Comparative Clinical Pathology (2022) 31:573–577
https://doi.org/10.1007/s00580-022-03364-y

BRIEF COMMUNICATION

Reproductive hormonal profile in Eastern Pacific green turtles captured in-water

Priscilla Howell[1] · Marcela Suárez-Esquivel[1] · Andrea Chaves[2] · Didiher Chacón[3] · Laura Castro-Ramírez[1] · Kinndle Blanco-Peña[4,5,6]

Received: 5 December 2021 / Accepted: 22 May 2022 / Published online: 17 June 2022

Abstract

The Eastern Pacific green turtle (EPGT) has been differentiating from the green turtle and is currently considered a separate population. This study determined the values of reproductive hormones and total thyroxine of 56 resident foraging green turtles captured between 2012 and 2014 at Golfo Dulce, Costa Rica. All of them underwent a physical examination and were deemed as healthy individuals. The progesterone values were 0.31–2.723 ng/ml, estradiol 25.54–104.129 pg/ml, testosterone 1.94–228.97 ng/dl, and thyroxine 0.5–0.9 µg/dl. Since our population did not show sexual dimorphism, and the curved carapace length sizes did not match previous reports, turtles' sex was determined based on high testosterone concentrations. Our results showed that 48 males (> 3 ng/dl), three females (< 2 ng/dl), and five yet undetermined individuals (2–3 ng/dl) were sampled. The green turtle populations at the Golfo Dulce seem to be formed by subpopulations at different stages of their reproductive cycle; among them could be males and females close to mating season, foraging females between seasons and sub-adults in their final developing habitat. The sampled population included 3 juveniles (one male and two not defined), 45 sub-adults (40 males, three females and one not determined), 7 adults (all males), and one undetermined individual (not sex determined either). The progesterone results indicated that none of the females were nesting; this might indicate that they nest far from the area. The information provided by this study is extremely valuable in the attempts to determine the health status for conservation programs of EPGT in the area.

Keywords Progesterone · Estrogen · Testosterone · Thyroxin · *Chelonia mydas* · Costa Rica

Introduction

The correct sex determination and hormonal reproductive and metabolic assessments are important goals to set a successful and integral turtle conservation program (Eckert et al. 2000). The endocrine analysis offers a minimally invasive approach to know the reproductive stage and population dynamics and benefit conservation efforts necessary for chelonians and similar species. In this sense, an endocrine profile typically includes the following hormones: testosterone (Testo), estradiol (E2), progesterone (P4), total thyroxine (T4), and corticosterone, as well as the relationship between these hormones and variables like size, maturity, or level of activity of the turtles, which are used to estimate the reproductive stage during nesting or mating.

Most studies on sea turtles analyze adult nesting females, accounting for one age group, one sex, and one life stage (Moon et al. 1998; Jessop et al. 1999; Hamann et al. 2002b; Al-Habsi et al. 2006; Mahmoud et al. 2011). However, if the

✉ Kinndle Blanco-Peña
kblanco@una.ac.cr

1 Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica

2 Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica

3 WIDECAST, San José, Costa Rica

4 Instituto Regional de Estudios en Sustancias Tóxicas (IRET), Universidad Nacional, Heredia, Costa Rica

5 Posgrado en Ciencias Veterinarias Tropicales (PCVET), Universidad Nacional, Heredia, Costa Rica

6 Escuela de Ciencias Biológicas, Universidad Nacional, Heredia, Costa Rica

capture is performed in the sea, the geographic location does not always predict their reproductive condition, especially in areas where foraging and mating populations share the space (Owens 1996).

The Golfo Dulce, located within the marine area of the Piedras Blancas National Park in the southern Pacific area of Costa Rica, is an important foraging area for different marine turtle species, including the Eastern Pacific green turtle (EPGT) (Chacón-Chaverri et al. 2015; Méndez-Salgado et al. 2020). Other initiatives to improve their conservation status are currently carried out (Chacón-Chaverri et al. 2015; Méndez-Salgado et al. 2020; Howell et al. 2021). Nevertheless, there is a considerable lack of knowledge about whether males use it or not, which could be related to reproductive activities. Because of these reasons, this study intends to fill gaps in the health and reproductive status determination of free turtle populations in Costa Rica by evaluating the hormonal profile of *Chelonia mydas*.

Materials and methods

EPGT were captured in-water at a known foraging site located in the Golfo Dulce, Costa Rica (8° 40′31.01¨N, 83° 26′ 48.65¨O) from 2012 to 2014, using a simple 100 × 7 m net, as previously described by Howell et al. (2021). All institutional and national guidelines for animal care and welfare were followed. Individuals showing diminished reflexes, any sort of body secretions, or injuries were excluded from the study.

The endocrine profile was determined at the Endocrinology and Reproductive Biotechnology Lab of the Veterinary School, Universidad Nacional, Costa Rica. E2, P4, Testo, and T4 determinations were performed with an Automated Immunoassay Analyzer AIA-360 (TOSOH®), using reagents from the same manufacturer. The methodology was validated by parallelism comparing three samples with high Testo quantity with a known Testo concentration. Serial dilutions were performed in multiples of three (1:3, 1:9, 1:27, over to 1:2187), following the manufacturer's recommendation.

Individuals were classified as male or female using previously reported values for immature green turtles from Hawaii: Individuals with Testo values lower than 2 ng/dl were considered females ($n = 3$), while those with values higher than 3 ng/dl were classified as males ($n = 48$). Animals with values between 2 and 3 ng/dl were indeterminate ($n = 5$) (Table 2; Wibbels et al. 1993).

Results

A total of 56 apparently healthy green turtles were sampled for the study. Sub-adult individuals were the most numerous, according to their curved carapace length (CCL) measurements (Table 1).

Table 1 Age groups and sex of the Eastern Pacific Green turtle (*Chelonia mydas*) (EPGT) captured in the Golfo Dulce, Costa Rica, during 2012–2014 according to their curved carapace length (CCL)

Age group	CCL		Sex (according to Testo levels)		
		n	Males	Females	ND
Hatchling	< 38 cm	0	0	0	0
Juvenile	38–68 cm	3	1		2
Sub-adult	68–88 cm	45	40	3	2
Adult	> 88 cm	7	7	0	0
Not determined		1	0	0	1
Total		**56**	**48**	**3**	**5**

According to Aguirre and Balazs (2000), following the adjustments suggested by Musick (2002)

We measured Testo hormone with no issues in all animals. We chose Testo over the other hormones because it showed higher results, allowing us to dilute without previous extraction; it is also the most helpful hormone for hormonal sexing (Fig. 1).

Most Testo results were higher than 43.21 ng/dl, which could correspond to males beginning their reproductive season. However, results lower than this value could be taken as females in preparation, males in base or mildly higher levels, or sub-adults closer to maturity in their final development habitat. According to the Testo levels, we sampled 48 males, 3 females and 5 undetermined individuals (Tables 1 and 2).

Fifty percent of the E2 data obtained were below the detection limit of the equipment used (25 pg/ml), resulting in 96% of the tested population under that threshold. Almost all individuals with Testo levels compatible with males showed E2 values below the detection limit, except for one sample with both hormones—mainly Testo—superior to basal levels. Similar to E2, more than half of the total thyroxine (T4) results were inferior to the detection limit (0.5 µg/dl). In this sense, the automated analyzer could only provide 37.5% of the T4 results, with a maximum of 0.9 µg/dl. The other samples were below 0.50 µg/dl, coinciding with previous reports (Moon et al. 1998).

P4 was the only hormone with 100% detection, showing low variability results in the lower range. Interestingly, by considering the P97, it is evident that most samples were much lower than the highest of the population.

Discussion

The variability in Testo results is similar to the values of previous reports (Hamann et al. 2005). This could be related to different aspects, mainly associated with the reproductive cycles in reptiles and many other animals (Blanvillain

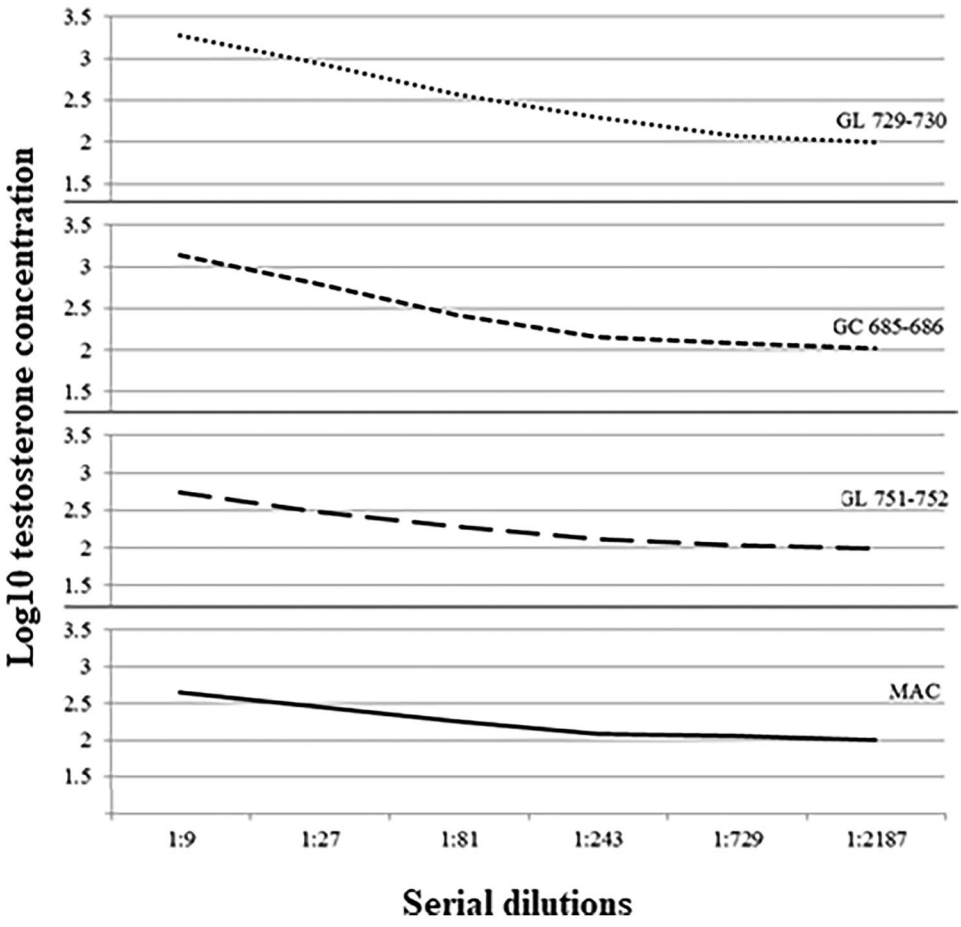

Fig. 1 Parallelism between testosterone (Testo) concentrations in three specimens and the multi-analyte control (MAC) through six serial dilutions

et al. 2011). Right before migration, females' Testo reaches its highest (30 ng/dl), while E2 levels fall abruptly (Owens 1996). After courting, it decreases significantly and shows smaller peaks on early nesting, before each attempt (successful or not) (Owens 1996; Hamann et al. 2002a). The more the nesting attempts, the more fluctuant the Testo is (Hamann et al. 2002a), which could affect our results. This mechanism is known in green turtles, Kemp's ridleys, and loggerheads, although the greens of Michoacan, Mexico, do not seem to be as strict (Owens 1996). Besides, males are known to show elevated Testo matching spermatogenesis and the beginning of migration, reaching levels up to 3000 ng/dl approximately, while Testo falls to basal levels when females nest and males return to the foraging site (Owens 1996; Rostal et al. 1998; Blanvillain et al. 2011).

On the other hand, the life state carries growing levels from juveniles to sub-adults and reproductive cycles once maturity is achieved. Testo and E2 levels rise with photoperiod and temperature during the adult stage until the migratory season arrives (Owens 1996). The samples over the 2200 ng/dl detection limit, likely correspond to males starting their reproductive season.

Fifty percent of the E2 data obtained were below the detection limit of the equipment used (25 pg/ml). This has occurred in geographically diverse areas using radioimmunoassay and similar techniques, being the most sensible technique, the high-performance liquid chromatography (HPLC). Besides, it is expected that females keep basal E2 levels until the subsequent migration is nigh. When they are still feeding on the foraging site, these hormones increase up to approximately 200 pg/ml and then fall abruptly, marking the beginning of the migration, along with Testo at its highest peak (Owens 1996; Blanvillain et al. 2011). It has also been suggested that this progressive increase is the main stimulus for vitellogenesis, so it would not be necessary to maintain levels that high during nesting when E2

Table 2 Hormonal measurements of the eastern Pacific green sea turtle (*Chelonia mydas agassizii*; $n = 56$) from Golfo Dulce, Costa Rica

Hormone	Min	Max	P3	P97
Testosterone (ng/dl)	< 1.5	> 2200	1.94	228.92
Estrogen (pg/ml)	< 0.25	111.5	25.54	104.13
Thyroxin (µg/dl)	< 0.5	0.9	0.5	0.9
Progesterone (ng/ml)	0.19	3.78	0.31	2.723

reaches levels so low that prove undetectable (Al-Habsi et al. 2006). Valente et al. (2011) detected more than 1000 pg/ml from an adult loggerhead female in captivity with marked folliculogenesis.

The only sample with both hormones—mainly Testo—superior to basal levels could correspond to a sub-adult female with unstable hormone concentrations. Animals with a lower concentration than the detection level may correspond to turtles between reproductive seasons, nesting, or immature sub-adults. It is plausible that these turtles are sub-adults or adults between seasons. In fact, two individuals had levels over 100 pg/ml—and some other closing in—which might be beginning preparations for migrating.

Previous studies have reported that T4 levels remained constant all year, both in males and females, inferring that it was due to the environmental temperature and the high-protein diet, compared to the diet subjected to availability in the wild (Licht et al. 1979; Owens 1996; Blanvillain et al. 2011). In our case, some individuals showed T4 values that match mainly with those reported in captivity (0.89–1.05 ug/dl) (Licht et al. 1979; Moon et al. 1998). Considering that all our captured individuals presented excellent body condition scores, it is likely that the diet they consume in this foraging site is of high quality, resulting in high T4 levels.

Interestingly, the highest concentrations of this hormone occurred in May and June, matching the beginning of the rainy season. Temperatures at the Golfo Dulce tend to be warm and steady throughout the year, shifting between 25 and 28 °C (Morales-Ramírez et al. 2015). The samples obtained in the following months likely correspond to mildly lower temperatures and, consequently, lower total T4 concentrations that the analyzer could not detect. This can agree with previous reports from the Cayman Islands, where the reproductive phase seems to alter T4 concentrations aside from environmental temperature and nutritional state (Licht et al. 1985).

P4 and luteinizing hormone peaks occur between 10 to 14 days before the first nesting attempt and in the next 48 h to each attempt, so it would only be detectable on the corresponding season and relatively close to the nesting beach (Owens 1996; Al-Habsi et al. 2006). So, our results are consistent with turtles before the reproductive season, with levels between 0.09 and 1.90 ng/ml. On the contrary, the Testo results were scattered over both detection limits, with two samples higher than 2200 ng/dl and one below 1.5 ng/dl.

Due to the lack of knowledge of this group, there may be errors discerning the life state due to the following factors: (i) the carapace length is not always helpful in separating adults from sub-adults; (ii) the hormonal values may not correspond to these chelonians; and (iii) most Testo results are not compatible with immature turtle sexing. During the sub-adult phase, turtles migrate to develop in a new habitat until their first reproductive migration. Besides, big individuals that are far from their reproductive period could be classified as adult females due to the absence of secondary sexual characteristics (Owens 1996). Since—to the authors' knowledge—it is still unknown if this population is between reproductive seasons or during one of them, or even sharing the foraging site with another coursing a different cycle, all hormonal mechanisms must be considered.

Finally, alterations in Testo levels have been described by raising the temperature in cases of cold stunning and a few hours after the stress of being captured. Therefore, the sampling should not exceed 15 min after capture (Owens 1996). In our case, the method used for capturing, sampling, and the Golfo Dulce temperatures could have influenced the values obtained. These possibilities must be considered in the following approaches.

Conclusions

- The automated immunoassay analyzer AIA-360 is suitable to detect most of the evaluated hormones. However, Testo, E2, and T4 in some cases fell beyond the equipment's detection levels.
- It was feasible to sex all individuals in this study. Since the curved carapace length sizes did not match our population, and in cases where there was no sexual dimorphism, the use of sex steroid measurements allowed the sex determination of most individual, with males showing higher Testo concentrations and females with elevated E2 concentrations.
- Green turtle populations at the Golfo Dulce seem to be formed by subpopulations in different stages of their reproductive cycle, with males and females close to mating season, foraging females between seasons and sub-adults in their final developing habitat.
- Finally, P4 results indicate that none of the females were nesting, so these turtles probably nest far from this area.

Recommendations

In the future, procedures like laparoscopy, testicular biopsy, mating, or nesting behavior observation should be included to confirm the reproductive hormones' results, especially in sexually immature animals, ideally using a population with known sizes associated with each life stage.

Author contribution All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Priscilla Howell, Kinndle Blanco-Peña, Marcela Suárez-Esquivel, Laura Castro-Ramírez, Andrea Chaves, and Didiher Chacón. The first draft of the manuscript was written by Kinndle Blanco-Peña,

Marcela Suárez-Esquivel, Laura Castro-Ramírez, and Priscilla Howell. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Fondo de Educación Superior Estatal del Consejo Nacional de Rectores de Costa Rica (CONARE), the Ministry of Science and Technology of Costa Rica (MICITT), and the Fishing Law of Costa Rica.

Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval The study was conducted following the current guidelines for animal welfare stipulated by the School of Veterinary Medicine, Universidad Nacional, Costa Rica. Samples were collected according to the following permit numbers: MINAET-SINAC-Costa Rica: SINAC-SE-GASP-PI-202.

Conflict of interest The authors declare no competing interests.

References

Aguirre A, Balazs G (2000) Blood biochemistry values of green turtles, *Chelonia mydas*, with and without fibropapillomatosis. Comp Haematol Int 10:132–137. https://doi.org/10.1007/s005800070004

Al-Habsi AA, Alkindi AYA, Mahmoud IY et al (2006) Plasma hormone levels in the green turtles Chelonia mydas during peak period of nesting at Ras Al-Hadd-Oman. J Endocrinol 191:9–14. https://doi.org/10.1677/joe.1.06790

Blanvillain G, Owens DW, Kuchling G (2011) Hormones and reproductive cycles in turtles. In: López N (ed) Hormones and reproduction of vertebrates: Reptiles. Academic Press, San Diego, USA, pp 277–303

Chacón-Chaverri D, Martínez-Cascante DA, Rojas D, Fonseca LG (2015) Golfo Dulce, Costa Rica, an important foraging ground for the Pacific hawksbill turtle (*Eretmochelys imbricata*). Rev Biol Trop 63:351–362

Eckert KL, Bjorndal KA, Abreu Grobois FA, Donnelly M (2000) Diseño de un programa de conservación. p. 6–8. In Técnicas de investigación y manejo para la conservación de las tortugas marinas. Consolidated Graphic Communications, Pennsylvania, USA. https://portals.iucn.org/library/sites/library/files/documents/1999-076-Es.pdf

Hamann M, Jessop T, Limpus C, Whittier J (2002a) Interactions among endocrinology, seasonal reproductive cycles and the nesting biology of the female green sea turtle. Mar Biol 140:823–830. https://doi.org/10.1007/s00227-001-0755-8

Hamann M, Jessop TS, Limpus CJ, Whittier JM (2005) Regional and annual variation in plasma steroids and metabolic indicators in female green turtles, *Chelonia mydas*. Mar Biol 148:427–433. https://doi.org/10.1007/s00227-005-0082-6

Hamann M, Limpus C, Whittier J (2002b) Patterns of lipid storage and mobilisation in the female green sea turtle (*Chelonia mydas*). J Comp Physiol B 172:485–493. https://doi.org/10.1007/s00360-002-0271-2

Howell P, Meneses A, Suárez-Esquivel M et al (2021) Blood parameters of the Eastern Pacific green turtle (*Chelonia mydas*) foraging in the Golfo Dulce, Costa Rica. Comp Clin Path. https://doi.org/10.1007/s00580-021-03274-5

Jessop TS, Limpus CJ, Whittier JM (1999) Plasma steroid interactions during high-density green turtle nesting and associated disturbance. Gen Comp Endocrinol 115:90–100. https://doi.org/10.1006/gcen.1999.7288

Licht P, Wood J, Owens DW, Wood F (1979) Serum gonadotropins and steroids associated with breeding activities in the green sea turtle *Chelonia mydas*: I. Captive Animals General and Comparative Endocrinology 39:274–289. https://doi.org/10.1016/0016-6480(79)90122-9

Licht P, Wood JF, Wood FE (1985) Annual and diurnal cycles in plasma testosterone and thyroxine in the male green sea turtle Chelonia mydas. Gen Comp Endocrinol 57:335–344. https://doi.org/10.1016/0016-6480(85)90212-6

Mahmoud IY, Alkindi AY, Khan T, Al-Bahry SN (2011) Detection of low plasma estradiol concentrations in nesting green turtles (*Chelonia mydas*) by HPLC/Ms-Ms. J Exp Zool A Ecol Genet Physiol 315:170–174. https://doi.org/10.1002/jez.665

Méndez-Salgado E, Chacón-Chaverri D, Fonseca LG et al (2020) Trophic ecology of hawksbill turtles (*Eretmochelys imbricata*) in Golfo Dulce, Costa Rica: integrating esophageal lavage and stable isotope (δ13C, δ15N) analysis. Lat Am J Aquat Res 48:114–130. https://doi.org/10.3856/vol48-issue1-fulltext-2230

Moon DY, MacKenzie DS, Owens DW (1998) Serum thyroid hormone levels in wild and captive sea turtles. Korean J Biol Sci 2(2):177–181. https://doi.org/10.1080/12265071.1998.9647404

Morales-Ramírez Á, Acuña-González J, Lizano O et al (2015) Oceanographic features of Golfo Dulce, Costa Rican Pacific: a review for decision-making in marine conservation. Revista de Biología Tropical 63:131–160. https://doi.org/10.15517/rbt.v63i1.23100

Musick JA (2002) Sea turtles. In K. E. Carpenter, K.E. (Ed.). The living marine resources of the Western Central Atlantic. (pp. 2018–2028). Rome, Italy. Food and Agriculture Organization of the United Nations

Owens DW (1996) Hormones on the life story of sea turtles. In: Lutz PL, Musick JA (eds) The biology of sea turtles. CRC Press, Boca Raton, Florida, pp 315–342

Rostal DC, Owens DW, Grumbles JS et al (1998) Seasonal reproductive cycle of the Kemp's ridley sea turtle (*Lepidochelys kempi*). Gen Comp Endocrinol 109:232–243. https://doi.org/10.1006/gcen.1997.7026

Valente ALS, Velarde R, Parga ML et al (2011) Reproductive status of captive Loggerhead sea turtles based on serum levels of gonadal steroid hormones, corticosterone and thyroxin. Vet J 187:255–259. https://doi.org/10.1016/j.tvjl.2009.11.022

Wibbels T, Balazs GH, Owens DW, Amoss MS (1993) Sex ratio of immature green turtles inhabiting the Hawaiian Archipelago. J Herpetol 27:327–329. https://doi.org/10.2307/1565155