results show that, as in other terrestrial chelonians [25,26], the female yellow-footed tortoise should be considered an opportunist seasonal breeder, capable of breeding across the year, but showing different reproductive yields according to climatic events. In the Napo River basin in particular, we found a seasonal concentration of gravid females between May and October.

4.2. Development of ovarian follicles and eggs

We observed all four reproductive phases in the yellow-footed tortoise. The presence of follicles at all stages of maturation in the same female suggests varying degrees of sensitivity to the ovulation-inducing gonadotropins, such as luteinizing hormone [27]. During vitellogenesis, selected follicles are activated to undergo follicular development and yolk accumulation, growing in average diameter from 12 to 35 mm, reaching a maximum follicular diameter of 58 mm. This follicular size is compatible with observations in *Geochelone nigra*, where follicles became preovulatory at a diameter of 40–42 mm [19].

The ovarian follicular hierarchy, characteristic of reptiles and birds, allows the sequential ovulation of few dominant follicles, improving the viability of each ovulated follicle and reducing the probable losses due to the simultaneous ovulation of several follicles, consequently decreasing the punctual energy investment and distributing it over time [22,27,28]. The ovarian follicular hierarchy also promotes atresia of non-dominant follicles [27]. Due to the difficulty of observation because atresia involves resorption of the follicle, we did not assess follicular atresia in experimental females.

After ovulation, the oocytes are collected by the oviduct's infundibulum and pass into the oviduct, where they are covered by a layer of albumin around the yolk [29]. As the oocyte passes through the oviduct, the layers of the shell membrane increase and thicken gradually. During this process, the egg increases in size, from an average preovulatory diameter of 35 mm to an average shelled egg diameter of 54 mm. After six to seven days, these shelled eggs undergo calcification and are deposited in the oviduct, where they remain for several days until they go to the cloaca and are oviposited [30].

The eggs of the yellow-footed tortoise were larger than those of similar sized species (*Testudines hermanni, T. graeca* and *T. marginata* with an average diameter of 32.9, 32.3 and 31.5 mm, respectively [31]), and similar to those of larger tortoises (*G. nigrans* with 55–68 mm [32]). Considering the value of the preovulatory follicle as a proxy for the size of the yolk, 62.7% of the egg is represented by yolk in the yellow-footed tortoise, similar to that observed in *G. nigra* (62.5% [32]), suggesting a comparable egg biometry in both species. Furthermore, these values suggest that the biometry of the preovulatory follicle is correct.

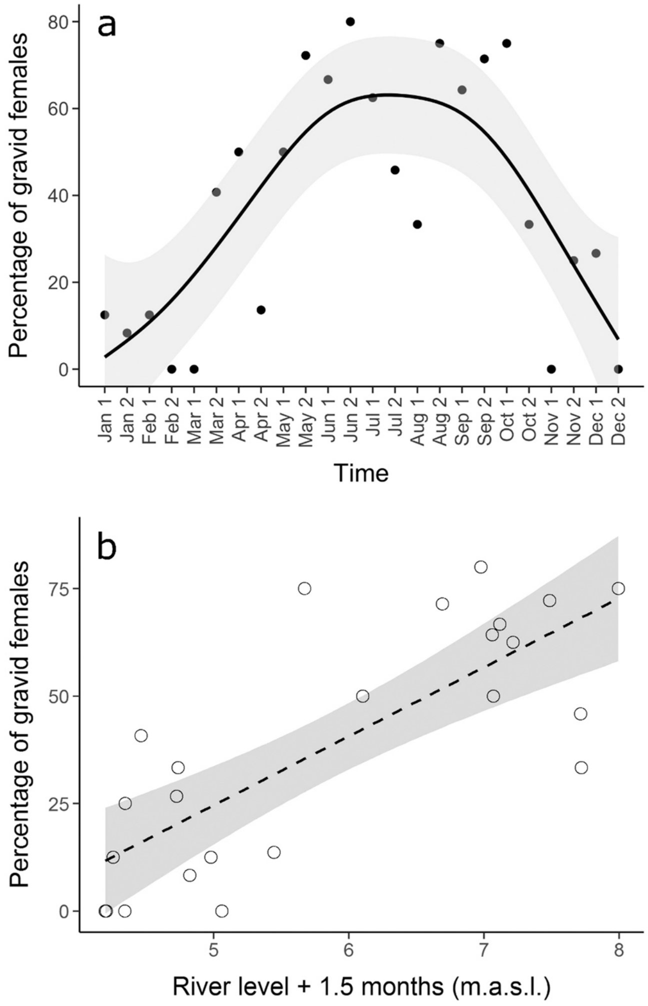

Fig. 7. Percentage of gravid yellow-footed tortoise (*Chelonoidis denticulatus*; n = 116) females distributed across the year; for two 15-d periods per month (a) and related with the rainfall + 1.5 month time-lag (b). Lines represent averages and grey areas indicates 95% confidence interval. We used fortnights (half-month) as our time scale. Percentage of gravid females was positively associated with the river water level + 1.5 month time-lag (gravid females: r = 0.667, p = 0.0003), and with rainfall + 3.5 month time-lag (r = 0.421, p = 0.04).

4.3. Corpora lutea and reproductive performance

Concomitantly with ovulation, the empty follicle collapses, the follicular cells hypertrophy and develop a cup-shaped CL about 15–25% of the diameter of the mature follicle [33]. In the yellow-footed tortoise, the CL had an average diameter of 13 mm, which represented 25.5% of the preovulatory diameter. Corpora lutea produce progesterone in response to the luteinizing hormone [34], and promote the production of albumin and the formation of external membranes in the oviducts [35]. As observed in our study, CL are present in gravid females [34], and although they regress almost immediately, they remain visible throughout the breeding season [36-38]. Thus, CL may be a good indicator of reproductive activity [39]; the potential reproductive production can be calculated as the sum of preovulatory follicles and CL [22]. In our study, the potential reproductive production in the yellow-footed tortoise was five eggs per gravid female. Although we cannot estimate the total egg production because the sampling strategy stopped the reproduction process, total egg production range between 3 and 15 eggs in other studies [11,12,14]. The lack of correspondence between the number of CL and shelled eggs suggests that this species is not retaining eggs for a unique clutch, but rather a continuous clutch. This information has been frequently communicated by local indigenous hunters [Mayor, pers. comm.].

4.4. Body size effect

We observed a strong positive relationship between carapace length of gravid females and diameter of follicles and shelled eggs. Previous studies report that egg size is determined by adult body size, pelvic opening morphology, resource availability, and temperature [40]. Therefore, larger females have appropriate energy reserves to develop larger eggs, which are related to higher yolk mass, good embryonic development, and higher offspring survival [41]. There was no relationship between the carapace length and the number of shelled eggs per gravid female.

4.5. Seasonal effects

For the yellow-footed tortoise, the rainy season coincides with the final phase of vitellogenesis, when large follicles are developed. This phase was parallel to the beginning of the annual rainfall peak; two months later the peak of preovulatory follicles occurred, and 3.5 months later the highest frequency of shelled eggs and gravid females was observed. Thus, intermediary follicles require two months to being preovulatory follicles, and additional 1.5 months to being ovulated and complete their egg development. In contrast, *Chrysemys picta, Chelonia mydas* and *Sternotherus odoratus* require only 10–30 days to transform follicles from a non-ovulable to an ovulable state [41].

The peak of shelled eggs and gravid females occurred 1.5 months after the highest level of the river water, which means that egg laying begins when the river water level drops. Considering an incubation period of 140 days (from 128 to 152 days) [14], the hatching period occurs during the low level of river water. These results are compatible with observations in *C. carbonarius* [42] and *C. denticulatus* [43] in the Amazon. The habitat preference of the yellow-footed tortoise varies with the time of year; during the dry season it prefers low and humid areas, while in the rainy season it moves to the *terra firme* [12,44]. This habitat preference may be related to the spawning season. For instance, *C. carbonaria* spawns in the lower areas of the swampy forest near rivers to maintain humidity and prevent desiccation during the incubation period [42]. Chelonians often enter the latent period, also known as quiescence, after extended periods of oviposition [8]. All non-vitellogenic females were found in the low reproductive period when rain and river water levels are low, thus females are probably in quiescence during the remaining months of dry season.

4.6. Methodological considerations

The yellow-footed tortoise is amongst the most hunted and traded vertebrates in South America, particularly in the Amazon [9]. Several studies have shown the potential of involving hunters in the collection of reproductive data on wild species [5], but to our knowledge none has attempted doing the same with vendors in wild meat markets. Our results show that the participatory collection of reproductive information by wild meat vendors is effective to provide valuable reproductive data on yellow-footed tortoises, and the same may be applicable to other traded species. However, we point out the importance of carrying out an adequate standardization of photographs, visualizing main structures, avoiding as much as possible the magnification due to different positions of structures studied. Finally, time lag between the capture and butcher may produce reproductive variations due to multiple factors, such as the stress of captivity, availability of resources, mates, territories [45]. Although this probable bias, our results showed a seasonal pattern of events related to follicular development and egg production.

4.7. Management considerations

The reproduction of the yellow-footed tortoise is characterized by the presence of follicular hierarchy and the ability to reproduce throughout the year, but presenting seasonal variations in percentages of gravid females, influenced by rainfall and river water level.

Based on our findings, to improve the sustainable use of this species, adult females should be harvested primarily from the end of the laying period in the late dry season, until the beginning of the vitellogenesis in the mid rainy season to avoid the harvests of many gravid females. However, this strategy may be challenging as ovaries with large follicles and eggs from chelonians are a valuable and traditional food resource for Amazonian societies. This study provides essential information on the reproductive biology of the yellow-footed tortoise that can be useful for the development of *in situ* and *ex situ* conservation strategies.

CRediT authorship contribution statement

Pedro Mayor: Conceptualization, Methodology, Project administration, Investigation, Formal analysis, Writing – original draft, Supervision. **Sara Hidalgo**: Investigation, Data curation, Writing – review & editing. **Hani R. El Bizri**: Conceptualization, Methodology, Formal Analysis, Writing – review & editing. **Thais Q. Morcatty**: Conceptualization, Methodology, Project administration, Investigation, Data curation, Formal analysis, Writing – original draft, Supervision.

Declaration of Competing of Interest

The authors declare no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.therwi.2023.100022.

References

[1] V. Nijman, C.R. Shepherd, Trade in non-native, CITES-listed, wildlife in Asia, as exemplified by the trade in freshwater turtles and tortoises (Chelonidae) in Thailand, Contrib. Zool. 76 (3) (2007) 207–211.
[2] H.E. El Bizri, T.Q. Morcatty, J. Valsecchi, P. Mayor, J.E.S. Ribeiro, C.F.A. Vasconcelos-Neto, J.S. Oliveira, K.M. Furtado, U.C. Ferreira, C.F.S. Miranda, C.H. Silva, V.L. Lopes, G.P. Lopes, C.C.F. Florindo, R.M. Chagas, V. Nijman, J.E. Fa, Urban wild meat consumption and trade in central Amazonia, Conserv. Biol. 34 (2) (2020) 438–448.
[3] Stanford C.B., Rhodin A.G.J., Van Dijk P.P., Horne B.D., Blanck T., Goode E., Hudson R., Mittermeier R., Currylow A., Eisemberg C., Frankel M., Georges A., Gibbons P., Juvik, J.O., Kuchling, G., Luiselli L., Haitao S., Singh S., Walde A.D., editors. Turtles in trouble: the world's 25+ most endangered tortoises and freshwater turtles - 2018. Turtle Conservation Coalition: IUCN SSC Tortoise and Freshwater Turtle Specialist Group, Turtle Conservancy, Turtle Survival Alliance, Turtle Conservation Fund, Chelonian Research Foundation, Wildlife Conservation Society; 2018.
[4] J.C. Wingfield, G.J. Kenagy, Natural regulation of reproductive cycles, in: M. Schreibman, R.E. Jones (Eds.), Vertebrate Endocrinology: Fundamentals And Biomedical Implications, Academic Press, New York, 1991, pp. 181–241.
[5] P. Mayor, H. El Bizri, R.E. Bodmer, M. Bowler, Assessment of mammal reproduction for hunting sustainability through community-based sampling of species in the wild, Conserv. Biol. 31 (4) (2017) 912–923.
[6] G. Caughley, Analysis of vertebrate populations, John Wiley & Sons, London, 1977.
[7] E.O. Moll, Reproductive cycles and adaptations, in: M. Harless, H. Morlock (Eds.), Turtles perspectives and research, John Wiley & Sons, New York, 1979, pp. 305–331.
[8] P. Licht, Endocrine patterns in the reproductive cycle of turtles, Herpetologica 38 (1) (1982) 51–61.
[9] T.Q. Morcatty, J. Valsecchi, Social, biological, and environmental drivers of the hunting and trade of the endangered yellow-footed tortoise in the Amazon, Ecol. Soc. 20 (3) (2015) art3.
[10] L. Parry, C.A. Peres, Evaluating the use of local ecological knowledge to monitor hunted tropical-forest wildlife over large spatial scales, Ecol. Soc. 20 (3) (2015).
[11] Ferrara C., Kurzmann Fagundes C., Morcatty T.Q., Vogt R.C. Quelônios Amazônicos. Guia de identificação e distribuição. Brasil. Manaus: World Conservation Society; 2017.
[12] A.M. Echeverry-Alcendra, N.M.A. Guzmán, P. Stevenson, J. Cortés-Duque III, Biologia y Conservación de las tortugas continentales de Colombia, in: V.P. Páez, M.A. Morales-Betancourt, C.A. Lasso, O.V. Castaño-Mora, B.C. Bock (Eds.), Biología y Conservación de las Tortugas Continentales de Colombia. Bogotá, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, 2012, pp. 417–423.
[13] Rueda-Almonacid J.V., Carr J.L., Mittenmeier R.A., Rodríguez-Mahecha J.V., Mast R.B., Vogt R.C., Rhodin A.G.J., de la Ossa-Velásquez J., Rueda J.N., Mittenmeier C. G. Las tortugas y los cocodrilianos de los países andinos del Trópico. Colombia: Conservación Internacional- Andes CBC; 2007.
[14] P.E. Vanzolini, A note on the reproduction of *Geochelone carbonaria* and *G. denticulata* (testudines, testudinidae), Rev. Bras. Biol. 59 (4) (1999) 593–608.
[15] T.Q. Morcatty, A.S. Tavares, V. Nijman, J. Valsecchi, Adapting a traditional hunting technique to improve capture rates for the endangered yellow-footed tortoise (*Chelonoidis denticulatus*) during ecological surveys in Amazonia, J. Ethnobiol. 40 (2) (2020) 252–267.
[16] N. D'Cruze, F.E. Rodriguez Galarza, O. Broche, H.R. El Bizri, S. Megson, A. Elwin, F. Carniel Machado, J. Norrey, E. Coulthard, D. Megson, Characterizing trade at the largest wildlife market of Amazonian Peru, Glob. Ecol. Conserv. 28 (2021) e01631.
[17] P. Mayor, H.R. El Bizri, T.Q. Morcatty, K. Moya, N. Bendayán, N. Solis, C.F.A. Vasconcelos-Neto, M. Kirkland, O. Arevalo, T.G. Fang, P.E. Pérez-Peña, R.E. Bodmer, Wild meat trade over the last 45 years in the Peruvian Amazon, Conserv. Biol. 36 (2022) e13801.
[18] P. Mayor, H.R. El Bizri, T.Q. Morcatty, K. Moya, S. Solis, R.E. Bodmer, Assessing the minimum sampling effort required to reliably monitor wild meat trade in urban markets, Front. Ecol. Evol. 7 (2019) 180.
[19] M. Casares, A. Rubel, R.E. Honegger, Observations on the female reproductive cycle of captive giant tortoises (*Geochelone* spp.) using ultrasound scanning, J. Zoo. Wildl. Med. 28 (3) (1997) 267–273.
[20] L. Vazquez, Y. Nizamaldin, C. Combescure, R. Nedir, M. Bischof, D. Ehrenfest, J.-P. Carrel, U.C. Belser, Accuracy of vertical height measurements on direct digital panoramic radiographs using posterior mandibular implants and metal balls as reference objects, Dentomaxillofac. Radiol. 42 (2) (2013) 20110429.
[21] R.L. Read, C.G. Duncan, A.D. Wallace, J.A. Perry, F.M. Duerr, Assessment of on-screen measurements, magnification, and calibration in digital radiography, J. Am. Vet. Med. Assoc. 241 (6) (2012) 782–787.
[22] J. de La Ossa Velasquez, R.C. Vogt, Ciclo ovárico y jerarquía folicular de *Peltocephalus dumerilianus* (Testudines: Podocnemididae), Acta Amaz. 41 (2) (2011) 243–250.
[23] SENAMHI. Datos Hidrometeorológicos a nivel nacional. Serv Nac Meteorol e Hidrol Dir Gen Hidrol y Recur hídricos del Perú. https://senamhi.gob.pe/mapas/mapa-estaciones/_dat_esta_tipo.php?estaciones=240111. [accessed 20 February 2019].
[24] S.C. Stearns, The Evolution of Life Histories, Oxford University Press, Oxford, 1992.
[25] M.D. Hofmeyr, Egg production in Chersina angulata: an unusual pattern in a Mediterranean Climate, J. Herpetol. 38 (2) (2004) 172–179.
[26] T. Henen, Reproductive effort and reproductive nutrition of female desert tortoises: essential field methods, Integr. Comp. Biol. 42 (2002) 43–50.
[27] R.J. Etches, J.N. Petitte, Reptilian and avian follicular hierarchies: models for the study of ovarian development, J. Exp. Zool. Suppl. 4 (1990) 112–122.
[28] N. Perez-Santigosa, C. Diaz-Paniagua, J. Hidalgo-Vila, The reproductive ecology of exotic *Trachemys scripta elegans* in an invaded area of southern Europe, Aquatic. Conserv.: Mar. Freshw. Ecosyst. 18 (7) (2008) 1302–1310.
[29] J. Blue, B. Heulin, C. Haussy, S. Meylan, M. Massot, Experimental evidence of early costs of reproduction in conspecific viviparous and oviparous lizards, J. Evol. Biol. 25 (2012) 1264–1274.
[30] Wyneken J. The Anatomy of Sea Turtles. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470. Miami, FL; 2001.
[31] A. Hailey, N.S. Loumbour, Egg size and shape, clutch dynamics, and reproductive effort in European tortoises, Can. J. Zool. 66 (7) (1987) 1526–1537.
[32] D.C. Rostal, T.R. Robeck, J.S. Grumbles, P.M. Burchfield, D.M. Owens, Seasonal Reproductive Cycle of the Galápagos Tortoise (*Geochelone nigra*) in Captivity, Zoo Biol. 17 (1889) 505–517.
[33] Mateus I.F. Patologia e Clínica de Animais Exóticos e Autóctones. Évora: Universidade de Évora; 2014.
[34] D.W. Owens, The comparative reproductive physiology of sea turtles, Am. Zool. 20 (1980) 547–563.
[35] P.D. Altland, Observations on the structure of the reproductive organs of the box turtle, J. Morphol. 89 (3) (1951) 599–621.
[36] L.J. Guillette, A.R. Woodward, Q. You-Xiang, M.C. Cox, J.M. Matter, T.S. Gross, Formation and regression of the Corpus Luteum of the American alligator (*Alligator mississipiensis*), J. Morphol. 224 (1995) 97–110.
[37] J.M. Legler, Natural history of the ornate box turtle, Terrapene ornata ornata Agassiz, Univ. Kans. publ., Mus. Nat. Hist 11 (1960) 527–669.
[38] M.R. Miller, The endocrine basis for reproductive adaptations in reptiles, in: A. Gorbman (Ed.), Comparative Endocrinology, John Wiley & Sons, New York, 1959, pp. 449–516.
[39] J.D.L.O. Velasquez, R.C. Vogt, Ciclo ovárico y jerarquía folicular de *Peltocephalus dumerilianus* (Testudines: Podocnemididae), Acta Amaz 41 (2) (2011) 243–250.
[40] G. Jorgewich-Cohen, R.S. Henrique, P.H. Dias, M.R. Sánchez-Villagra, The evolution of reproductive strategies in turtles, PeerJ 10 (2022) e13014.
[41] B.P. Wallace, P.R. Sotherland, P. Santidrian-Tomillo, S.S. Bouchard, R.D. Reina, J.R. Spotila, F.V. Paladino, Egg components, egg size, and hatchling size in

leatherback turtles, Comp. Biochem. Physiol. A Mol. Integr. Physiol. 145 (4) (2006) 524–532.

[42] O. Hernandez, Reproducción y crecimiento del morrocoy, *Geochelone (chelonoidis) carbonaria* (spix, 1824) (Reptilia, Testudinidae), Biollania 13 (1997) 165–183.

[43] C.A. Haugaasen, T. Peres, Tree phenology in adjacent amazonian flooded and unflooded forests, Biotropica 37 (4) (2005) 620–630.

[44] Páez V.P., Morales-Betancourt M.A., Lasso C.A., Castaño-Mora O.V., Bock B.C. Biología y Conservación de las Tortugas Continentales de Colombia. Serie Editorial Recursos Hidrobiológicos y Pesqueros Continentales de Colombia. Bogotá; Instituto de Investigación de Recursos Biológicos Alexander von Humboldt; 2012.

[45] R. Goodman, Seasonal reproduction, mammals, in: E. Knobil, J.D. Neill (Eds.), Encyclopedia of reproduction, Academic Press, San Diego, California, 1999, pp. 341–352.