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Breeding Biology of Green Sea Turtles Stranded in Potiguar Basin, Northeastern Brazil

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Juvenile green sea turtles can be found along the Brazilian coast accounting for the higher number of occurrences (strandings, sightings and bycatch), and in pelagic areas along the northeast coast (Sales *et al*. 2008; Santos *et al*. 2011). In Brazil, their principal nesting areas are the Island of Trindade, Espírito Santo (mean curved carapace length or CCL of nesting females = 116.8 cm), Atol das Rocas Biological Reserve, Rio Grande do Norte (mean CCL = 115 cm), and Fernando de Noronha Archipelago, Pernambuco (Moreira *et al*. 1995; Bellini *et al*. 1996; Bellini & Sanches 1996; Grossman *et al*. 2003). Previous studies predicted their age at maturation of 25-50 years in Australia (Chaloupka *et al*. 2004); from 35 to >50 years in the Hawaiian Islands (Balazs & Chaloupka 2004); and 30-44 years (28 years were required to reach the minimum size) in Florida, Costa Rica and Mexico (Goshe *et al*. 2010).

Green sea turtles can be considered prepubescent when they reach 50-73 cm, straight carapace length, pubescent at 71-79 cm, and mature at 82 cm (Miller & Limpus 2003). Data on carapace length of the smallest recorded size for nesting females can be used to estimated size at sexual maturity (Marcovaldi *et al*. 1999; Sanches *et al*. 1999). However, carapace length is not a suitable measure for an accurate identification of an individual's maturation stage, such as prepubescent individual, pubescent individual or subadult, non- breeding adult, breeding adult and senescent adult (Miller 1997; Miller & Limpus 2003; Pérez *et al*. 2010).

The absence of dimorphic sexual chromosomes in marine turtles precludes sex determination through genetic methods, and there is no sexual dimorphism in juvenile green turtles (Godley *et al*. 2002; Rosa 2009). Various techniques have been applied to determine the sex of immature marine turtles, such as radioimmunoassay, laparoscopy, and ultrasonography (Wibbels 2003; Ceriani & Wyneken 2008). According to Otsuka *et al*. (2008), there is limited knowledge about histological characterization of testes and epididymis of juvenile green sea turtles, and the information available is insufficient to provide a detailed understanding of the gonadal changes in sea turtles. Since then, Rosa (2009) analyzed gonad samples from both sexes of juvenile green sea turtles in Brazil, and suggested that characteristics based on texture and adherence of gonads are not adequate for sex classification in individuals with 27.5-68 cm CCL. Pérez *et al*. (2010) analyzed gonads from Cuban green and hawksbill (*Eretmochelys imbricata*) sea turtles in different stages of development (prepubescent, pubescent and mature), and verified
that ovarian follicular development is an essential process in the classification of maturation stages associated with size. However, we found few studies focused on histological features of gonads, most research focused on nesting areas that provide knowledge on marine turtle breeding biology, including nesting behavior, interseasonal

nesting movements, number of nests, size of nester and clutch, egg size and hatching success (Hirth 1980; Bjorndal & Meylan 1983; DeGroot & Shaw 1993; Garduño-Andrade *et al*. 1999; Wang & Cheng 1999; Grossman *et al*. 2003; Moura *et al*. 2014).

Studies on the gonadal histology of sea turtles will serve to improve methodology in assessment of sexual maturity, and reinforce conservation efforts, such as long-term management programs involving researchers, government, non-governmental organizations, and local community; regional management plans for coastal fishing activity, which play a role and an impact on the marine turtle populations. Our aims were to: (1) determine the sex ratio of green turtles stranded in Potiguar Basin, Brazil, identifying their stages of sexual maturity based on macro- and microscopic examination of the gonads; and (2) correlate these stages of maturity to the size of examined individuals.

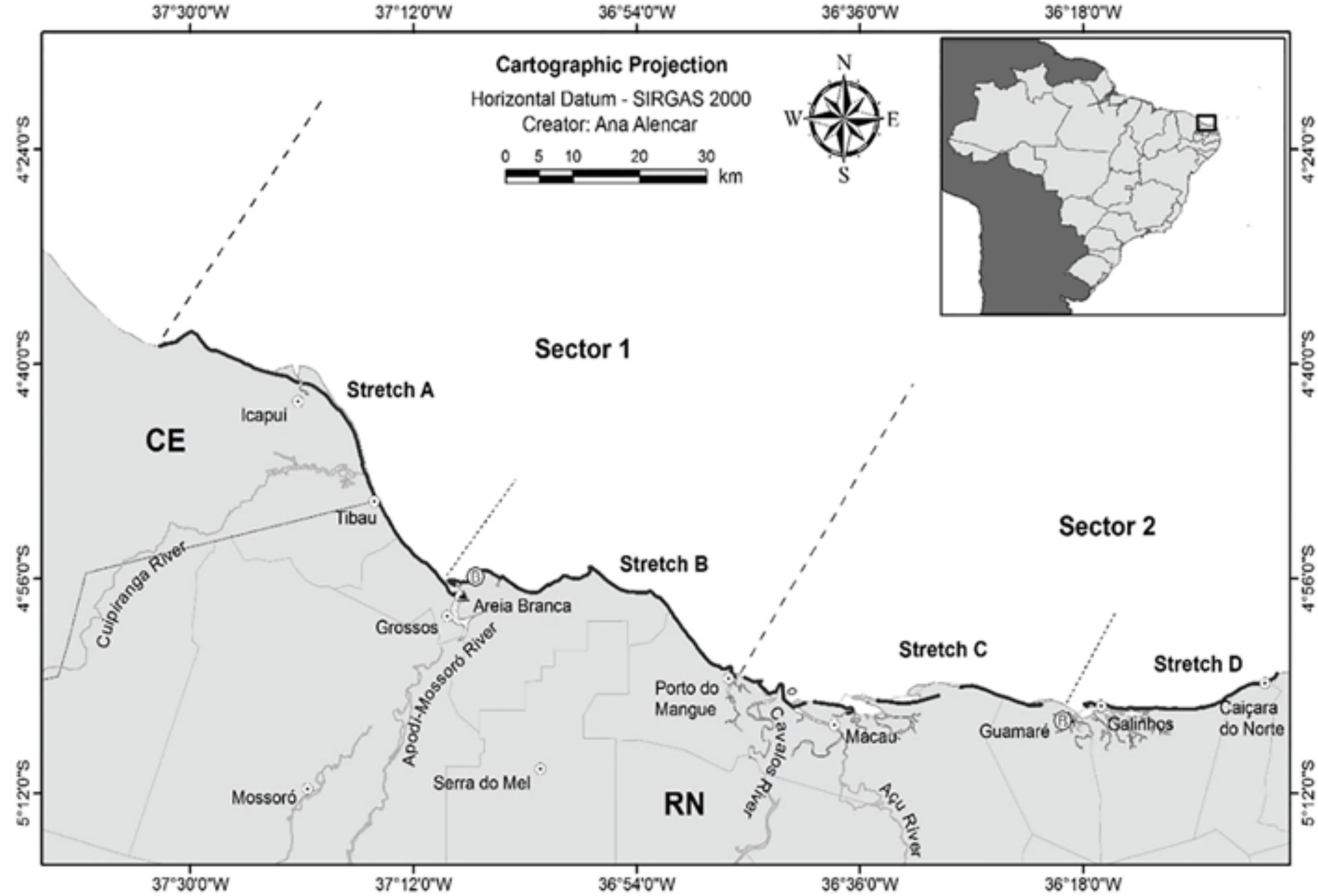

Figure 1. Geographical distribution of the study area. Source: Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN). RN: Rio Grande do Norte, and CE: Ceará.

This research was carried out in northeastern Brazil, between Caiçara do Norte (5.06699° S; 36.07678° W) in Rio Grande do Norte state (RN) and Icapuí (4.64674° S; 37.54780° W) in Ceará state (CE) covering an approximately distance of 300 km (Fig. 1). This region is known as the Potiguar Basin and it is considered an important feeding ground for green turtles (Gavilan-Leandro *et al*. 2016; Farias *et al*. 2019). Since 2010, the Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN) in Brazil has conducted the Beach Monitoring Program in the Potiguar Basin (*Programa de Monitoramento de Praias da Bacia Potiguar* - PMP-BP). The PMP-BP is part of an environmental constraint compliance required by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) associated with the oil exploitation operated by PETROBRAS (Petróleo Brasileiro S.A., Agreement Number 2500.005657510.2).

Our research was based on data obtained between 2011-2015, with daily monitoring conducted by a trained field team of PCCB- UERN. A portable GPS was used to record the location of both live and dead stranded green turtles. Live turtles were taken to a rehabilitation center in Areia Branca/RN and were evaluated clinically by veterinarians. Individuals found to be mildly or moderately decomposed (condition codes D2 and D3, Flint *et al*. 2009) and those that died during rehabilitation were necropsied, and representative gonad samples were obtained and fixed with 10% formalin. Gonad samples from 78 green turtles (53 females, CCL 29-107 cm and 25 males, CCL 27-114 cm) were dehydrated, diaphanized and embedded in paraffin, and serial 5 µm sections were prepared and stained with hematoxylin-eosin (HE).

We recorded CCL from nuchal to notch between supra-caudal scales, following Bolten (1999), using a flexible tape and green sea turtles were binned according to the following size classes (cm): 20-39.9, 40-59.9, 60-79.9, 80-99.9, 100-119.9, and 120-139.9. The sex of examined individuals was determined through macroscopic (visual) analysis of the gonads during necropsy. Based on Ceriani & Wyneken (2008), we analyzed texture, adherence, shape, and presence of paramesonephric ducts. We also observed external morphological characteristics that determine sexual dimorphism in the adult individuals: males have smaller CCL and larger front flipper claws than females; and also have curvature in their forelimbs and long prehensile tails, with the cloacal opening in the terminal portion of the tail (Wyneken 2001; Godley *et al*. 2002). Through microscopic examination, attention was given to the maturation stages based on characteristics of germinative cells present in the gonads. The stages of female maturation observed followed Miller & Limpus (2003) and reports from Pérez-Bermúdez *et al*. (2012): (1) prepubescent: predominant characteristics are homogeneous oocytes without expanded stroma or vitellogenic follicles; (2) pubescent: ovaries with little expansion, and previtellogenic follicles in different developmental stages; and (3) mature: presence of vascularized expanded stroma, previtellogenic and vitellogenic follicles. In regards to males, the determination of maturity stages followed classification from Wibbels *et al*. (1990) and Otsuka *et al*. (2008): (1) prepubescent: seminiferous tubules with small diameter, presence of spermatogonias, and well-developed interstitial connective tissue; (2) pubescent: seminiferous tubules with spermatogonias and spermatocytes, expansion in seminiferous tubules and reduction in interstitial connective tissue; and (3) mature: spermatogenesis approaches maximum, larger seminiferous tubules contain more layers of cells in the germinal epithelium, and abundant sperm in the lumen. Through microscopic analysis we confirmed the sex of some individuals and classified their maturation stages based on characteristics of germinative cells present in their gonads. We used the X^2 test to evaluate for statistically significant differences between number of sampled females and males. The significance level (alpha) was 5% ($P < 0.05$) and statistical analyzes were performed with Statistica software, version 7.0.

During the study period, 3,337 stranded green turtles were recorded (range = 23-132 cm CCL), and the macroscopic determination of sex was possible in 1,130 individuals considering their state of decomposition. Seventy-eight percent of the turtles were females (N = 877), and 22% were males (N = 253) (X^2 = 344.54; g.l. = 1; $P < 0.05$) (sex ratio = 3.46F:1M). These results are consistent with a study conducted in Rio Grande do Sul - Brazil that also found more females than males (2.8F:1M; Duarte *et al*. 2011). Research has indicated higher rates of females being produced from different nesting areas over the years (Wibbels 2003; King *et al*. 2013; Marcovaldi *et al*. 2014). Climate change may be linked to these results, and an increase of 2 °C in some nesting areas is predicted to result in fully feminized nests, while 3 °C increase would be considered a lethal incubation temperature (Hawkes *et al*. 2007). Studies conducted in the NE Caribbean suggest that in the last several decades only 15.5% of green sea turtles produced each year were male, and global warming may exacerbate this trend (Laloë *et al*. 2016).

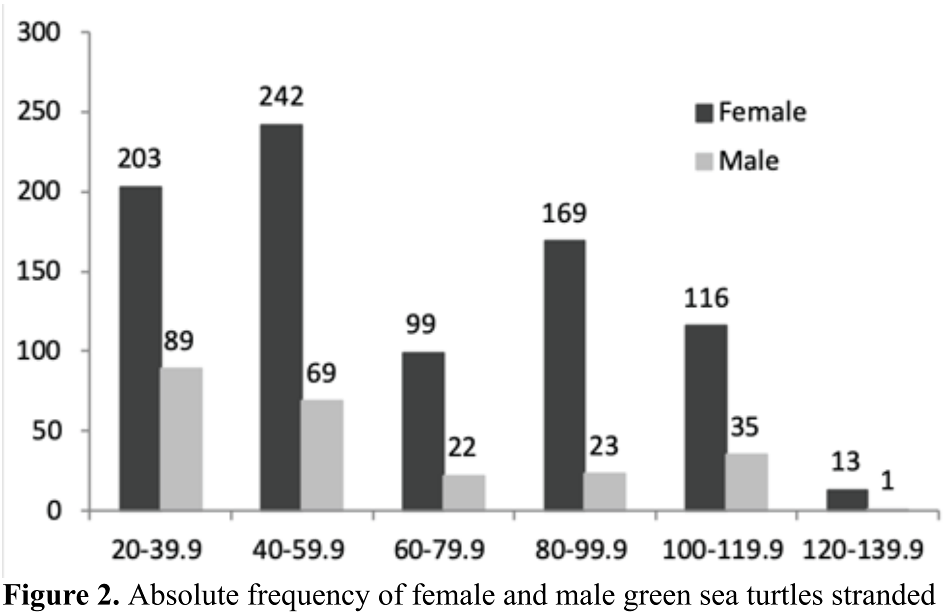

Figure 2. Absolute frequency of female and male green sea turtles stranded in the Potiguar Basin/RN-CE between 2011 and 2015 according to their size range.

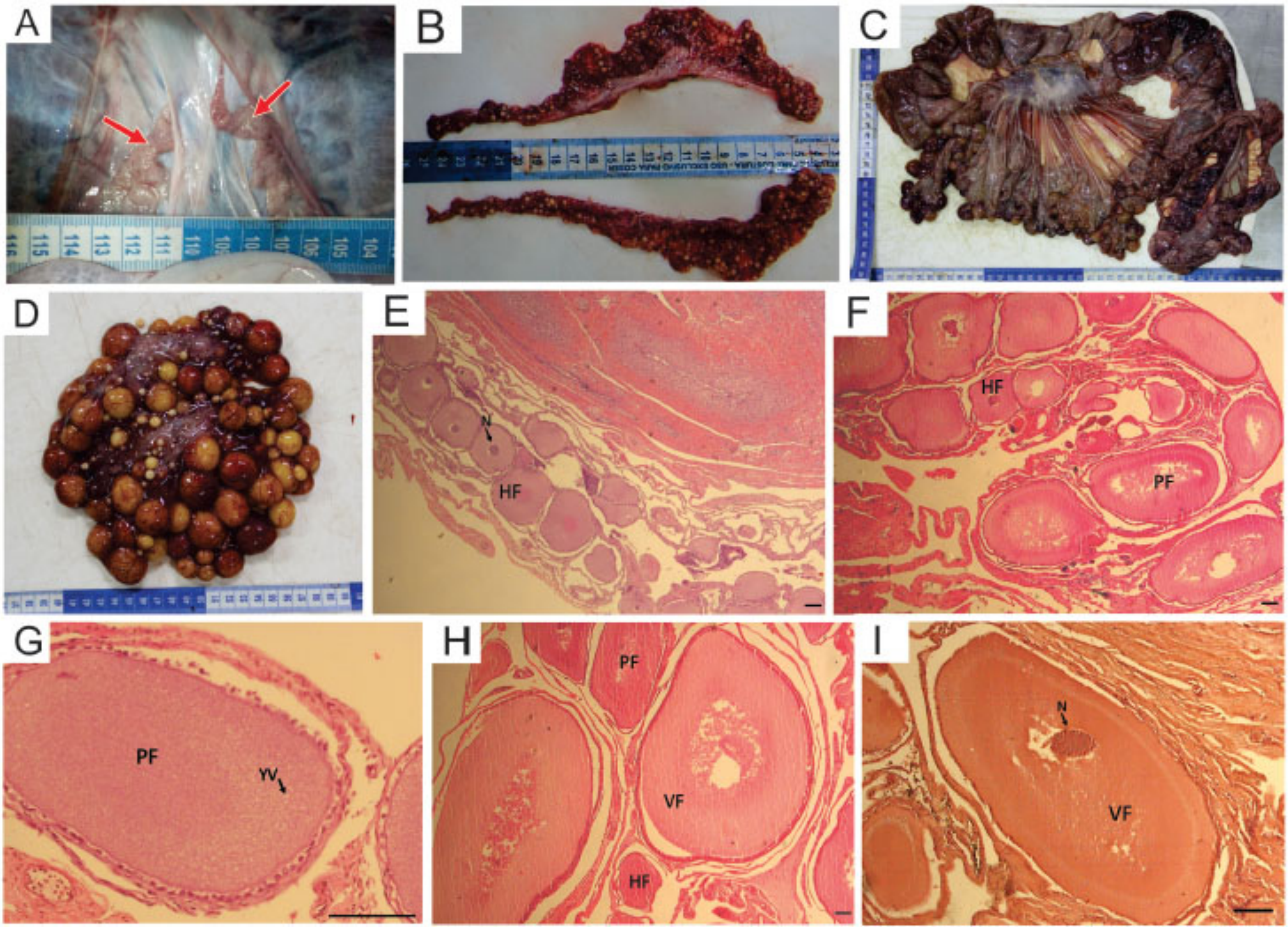

Figure 3. Maturation stages of female green sea turtles: (A) Prepubescent (red arrow: ovaries); (B) Pubescent; (C and D) Mature. Histological section of ovaries: (E) Prepubescent (CCL = 42 cm); (F and G) Pubescent (CCL = 62.1 cm and 64 cm, respectively); (H and I) Mature (CCL = 107 cm and 89 cm, respectively); N: nuclei; HF: homogeneous follicles; PF: previtellogenic follicles; YV: yolk vesicles, and VF: vitellogenic follicles. Bar = 100 µm.

Some individuals were found with broken carapaces, and we could not accurately classify their size. Therefore, a total of 1,130 green sea turtles were examined macroscopically and 1,081 were classified into size bins. The number of females was higher than males in all size categories (Fig. 2): 20-39.9 (N = 292; 69.5% females), 40-59.9 (N = 311; 77.8% females), 60-79.9 (N = 121; 81.8% females), 80-99.9 (N = 192; 88% females), 100-119.9 (N = 151; 76.8% females), and 120-139.9 (N = 14; 92.9% females). Data obtained between 1 January 2010 and 31 December 2012 in Potiguar Basin revealed 1,142 stranded green turtles; 71% were under 60 cm CCL, and thus were classified as juveniles (Gavilan-Leandro *et al*. 2016). Another study carried out during 2007-2012 in Anchieta - Espírito Santo state, Brazil, reported that 97% of 1,094 stranded green turtles were juveniles (Berrêdo *et al*. 2013). In southern Brazil, green sea turtles begin their coastal development period at around 30-40 cm carapace length (Lenz *et al*. 2017). Our findings suggest a strong probability that juvenile green turtles use Potiguar Basin and its adjacent areas as their feeding ground.

Macroscopic analysis of gonads revealed differences in appearance for females at different stages of maturity. Gonads of females in the prepubescent stage were pinkish in color with low vascularization, grainy texture, and smaller than mature gonads (Fig. 3A). Pubescent female gonads were pink in color, irregularly shaped, elongate, fusiform in outline, and more vascularized and granular than prepubescent ones (Fig. 3B). In mature ovaries, we observed in numerous round intensely yellow oocytes (Figs. 3C and 3D). Through histological examination, we observed that prepubescent females had ovaries with small follicles and homogeneous cytoplasm, without expanded stroma and reserve material (Fig. 3E), as described by previous studies (Rosa 2009; Pérez *et al*. 2010). Pubescent ovaries presented previtellogenic follicles with increased cytoplasm containing some lipid vesicles indicating the reserve material production, and could be characterized as initial vitellogenesis (Figs. 3F and 3G); these features were also found by Pérez *et al*. (2010). The presence of lipids in the oocyte cytoplasm in previtellogenic stages has been described in other reptiles and marine turtles, indicating that at this stage the oocyte grows rapidly due to the transcriptional activity of chromosomes and the strong metabolism associated with lipid synthesis (Guraya 1989; Pérez- Bermúdez *et al*. 2012). In mature ovaries, follicles were larger in diameter than in previous stages, and yolk platelets, vitellogenic follicles and follicles in different developmental stages were present (Figs. 3H and 3I). Pérez-Bermúdez *et al*. (2012) also described these structures, indicating that in vitellogenesis the yolk platelets progressively increase in diameter occupying all the cytoplasm. Several sizes of vitellogenic and previtellogenic follicles coexist in adult reproduction, which may be indicative of different clutches produced during a single nesting season or groups of follicles that will initiate vitellogenesis before the next breeding season.

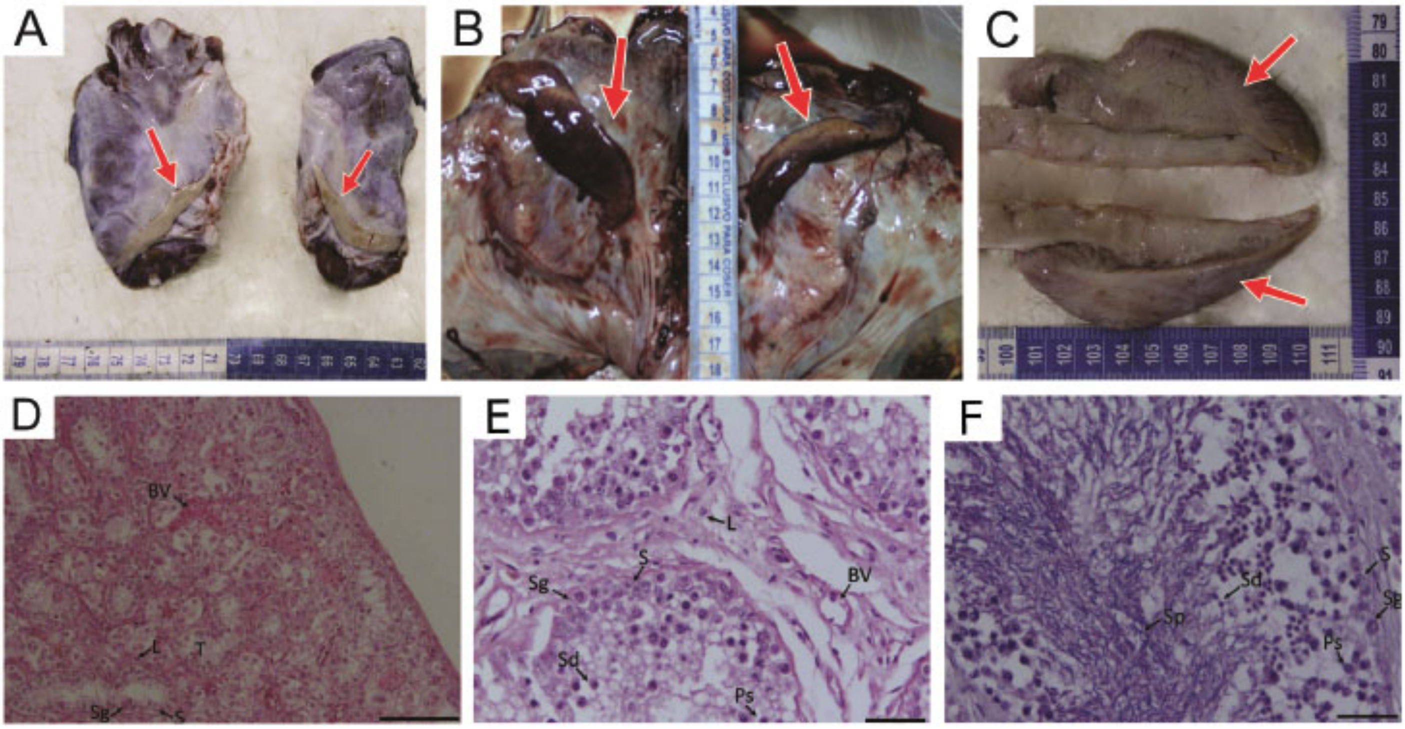

Figure 4. Maturation stages of male green sea turtles: (A) Prepubescent; (B) Pubescent; (C) Mature. Red arrows: testes. Histological section of testes: (D) Prepubescent (CCL = 32.3 cm) (Bar = 100 µm); (E) Pubescent (CCL = 93 cm) (Bar = 20 µm); (F) Mature (CCL = 105 cm) (Bar = 20 µm). T: seminiferous tubule; S: Sertoli cell; L: Leydig cell; BV: blood vessels; Sg: spermatogonia; Ps: primary spermatocyte; Sd: spermatid; Sp: sperm.

In males, the prepubescent gonads were white in color, smooth in texture, and presented low vascularization (Fig. 4A). The pubescent testes were more vascularized and enlarged than prepubescent ones (Fig. 4B). The mature gonads were highly vascularized and attached to the body wall by its flat dorsal surface (Fig. 4C). Histologically, most males were classified as prepubescent and presented seminiferous tubules with smaller diameter than mature testes, and seminiferous epithelium composed of Sertoli cells and Leydig cells within interstitial connective tissue (Fig. 4D); these characteristics were also described by Otsuka *et al*. (2008). Pubescent testes had expanded seminiferous tubules and layers of cells composed by spermatogonia, primary spermatocytes, spermatids and Sertoli cells, blood vessels, and Leydig cells in interstitial connective tissue (Fig. 4E). This is similar to a study conducted by Pérez *et al*. (2010), that reported that primary and secondary spermatocytes, spermatids, and sperm were scarce in the seminiferous tubules. Finally, the only individual classified as a mature male (CCL = 105 cm) had sperm within the seminiferous tubular lumen, larger diameter than previous stages, and germinative epithelium with several layers of different types of cells (Fig. 4F). According to Wibbels *et al*. (1990), these features indicate that a male has already developed the entire spermatogenic cycle.

We verified that macroscopic analysis was not appropriate to determine sex of prepubescent females and males because of the gross similarity in their gonads, and we suggest using the microscopic analysis as described by Wibbels (2003) and Ceriani & Wyneken (2008). During the years before sea turtles reaches puberty, gonads increase in size, and their morphological differences become increasingly more visible (Miller & Limpus 2003). In the puberty stage, hormonal changes increase the size and structure of ovaries and oviduct; and during spermatogenesis, testosterone influences the differentiation from Sertoli cells into seminiferous tubules (Hamann *et al*. 2003). A previous study on Brazilian juvenile green sea turtles at 27.5-68 cm, revealed that gonads of both sexes can present mixed textures and the same shape: 80% of females had ovaries with grainy texture, while 71% of males presented testes smooth in texture; 24 gonads were considered attached to the body wall (21 from males and 3 from females), and 72 were characterized as non-attached (49 ovaries and 23 testes; Rosa 2009). In our research, microscopic analysis was useful to confirm the sex of five males (27, 29, 31, 85 and 93 cm CCL) and two females (61 and 65 cm CCL), which could not be classified according to macroscopic characteristics such as texture or adherence.

Stage of gonadal maturation	Sex	Curved carapace length (cm)				
		Number	Minimum	Maximum	Mean ±SD	Median (Q1; Q3)
Prepubescent	F	35	29	59	37.4 ±7.1	36 (33; 40)
	M	17	27	60.5	39.5 ±10.7	35 (31.5; 48.4)
Pubescent	F	12	61	95	77.5 ±12.6	80 (64.25; 90.5)
	M	7	66	114	89.9 ±17.8	93 (69; 104.5)
Mature	F	6	89	107	100.8 ±6.8	103 (95; 106.25)
	M	1	-	-	105*	105*

Table 1. Curved carapace length of green sea turtles stranded in the Potiguar Basin/Rio Grande do Norte-Ceará, Brazil (2011-2015) according to their stages of gonadal maturation. F: Female; M: Male; SD: Standard deviation; Q1: 25th percentile; Q3: 75th percentile. *Absolute value.

In Brazil, minimum size of reproductive maturation in green sea turtles is based on the smallest documented carapace length for nesting sea turtle females in the largest nesting area within close proximity (CCL = 90 cm for *C. mydas*; Almeida *et al*. 2011). Our study considered CCL associated with stages of gonadal maturation (Table 1). We observed one female under 90 cm CCL (89 cm CCL) with histological characteristics described for an adult, and three other individuals (CCL > 90 cm) that were considered pubescent according to histological description of their ovaries. Research carried out in Paraná state, Brazil revealed that the gonadal development of green turtles was concomitant with their carapace growth (Rosa 2009). However, studies conducted in Cuba concluded that the follicular phases found in ovaries of hawksbill turtles were statistically independent of their carapace length (Pérez-Bermúdez *et al*. 2012). With regards to males, we classified one individual as pubescent (CCL = 114 cm) based on the absence of sperm within seminiferous tubules; whereas the only individual classified as mature was smaller (CCL = 105 cm). Pubescent hawksbill turtles demonstrated signs of spermatogenic activity upon histological analysis but lacked a fully developed penis, indicating asynchrony between testicular and penile developments; and another individual was considered adult male according to phenotypic characteristics (carapace length and penis size) but without histological structures consistent with sexual maturity (Pérez *et al*. 2010). As stated by Otsuka *et al*. (2008), the straight carapace length is not an adequate indicator of maturity, but histological analysis is more definitive.

It is well known that age and size of marine turtles can vary due to environmental temperatures and food availability in foraging grounds, and populations with high genetic variation are more resistant to environment changes that can interfere in development (Limpus & Nicholls 2000; Joseph & Shaw 2011). There is a paucity of research on maturation stages associated with carapace length, perhaps due to the logistic challenges associated with finding and capturing males for research. In the last decade, the required implementation of the Beach Monitoring Program in Brazil has led to an increase in research on the biology of marine vertebrates, especially sea turtles. Our results indicate that carapace length is not appropriate to identify maturation stage, and histological examination should be used, when possible, as a suitable technique for this purpose. This study also provided sex ratio data for green turtles stranded in Potiguar Basin, and important information about gonad morphology in different phases of sexual development.

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ALMEIDA, A.P., A.J.B. SANTOS, J.C.A. THOMÉ, C. BELINI, C. BAPTISTOTTE, M. A. MARCOVALDI, A.S. SANTOS & M. LOPEZ. 2011. Avaliação do estado de conservação da tartaruga marinha *Chelonia mydas* (Linnaeus, 1758) no Brasil. Biodiversidade Brasileira 1: 12-19.

BALAZS, G.H. 1995. Growth rates of immature green turtles in the Hawaiian Archipelago. In: Bjorndal, K.A. (Ed.). Biology and Conservation of Sea Turtles. Revised Edition. Smithsonian Institution, Washington, D.C. pp. 489-511.

BALAZS, G.H. & M. CHALOUPKA. 2004. Spatial and temporal variability in somatic growth of green sea turtles (*Chelonia mydas*) resident in the Hawaiian Archipelago. Marine Biology 145: 1043-1059.

BELLINI, C. & T.M. SANCHES. 1996. Reproduction and feeding of marine turtles in the Fernando de Noronha Archipelago, Brazil. Marine Turtle Newsletter 74:12-13.

BELLINI, C., M.A. MARCOVALDI, T.M. SANCHES, A. GROSSMAN & G. SALES. 1996. Atol das Rocas Biological Reserve: second largest Chelonia rookery in Brazil. Marine Turtle Newsletter 72:1-2.

BERRÊDO, R., M. ROSA, B. GIFFONI, G. SALES, M. BRITTO, J. THOMÉ & N. LEITE JR. 2013. Encalhes e interação da pesca costeira com tartarugas marinhas em Anchieta, Espírito Santo, Brasil. VI Jornada y VII Reunión De Conservación E Investigación De Tortugas Marinas en el Atlántico Sur Occidental (ASO). pp. 59-64.

BJORNDAL, K.A. & A.B. MEYLAN. 1983. Sea turtles nesting at Melbourne Beach, Florida, I. Size, growth and reproductive biology. Biological Conservation 26: 65-77.

BOLTEN, A.B. 1999. Techniques for measuring sea turtles. In: Eckert, K.L., K.A. Bjorndal, F.A. Abreu-Grobois, M. Donnelly (Eds.). Research and Management Techniques for the Conservation of Sea Turtles. IUCN SSC Marine Turtle Specialist Group, Washington, D.C. pp. 110-114.

CERIANI, S.A. & J. WYNEKEN. 2008. Comparative morphology and sex identification of the reproductive system in formalin- preserved sea turtle individuals. Zoology 111: 179-187.

CHALOUPKA, M., LIMPUS, C. & J. MILLER. 2004. Green turtle somatic growth dynamics in a spatially disjunct Great Barrier Reef metapopulation. Coral Reefs 23: 325-335.

DeGROOT, K.A. & J.H. SHAW. 1993. Nesting activities by the loggerhead (*Caretta caretta*) at Back Bay National Wildlife Refuge, Virginia. Proceedings of the Oklahoma Academy of Science 73: 15-17.

DUARTE, D.L., D.D.S. MONTEIRO, R.D. JARDIM, J.C. SOARES & A.S. VARELA-JUNIOR. 2011. Determinação sexual e maturação gonadal de fêmeas da tartaruga-verde (*Chelonia mydas*) e tartaruga-cabeçuda (*Caretta caretta*) no extremo sul do Brasil. Acta Biológica Paranaense 40: 87-103.

FARIAS, D.S.D., A.E.B. ALENCAR, A.C. BOMFIM, A.B.L. FRAGOSO, S. ROSSI, G.J.B. MOURA, S.A. GAVILAN & F.J.L. SILVA. 2019. Marine turtles stranded in Northeastern Brazil: Composition, spatio-temporal distribution and anthropogenic interactions. Chelonian Conservation & Biology 18: 1-8.

FLINT, M., J. PATTERSON-KANE, P. MILLS & C. LIMPUS. 2009. A veterinarian's guide for sea turtle post mortem examination and histological investigation. Brisbane, Australia: The University of Queensland. 56p.

GARDUÑO-ANDRADE, M., V . GUZMÁN, E. MIRANDA, R. BRISEÑO-DUEÑAS, & F.A. ABREU-GROBOIS. 1999. Increases in hawksbill turtle (*Eretmochelys imbricata*) nesting in the Yucatán Peninsula, Mexico, 1977-1996: Data in support of successful conservation? Chelonian Conservation & Biology 3: 286-295.

GAVILAN-LEANDRO, S.A.C., F.J.L. SILVA, D.S.D. FARIAS, A.B.L. FRAGOSO, T.E. BEZERRA & A.E.B. ALENCAR. 2016. Pesquisa e Conservação de Tartarugas Marinhas na Bacia Potiguar, Rio Grande do Norte, Nordeste do Brasil. In: Correia, J.M.S., E.M. Santos & G.J.B. Moura (Eds.). Conservação de Tartarugas Marinhas no Nordeste do Brasil: Pesquisas, Desafios e Perspectivas. Volume 1. Recife: Editora Universitária da UFRPE. pp. 71-89.

GODLEY, B.J., A.C. BRODERICK, R. FRAUENSTEIN, F. GLEN & G.C. HAYS. 2002. Reproductive seasonality and sexual dimorphism in green turtles. Marine Ecology Progress Series 226: 125-133.

GOSHE, L.R., L. AVENS, F.S. SCHARF & A.L. SOUTHWOOD. 2010. Estimation of age at maturation and growth of Atlantic green turtles (*Chelonia mydas*) using skeletochronology. Marine Biology 157: 1725-1740.

GROSSMAN, A., C. BELLINI & M.A. MARCOVALDI. 2003. Reproductive biology of the green sea turtle at the Biological Reserve of Atol das Rocas off northeast Brazil. 2002. In: Seminoff, J.A. (Comp.). Proceedings of the 22nd Annual Symposium on Sea Turtle Biology and Conservation. NOAA Tech Memo NMFS- SEFSC-503, p. 136.

GURAYA, S.S. 1989. Ovarian follicles in reptiles and birds. Berlin, Heidelberg: Springer. 285p.

HAMANN, M., C.J. LIMPUS & D.W. OWENS. 2003. Reproductive cycles of males and females. In: Lutz, P.L., J.A. Musick & J. Wyneken (Eds.). The Biology of Sea Turtles. Volume 2. CRC Press, Boca Raton, FL. pp. 135-164.

HAWKES, L.A., A.C. BRODERICK, M.H. GODFREY & B.J. GODLEY. 2007. Investigating the potential impacts of climate change on a marine turtle population. Global Change Biology 13: 923-932.

HIRTH, A.F. 1980. Some aspects of the nesting behavior and reproductive biology of sea turtles. American Zoologist 20: 507-523.

JOSEPH,J.&P.W.SHAW.2011.Multiple paternity in egg clutches of hawksbill turtles (*Eretmochelys imbricata*). Conservation Genetics 12: 601-605.

KING, R., W.H. CHENG, C.T. TSENG, H. CHEN & I.J. CHENG. 2013. Estimating the sex ratio of green sea turtles (*Chelonia mydas*) in Taiwan by the nest temperature and histological methods. Journal of Experimental Marine Biology and Ecology 445: 140-147.

LALOË, J.O., N. ESTEBAN, J. BERKEL & G.C. HAYS. 2016. Sand temperatures for nesting sea turtles in the Caribbean: Implications for hatchling sex ratios in the face of climate change. Journal of Experimental Marine Biology and Ecology 474: 92-99.

LENZ, A.J., L. AVENS & M. BORGES-MARTINS. 2017. Age and growth of juvenile green turtles *Chelonia mydas* in the western South Atlantic Ocean. Marine Ecology Progress Series 568: 191-201.

LIMPUS, C. & N. NICHOLLS. 2000. ENSO regulation of Indo-Pacific green turtle populations. In: Hammer, G.L., N. Nicholls & C. Mitchell (Eds.). Applications of Seasonal Climate Forecasting in Agricultural and Natural Ecosystems. Volume 21. Dordrecht: Springer. pp. 399-408.

MARCOVALDI, M.A., A.J. SANTOS, A.S. SANTOS, L.S. SOARES, G.G. LOPEZ, M.H. GODFREY & M.M.P.B FUENTES. 2014. Spatio-temporal variation in the incubation duration and sex ratio of hawksbill hatchlings: implication for future management. Journal of Thermal Biology 44: 70-77.

MARCOVALDI, M.A., C.F. VIEITAS & M.H. GODFREY. 1999. Nesting and conservation management of hawksbill turtles (*Eretmochelys imbricata*) in northern Bahia, Brazil. Chelonian Conservation & Biology 3: 301-307.

MILLER, J.D. 1997. Reproduction in sea turtles. In: Lutz, P.L. & J.A. Musick (Eds.). The Biology of Sea Turtles. CRC Press, Boca Raton, FL. pp.51-81.

MILLER, J.D. & C.J. LIMPUS. 2003. Ontogeny of marine turtle gonads. In: Lutz, P.L., J.A. Musick & J. Wyneken (Eds.). The Biology of Sea Turtles. CRC Press, Boca Raton, FL. pp.199-224.

MOREIRA, L.M.P., C. BAPTISTOTTE, J. SCALFONI, J.C. THOMÉ & A.P.L.S. ALMEIDA. 1995. Occurrence of *Chelonia mydas* on the Island of Trindade, Brazil. Marine Turtle Newsletter 70:2.

MOREIRA, L.D.P. 2003. Ecologia reprodutiva e estimativa de ninhos da tartaruga verde aruanã - *Chelonia mydas* (Linnaeus, 1758) (Testudines, Reptilia) na ilha da Trindade, Espírito Santo, Brasil. MSc Thesis, Universidade Federal do Espírito Santo, Espírito Santo, Brazil. 63p.

MOURA, C.C.M., G.J.B. MOURA, E.B.F. LISBOA & V.L.F. LUZ. 2014. Distribuição geográfica e considerações ecológicas sobre a fauna de Testudines da Região Nordeste do Brasil. SITIENTIBUS Série Ciências Biológicas 14: 1-20.

OTSUKA, S., M. SUZUKI, N. KAMEZAKI, T. SHIMA, M. WAKATSUKI, Y. KON & N. OHTAISHI. 2008. Growth related changes in histology and immunolocalization of steroid hormone receptors in gonads of the immature male green turtle (*Chelonia mydas*). Journal of Experimental Zoology A 309: 166-174.

PÉREZ-BERMÚDEZ, E., A. RUIZ-URQUIOLA, I. LEE GONZÁLEZ, B. PETRIC, N. ALMAGUER-CUENCA, A. SANZ-OCHOTORENA & G. ESPINOSA-LÓPEZ. 2012. Ovarian follicular development in the hawksbill turtle (Cheloniidae: *Eretmochelys imbricata* L). Journal of Morphology 273: 1338-1352.

PÉREZ, E., A. RUIZ, G. ESPINOSA & I. LEE. 2010. Histología gonadal y criterios fenotípicos de maduración en las tortugas
marinas *Chelonia mydas* y *Eretmochelys imbricata* (Testudines: Chelonidae) de Cuba. Revista de Biología Tropical 58: 287-298.

SANCHES, T.M., C. BELLINI & S.J.R. NETO. 1999. Primeiros registros das tartarugas marinhas *Dermochelys coriacea* e *Caretta caretta* no Rio Grande do Norte, Brasil. Resumos do Oitavo Congresso Nordestino de Ecologia 31: 27.

ROSA, L. 2009. Estudo da biologia reprodutiva na fase juvenil da tartaruga-verde (*Chelonia mydas*) no litoral do estado do Paraná. MSc Thesis. Universidade Federal do Paraná, Brasil. 69p.

SALES, G., GIFFONI, B. & P.C.R. BARATA. 2008. Incidental catch of sea turtles by the Brazilian pelagic longline fishery. Journal of the Marine Biological Association UK 88: 853-864.

SANTOS, A.S., A.P. ALMEIDA, A.J.B. SANTOS, B. GALLO, B. GIFFONI, C. BAPTISTOTTE, C.A. COELHO, E.H.S.M. LIMA, G. SALES, G.G. LOPEZ, G. STAHELIN, H. BECKER, J.C. CASTILHOS, J.C.A. THOMÉ, J. WANDERLINDE, M.Â.A.G.D. MARCOVALDI, M.D.L.M.L. MENDILAHARSU, M.T. DAMASCENO, P.C.R. BARATA & R. SFORZA. 2011. *Chelonia mydas* (Linnaeus, 1758). In: (Marcovaldi, M.Â.A.G.D., A.S. Santos & G. Sales (Orgs.). Plano de Ação Nacional para Conservação das Tartarugas Marinhas Instituto Chico Mendes de Conservação da Biodiversidade, Brasília. pp. 30-33.

WANG, H.C. & I.J. CHENG. 1999. Breeding biology of the green turtle, *Chelonia mydas* (Reptilia: Cheloniidae), on Wan-An Island, PengHu archipelago. II. Nest site selection. Marine Biology 133: 603-609.

WIBBELS, T., D.W. OWENS, C.J. LIMPUS, P.C. REED & J.M.S. AMOSS. 1990. Seasonal changes in serum gonadal steroids associated with migration, mating, and nesting in the loggerhead sea turtle (*Caretta caretta*). General and Comparative Endocrinology 79: 154-164.

WIBBELS, T. 2003. Critical approaches to sex determination in sea turtles. In: Lutz, P.L., J.A. Musick & J. Wyneken (Eds.). The Biology of Sea Turtles. Volume 2. CRC Press, Boca Raton, FL. pp.103-134.

WYNEKEN, J. 2001. The Anatomy of Sea Turtles. NOAA Tech Memo NMFS-SEFSC-470. 172p.