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Blood Biochemistry of the Breeding Population of Green Turtles (*Chelonia mydas*) in the Aves Island Wildlife Refuge, Venezuela

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Abstract. We determined the blood biochemistry parameters for the breeding population of green turtles (*Chelonia mydas*) in the Aves Island Wildlife Refuge (Venezuela), the second largest breeding colony of relevance in the Caribbean. We collected 59 blood samples (48 nesting females and 11 adult males) by puncturing the dorsal cervical sinus. Sexual maturity was estimated by measuring curved carapace length, curved carapace width, and tail length (only in males). We used colorimetric techniques for biochemical analysis. Mineral concentrations in the samples were determined by atomic absorption (Ca, Mg, Zn, Cu, and Mg) and emission (Na and K) spectrometry. This study provides valuable biochemistry reference values for breeding populations of green turtles from Venezuela and the Caribbean. The population is considered healthy, with parameter values coinciding with previously reported reference ranges of species in the Caribbean and Atlantic populations. We conclude that, for the population in the Aves Island Wildlife Refuge, variability in the values obtained for total protein, albumin, blood urea nitrogen, alanine aminotransferase, glucose, triglycerides, HDL-cholesterol, calcium, phosphorus, magnesium, and copper was directly related to factors such as sex and animal size.

Keywords. Aves Island; Blood Biochemistry; *Chelonia mydas;* Green Turtle; Venezuela.

Resumen. Determinamos los parámetros de la bioquímica sanguínea para la población reproductiva de tortuga verde (*Chelonia mydas*) en el Refugio de Fauna Silvestre Isla de Aves, Venezuela, la segunda colonia anidadora de mayor importancia en el Caribe. Se colectaron 59 muestras sanguíneas (48 hembras anidadoras y 11 machos adultos) mediante punción de los senos cervicales. La madurez sexual de los animales fue determinada midiendo el largo curvo del caparazón, ancho curvo del caparazón y la longitud de la cola (solo en los machos). Fueron utilizadas técnicas colorimétricas para los análisis bioquímicos. La concentración mineral en las muestras fue determinada por espectrofotometría de absorción (Ca, Mg, Zn, Cu y Mg) y emisión (Na y K) atómica. Este trabajo provee valiosa información sobre intervalos de referencia de parámetros bioquímicos sanguíneos en poblaciones reproductivas de tortugas verde en Venezuela y el Caribe. Los resultados obtenidos permiten la clasificación de la población como saludable, coincidiendo con los rangos de referencias previamente reportados para la especie en poblaciones del Caribe y el Atlántico. Fue concluido que, para la población en el Refugio de Fauna Silvestre Isla de Aves, la variabilidad en los valores obtenidos para proteínas totales, albúmina, nitrógeno ureico en la sangre, alanina aminotransferasa, glucosa, triglicéridos, HDL-colesterol, calcio, fósforo, magnesio y cobre estuvieron directamente relacionadas a factores como el sexo y tamaño de los animales.

INTRODUCTION

Blood biochemistry represents a valuable tool for monitoring the health of wildlife, which allows for renal, hepatic, cellular, and muscular assessments of individuals (Montilla *et al.,* 2008). These evaluations are important to characterize distinctive physiological parameters and specific value types of disease (Aguirre and Balazs, 2000; Kakizoe *et al.,* 2007). Changes in blood biochemical values can be indicators of chronic or pathological conditions (Aguirre *et al.,* 1995). Currently, there is a need for more specific studies to establish reference values for healthy animals of each species from different geographic locations and age classes.

Biological data collection and management actions for threatened species of marine wildlife must be spread over spatial and temporal scales that complement the species' ecological scale (Hamann *et al.,* 2006; Seminoff, 2004). Previous research has suggested that blood chemistry variation can be associated with geographic areas, habitats and population genetics (Herbst and Jacobson, 2003); maturity and sex of individuals (Hamann *et al.,* 2006); and migratory conditions and diets (Stamper *et al.,* 2005; Whiting *et al.,* 2007). Yet, due to a variety of reasons, baseline health data are not available for many populations throughout the world, including threatened populations of the green turtle *Chelonia mydas* (Hamann *et al.,* 2006; Flint *et al.,* 2010). Currently, studies are principally restricted to some green turtle populations in the Southern Bahamas (Bolten *et al.,* 1992), Hawaii (Aguirre *et al.,* 1995; Aguirre and Balazs, 2000), United Arab Emirates (Hasbún *et al.,* 1998), EE.UU. (Stamper *et al.,* 2005), Venezuela (Montilla *et al.,* 2008), Uruguay (Ferrando, 2010), and Australia (Anderson *et al.,* 2011).

In Venezuela, the green turtle is one of five sea turtle species distributed along different areas of the continental

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coast (about 2000 km, from the peninsula of "La Guajira" in the west to "Punta Barima" in the east) and its insular systems that include Aves Island. This large distribution provides different habitats for the development, feeding, breeding, refuge, and nesting of the species (Guada and Solé, 2000). Aves Island Wildlife Refuge (Refugio de Fauna Silvestre Isla de Aves in Spanish, or RFSIA) is the main nesting site for green turtles in the country and second most important nesting colony in the Caribbean with a total of 300 to 600 nesting females between July and September (peak of the reproductive season; Pritchard, 1984; Vera and Buitrago, 2012). Since 1979 a mark-recapture program of green turtles has been ongoing at RFSIA. However, to establish a successful conservation program for this green turtle population, collection of blood samples is essential, providing baseline values for hematologic and blood chemistry parameters. This would assist in evaluating the health and well being of the population, increasing the ability to make necessary and timely management decisions that consider spatial and temporal scales (Aguirre and Balazs, 2000; Harris *et al.,* 2011; Prieto-Torres, 2011; Prieto-Torres *et al.,* 2012).

The aims of this study were to 1) obtain blood chemistry reference values of the breeding population of green turtles from Aves Island Wildlife Refuge, 2) compare the blood chemistry values with data previously reported for sea turtle populations, and 3) determine blood chemistry differences of the RFSIA population according to sex, size, and reproductive state of the animals.

MATERIALS AND METHODS

Study period and site

To assess blood biochemistry values we collected blood samples from the breeding population of green turtles on Aves Island (Republic Bolivarian of Venezuela) on 31 July and 10 September 2010. Aves Island is a Federal entity located in the Caribbean Sea (15°40'33"N, 63°36'27"W, Fig. 1) and was declared a Wildlife Refuge in 1972 by Decree No. 1069 of the Official Gazette No. 29888. Since 1979, a turtle protection program has been ongoing, led by the Venezuelan Ministry of the Environment (Prieto-Torres, 2011; Vera and Buitrago, 2012).

Morphometry and physical examination

All turtles were tagged on their flippers with Inconel metal tags (No. 681, National Band & Tag Company) and released in the vicinity of capture. Sexual maturity of animals was estimated measuring curved carapace length (CCL), curved carapace width (CCW), and tail length (TL, only in males) (Bolten, 1999). We performed a physical examination on each green turtle captured to assess behavior, movement, body condition, and lesions on skin and carapace (Harris *et al.,* 2011).

Sample size and blood collection

For this study, we collected 59 blood samples from 48 nesting females captured passively during oviposition and 11 adult males captured manually through diving. Nesting females were approached approximately 5 min after nest-building behavior ceased and egg-laying activity had begun. If the female did not appear to be in an egg-laying trance at the time of the first approach, we waited an additional 5 min to avoid disturbing of egg-laying activity (Deem *et al.,* 2006).

Approximately 5 ml of blood was collected from the dorsal cervical sinus (Owens and Ruiz, 1980) using a 22 × 1.5 inch gauge needle and dispensed directly into medium (5 ml) Vacutainer® tubes containing lithium heparin (Corvac, Sherwood Medical, Saint Louis, Missouri, USA). After blood collection, pressure was applied and the affected area was treated to avoid hematoma formation (Aguirre *et al.,* 1995). Sample tubes were kept on wet ice in a cooler (no more than one hour) before their processing. Plasma was separated by centrifugation at 13000 rpm × 5 minutes (Aguirre *et al.,* 1995; Hasbún *et al.,* 1998) and divided among 2–3 vials. There was no evidence of hemolysis. Vials were stored in a freezer at -50°C until tested at the Laboratorio de Investigación y Desarrollo en Nutrición (Universidad del Zulia).

Biochemical analysis and mineral concentrations

Plasma was used in preference to serum because in reptiles clot formation can be unpredictable, changing biochemical values and occasionally producing hemolysis in the blood samples (Bolten *et al.,* 1992, Campbell, 2004). This study looked at 25 chemical parameters that can be separated into three general groups: nutrients and metabolites, enzymes, and elements and electrolytes (Whiting *et al.,* 2007). Commercial kits (Wiener Lab. Group, Argentina) were used for biochemical analyses. Total plasma protein and albumin were measured using the Lowry and Bromocresol colorimetric methods, respectively; globulin and albumin/globulin proportion (A/G) were derived from these values. Alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (GGT) were measured at 37°C conditions. Also measured were phosphorus (phosphomolybdate method), creatinine, uric acid, cholesterol, HDL-cholesterol, glucose, triglycerides and blood urea nitrogen (BUN) (enzymatic colorimetric

assay). All plasma biochemical analyses were performed using an automated chemistry analyzer (METROLAB 2300 Plus, Wiener Lab. Group, Argentina) in the Centro de Investigaciones Endocrino Metabólicas "Dr. Félix Gómez" (Faculty of Medicine, Universidad del Zulia).

Mineral concentrations were determined using a Perkin Elmer 3100 (EUA) spectrophotometer in the Laboratorio de Instrumentación Analítica (Faculty of Science-Universidad del Zulia). Calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu) and iron (Fe) were measured using flame atomic absorption spectroscopy (FAAS). Detection limits for FASS were 0.003 ppm for Ca, Fe, and Cu; and 0.006 ppm for Mg and Zn. Sodium (Na) and Potassium (K) were analyzed using atomic emission spectroscopy (AES) (Aguirre *et al.,* 1995; Aguirre and Balazs, 2000; Montilla *et al.,* 2008; Harris *et al.,* 2011).

Remigration periods

For each nesting female, we determined the remigration period, defined as the number of years between consecutive nesting season (Alvarado and Murphy, 1999). We used as references the documentation of the last nesting event for each female that appeared in the database of the Ministry of the Environment's conservation project (Prieto-Torres, 2011). Nesting females were classified as neophytes (new recruits, without report of previous nesting), remigrant females with a remigration period of two years, and remigrant females with a remigration period longer than three years.

Statistical analyses

Data were expressed as mean, standard deviation (SD), and reference intervals for each chemical parameter. To evaluate data normality we used the Shapiro-Wilk statistic. Reference interval values were only calculated for nesting females due to the small sample of males. We calculated these values in two ways (Farver, 2008): 1) all values between the mean and two SDs were included for normally distributed variables; 2) data found between the 2.5th and 97.5th percentiles were selected for variables that were not normally distributed. The relation between body size (CCL and CCW) and blood chemistry values in individuals was evaluated using linear regression analysis. We used either Student's *t* or the U-Mann-Whitney test to identify differences between values for male and female turtles. The Kruskal-Wallis or ANOVA test was used to identify differences between females according to remigration period (considered a reproductive state indicator). All analysis was performed using a significance level of 95% ($\alpha = 0.05$) in SPSS 19.0 for Windows.

RESULTS

Morphometry and physical examination

Captured animals were classified as adults (Aguirre and Balazs, 2000; Montilla *et al.,* 2008). For nesting females, mean CCL was 112.35 ± 5.94 (101.50–125.40) cm and mean CCW was 101.55 ± 6.11 (87.00–116.70) cm; for

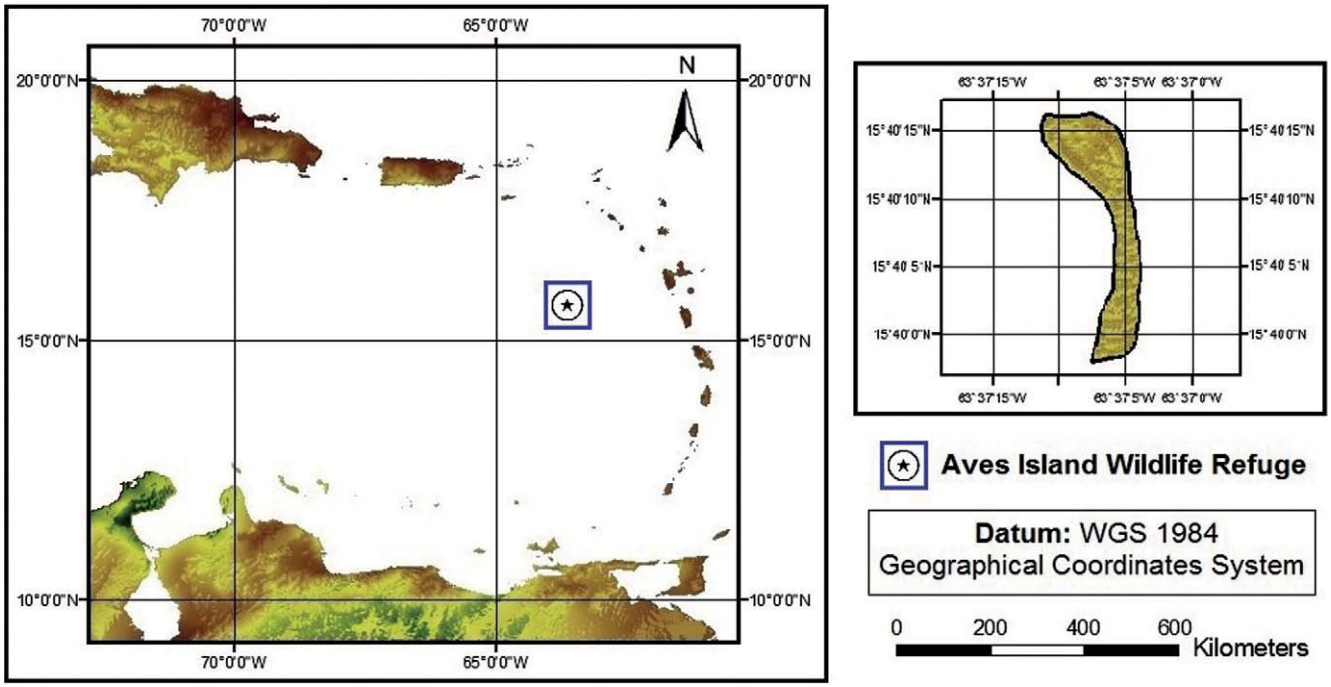

Figure 1. Aves Island Wildlife Refuge locality map.

Table 1. Blood chemistry values for breeding population of green turtles, *Chelonia mydas,* in the Aves Island Wildlife Refuge. Single asterisks (*) indicates parameters analyzed with Student's *t* test. Double asterisks (**) indicate parameters analyzed with U-Mann-Whitney test. A solid square (■) indicates significance at the 0.05 level

Parameters	Population (*n* = 59)	Nesting females (*n* = 48)			Adult males (*n* = 11)	
	Mean ± SD	Mean ± SD	Reference intervals	Min.–Max.	Mean ± SD	Min.–Max.
Nutrients and Metabolites						
Total protein (g/dL)*	4.83 ± 0.61	4.74 ± 0.56	3.62–5.86	3.30–5.90	5.24 ± 0.69	4.10–6.40
Albumin (g/dL)**■	2.18 ± 0.40	2.24 ± 0.41	1.30–3.41	1.30–3.45	1.91 ± 0.61	1.50–2.20
Globulin (g/dL)*	2.70 ± 0.61	2.55 ± 0.50	1.27–3.71	1.20–3.72	3.33 ± 0.65	2.60–4.60
A/G ratio**■	0.87 ± 0.36	0.92 ± 0.37	0.49–2.39	0.46–2.46	0.60 ± 0.13	0.37–0.85
Creatinine (mg/dL)**	0.48 ± 0.40	0.49 ± 0.43	0.08–2.51	0.07–2.80	0.46 ± 0.21	0.10–0.78
BUN (mg/dL)**■	24.09 ± 13.58	21.16 ± 9.00	4.68–41.33	4.00–42.00	36.91 ± 21.59	14.00–80.00
Uric acid (mg/dL)*	0.97 ± 0.53	1.08 ± 0.52	0.04–2.12	0.22–2.40	0.49 ± 0.29	0.16–1.00
Glucose (mg/dL)**■	76.14 ± 23.51	71.46 ± 23.01	25.44–117.48	32.40–136.67	96.58 ± 12.35	71.55–116.67
Cholesterol (mg/dL)*	329.20 ± 70.18	322.26 ± 66.45	189.36–455.16	133.90–497.40	359.47 ± 81.09	250.20–484.10
Triglycerides (mg/dL)**■	709.82 ± 359.88	835.91 ± 267.03	165.42–1395.15	146.90–1401.00	159.62 ± 82.22	85.00–335.60
HDL-cholesterol (mg/dL)**■	35.69 ± 22.84	28.89 ± 11.70	10.23–56.78	10.00–57.00	65.36 ± 34.50	10.00–122.00
Enzymes						
ALKP (U/L)**	123.65 ± 55.46	126.84 ± 55.74	50.00–312.61	50.00–330.20	109.75 ± 54.53	61.00–246.80
ALT (U/L)**■	25.29 ± 30.48	30.52 ± 31.55	1.64–141.37	1.57–144.10	2.68 ± 1.55	1.31–6.27
AST (U/L)*	65.71 ± 31.53	65.59 ± 29.32	6.95–124.23	4.60–131.00	66.25 ± 41.54	5.50–167.00
GGT (U/L)**	2.79 ± 2.85	2.73 ± 3.04	0.00–13.73	0.00–13.90	3.09 ± 1.78	1.16–5.79
Elements and Electrolytes						
Sodium (meq/L)**	200.72 ± 26.48	203.22 ± 25.58	152.06–254.38	144.27–269.32	189.94 ± 28.99	144.27–229.45
Potassium (meq/L)*	3.45 ± 1.03	3.39 ± 0.95	1.35–6.11	1.35–6.15	3.71 ± 1.31	1.35–5.02
Calcium (mg/dL)**■	11.41 ± 4.15	12.79 ± 3.28	6.23–19.35	6.52–21.77	5.50 ± 1.14	4.37–7.13
Phosphorus (mg/dL)**■	8.94 ± 3.17	9.45 ± 3.17	4.71–21.30	4.70–21.50	6.60 ± 1.97	4.40–11.30
Calcium/Phosphorus ratio*	1.33 ± 0.44	1.43 ± 0.41	0.61–2.25	0.30–2.33	0.90 ± 0.31	0.42–1.40
Magnesium (mg/L)**■	7.35 ± 1.81	7.62 ± 1.86	4.11–12.85	4.10–13.04	6.18 ± 0.95	3.79–7.29
Zinc (mg/L)**	1.29 ± 0.59	1.32 ± 0.64	0.70–3.66	0.70–3.70	1.14 ± 0.30	0.69–1.63
Copper (mg/L)**■	0.30 ± 0.17	0.28 ± 0.15	0.03–0.58	0.03–0.60	0.39 ± 0.24	0.05–0.80
Zinc/Copper ratio**	6.94 ± 10.30	7.32 ± 11.07	1.32–68.83	1.30–72.92	5.34 ± 6.34	1.36–22.62
Iron (ug/dL)**	49.75 ± 51.54	50.48 ± 55.81	4.05–196.27	4.05–279.28	46.09 ± 21.55	19.34–65.21

adult males, mean CCL was105.7 ± 3.75 (98.6–110.0) cm, mean CCW was 96.3 ± 4.38 (87.4–100.6) cm, and mean TL was 34.3 ± 3.29 (27.0–38.5) cm. All individual green turtles were alert and active during capture and were considered clinically healthy upon physical examination (Thomson *et al.,* 2009; Ferrando, 2010; Harris *et al.,* 2011).

Nesting females appeared healthy according to physical examination criteria. All females were strong enough to navigate from the water to their nesting sites, and undergo the rigorous process of digging, laying eggs, and covering the nest without any unusual incident. Many of the nesting turtles had evidence of old scars and healed injuries, none of which appeared to affect their behavior. Only two females showed evidence of possible fibropapillomas in low quantities on the front flippers and neck region, but their biochemical values were within the range found for other individuals, and their samples were not excluded from the analysis.

Plasma biochemistry values

Descriptive statistics of biochemical analysis for the population and each sex are presented in Table 1. Biochemical analysis for 10 samples could not be completed across all parameters due to limited sample volume. Iron concentration values for five samples were less than the detection limit of the technique employed.

For the first time in the clinical evaluation of sea turtles, we report HDL-cholesterol, Ca/P ratio, copper, and Zn/Cu ratio concentrations to complement the assessment of lipid profile and nutritional status in these animals.

Differences within the population

Seven blood parameters were significantly correlated with turtle size in the population, of which four were inversely correlated (Table 2). The variability (R^2) accounted for body size in seven parameters, ranging from 6.6–19.2%. Additionally, 11 blood chemistry parameters differed significantly between nesting females (with higher values of albumin, A/G ratio, ALT, triglycerides, calcium, phosphorus, and magnesium) and adult males (with higher values for BUN, glucose, HDL-cholesterol, and copper).

We observed 22 neophyte and 23 remigrant females (12 females with remigration intervals of 2 years and 11 females with intervals longer than 3 years). Three females were observed with tags from another Caribbean conservation program, but without previously reports of nesting in RFSIA. There were no significant differences between the biochemical parameters studied for female nesting subgroups (Table 3).

Table 2. Relationship between body size (CCL and CCW) and blood chemistry parameters of green turtles, *Chelonia mydas,* in the Aves Island Wildlife Refuge. Bold numbers indicate significant correlation. Asterisks marks blood parameters that were inversely correlated with turtle size

Parameters	*Curved Carapace Length*		*Curved Carapace Width*	
	R^2	*p*	R^2	*p*
Nutrients and Metabolites				
Total protein (g/dL)*	0.091	**0.020**	0.085	**0.029**
Albumin (g/dL)	0.019	0.302	<0.001	0.922
Globulin (g/dL)	0.119	**0.007**	0.070	**0.043**
A/G ratio	0.006	0.557	<0.001	0.968
Creatinine (mg/dL)	0.011	0.428	0.055	0.074
BUN (mg/dL)*	0.071	**0.042**	0.075	**0.036**
Uric Acid (mg/dL)	0.022	0.262	0.002	0.634
Glucose (mg/dL)	0.010	0.451	0.001	0.808
Cholesterol (mg/dL)	0.007	0.529	0.016	0.333
Triglycerides (mg/dL)	0.093	**0.019**	0.037	0.142
HDL-cholesterol (mg/dL)*	0.066	**0.049**	0.050	0.088
Enzymes				
ALKP (U/L)	0.020	0.288	0.032	0.176
ALT (U/L)	0.006	0.552	<0.001	0.941
AST (U/L)	0.026	0.218	0.036	0.147
GGT (U/L)	0.046	0.102	0.061	0.059
Elements and Electrolytes				
Sodium (meq/L)	0.007	0.556	0.002	0.749
Potassium (meq/L)	0.003	0.723	0.020	0.323
Calcium (mg/dL)	0.012	0.441	0.001	0.819
Phosphorus (mg/dL)	0.008	0.540	<0.001	0.977
Calcium/phosphorus ratio	0.018	0.356	0.001	0.852
Magnesium (mg/L)	0.085	**0.034**	0.070	0.055
Zinc (mg/L)	<0.001	0.987	0.001	0.828
Copper (mg/L) *	0.143	**0.006**	0.192	**0.001**
Zinc/copper ratio	0.045	0.132	0.057	0.090
Iron (ug/dL)	0.003	0.712	0.002	0.749

DISCUSSION

Nutrients and metabolites

For the breeding population at RFSIA, most nutrients and metabolites analyzed were consistent with previously reported values for green turtle populations in the Caribbean and the Atlantic Ocean (Bolten and Bjorndal, 1992; Hasbún *et al.,* 1998; Aguirre and Balazs, 2000; Whiting *et al.,* 2007; Montilla *et al.,* 2008; Ferrando, 2010; Anderson *et al.,* 2011), with the exception of glucose, cholesterol, and triglyceride concentrations.

The mean glucose values obtained in this study were low relative to the reference values (70 to 120 mg/dL) of healthy individual green turtles but were consistent with those reported for nesting females of other sea turtle species (Deem *et al.,* 2006; Santoro and Meneses, 2007; Casal *et al.,* 2009; Goldberg *et al.,* 2011; Perrault *et al.,* 2012). This result could be associated with reduce food intake during the nesting season (Casey *et al.,* 2010; Anderson *et al.,* 2011), and the high energy required to complete the nesting process by females (Santoro and Meneses, 2007). Otherwise, differences between nesting females and adult males suggest that the capture technique and blood sample extraction process (capture by diving vs. passive capture) can generate stress conditions in the animals and promote increased glucose values (Aguirre *et al.,* 1995; Aguirre and Balazs, 2000; Montilla *et al.,* 2008).

High plasma lipid values obtained for the population can be explained by catabolism of energy reserves during the reproductive stage (Derickson, 1976; Bonnet, 1979; Goldberg *et al.,* 2011), egg formation by nesting females during vitellogenesis (Bonnet, 1979; Kakizoe *et al.,* 2007; Deem *et al.,* 2009; Goldber *et al.,* 2011), and testosterone production in males (Kasikoe *et al.,* 2007).

Enzymes

The ALKP concentrations obtained in the population are consistent with previous results (Montilla *et al.,* 2008; Casal *et al.,* 2009), which demonstrated that levels were expected to be higher in adults than in juveniles and subadults. In mammals, it is a sensitive indicator of bile obstruction, which increases in growing individuals. However, this enzyme is not tissue-specific in reptiles, making the interpretation of this finding difficult (Lowell, 1998). On the other hand, the high activity of ALKP obtained in this study may be related to an increase in osteoblastic activity for egg formation by nesting turtles (Campbell, 2004).

ALT and AST concentrations were slightly different from previous results observed in other populations of

Table 3. Blood chemistry values of female nesting subgroups of green turtle, *Chelonia mydas,* in the Aves Island Wildlife Refuge. Single asterisks (*) indicate parameters analyzed with ANOVA. Double asterisks (**) indicate parameters analyzed with the Kruskal-Wallis test.

Parameters	New recruits (*n* = 22)	Remigrant females	
		two years (*n* = 12)	> three years(*n* = 11)
	Mean ± SD	Mean ± SD	Mean ± SD
Nutrients and Metabolites			
Total protein (g/dL)*	4.72 ± 0.49	4.52 ± 0.67	4.93 ± 0.61
Albumin (g/dL)**	2.13 ± 0.40	2.22 ± 0.35	2.49 ± 0.48
Globulin (g/dL)*	2.59 ± 0.32	2.31 ± 0.73	2.44 ± 0.94
A/G ratio**	0.84 ± 0.20	1.10 ± 0.55	0.94 ± 0.43
Creatinine (mg/dL)**	0.46 ± 0.22	0.41 ± 0.27	0.69 ± 0.79
BUN (mg/dL)**	20.82 ± 8.26	17.91 ± 7.85	23.23 ± 10.41
Uric Acid (mg/dL)*	1.06 ± 0.58	0.90 ± 0.42	1.22 ± 0.50
Glucose (mg/dL)**	71.30 ± 25.94	75.93 ± 18.16	71.59 ± 24.65
Cholesterol (mg/dL)*	320.47 ± 53.38	305.45 ± 82.37	319.44 ± 64.23
Triglycerides (mg/dL)**	805.12 ± 264.41	834.44 ± 217.49	843.08 ± 346.46
HDL-cholesterol (mg/dL)**	31.96 ± 10.99	27.14 ± 12.82	23.55 ± 10.74
Enzymes			
ALKP (U/L)**	113.64 ± 40.05	121.53 ± 65.65	161.06 ± 70.25
ALT (U/L)**	34.55 ± 41.74	18.68 ± 15.15	38.04 ± 21.52
AST (U/L)*	69.28 ± 24.38	71.87 ± 30.48	48.41 ± 33.81
GGT (U/L)**	2.32 ± 2.52	2.61 ± 3.22	3.73 ± 4.26
Elements and Electrolytes			
Sodium (meq/L)**	199.53 ± 31.07	208.85 ± 27.25	202.49 ± 11.07
Potassium (meq/L)*	3.12 ± 1.03	3.42 ± 1.08	3.77 ± 0.67
Calcium (mg/dL)**	12.93 ± 3.28	11.74 ± 4.23	13.12 ± 1.40
Phosphorus (mg/dL)**	9.15 ± 2.15	10.46 ± 5.41	9.06 ± 2.36
Calcium/Phosphorus ratio*	1.43 ± 0.33	1.37 ± 0.60	1.41 ± 0.32
Magnesium (mg/L)**	7.47 ± 1.99	8.01 ± 2.04	7.54 ± 1.71
Zinc (mg/L)**	1.36 ± 0.73	1.18 ± 0.38	1.21 ± 0.42
Copper (mg/L)**	0.29 ± 0.18	0.20 ± 0.11	0.32 ± 0.11
Zinc/Copper ratio**	8.92 ± 16.01	7.68 ± 4.61	4.13 ± 1.88
Iron (ug/dL)**	42.57 ± 41.22	29.26 ± 32.09	56.74 ± 61.83

this species (Campbell, 1996; Wilkinson, 2004; Whiting *et al.,* 2007; Ferrando, 2010; Anderson *et al.,* 2011; Goldberg *et al.,* 2011; Harris *et al.,* 2011). The underlying cause is uncertain, but it might be due to fasting during the reproductive season because ALP, AST, ALT and amylase values increase significantly in green turtles while feeding in foraging areas (Anderson *et al.,* 2011). The different ALT and AST concentrations might also be related to morphological variation among the specimens (Stamper *et al.,* 2005; Goldberg *et al.,* 2011), suggesting that animals with higher mass have higher liver enzyme activity. Activity of the serum GGT was similar to values reported by Aguirre and Balazs (2000), but is not a parameter frequently used in evaluating hepatic biliar function in sea turtles, since it is normally low.

Elements and electrolytes

Differences in Ca and P values between sexes are due to mineral mobilization directed by egg production by females during the reproductive stage (Wilkinson, 2004; Deem *et al.,* 2006). The Ca/P ratio obtained was 1.33, but this value had not been determined before for green turtle populations in Venezuela (Montilla *et al.,* 2008); further studies are needed to determine Ca/P proportions in nesting and non-nesting populations. Additionally, we documented for the first time reference values for Cu concentrations and the Zn/Cu ratio, which were used to determine the nutritional status of individuals (Montilla *et al.,* 2008) for healthy sea turtle populations. Cu and Zn are important trace elements that play an active role in enzymatic functions, cellular defense mechanisms, and hormone metabolism (Mayes, 2000).

Differences within the population

It is known that biochemical values in reptiles can be influenced by many variables, including age, sex, diet, nutritional status, and seasonal changes (Bolten and Bjorndal, 1992; Aguirre and Balazs, 2000; Herbst and

Jacobson, 2003; Campbell, 2004; Stamper *et al.,* 2005; Hamann *et al.,* 2006; Whiting *et al.,* 2007). During this study, we attempted to randomly capture turtles of similar age/size class (adults) during the same reproductive season, so we did not expect great variation in blood chemistry among the animals due to environmental changes. However, it is important to understand that in this study breeding condition—sex and size—do affect biochemical value, producing changes in some parameters such as total protein, albumin, BUN, ALT, glucose, triglycerides, HDL-cholesterol, Ca, P, Mg, and Cu. Results obtained for the breeding population of green turtles in RFSIA, according to those previously reported for the species, confirmed that baseline data should be collected considering geographic area, seasonal variability, and foraging or reproductive populations (Whiting *et al.,* 2007; Anderson *et al.,* 2011).

In conclusion, this study allowed for the establishment of reference intervals for a wide range of biochemical plasma variables in green turtles at the Aves Island Wildlife Refuge, which may be valid for assessment of other populations in the tropical regions. Results obtained showed that this population could be defined as healthy and in good nutritional condition for all parameters evaluated (Bolten and Bjorndal, 1992; Aguirre and Balazs, 2000; Whiting *et al.,* 2007; Montilla *et al.,* 2008; Ferrando, 2010; Anderson *et al.,* 2011). Discussion about biochemical parameter differences provide a complete assessment and clinical diagnosis of sea turtles; and can contribute to the development of appropriate management for the conservation of *Chelonia mydas* in RFSIA, Venezuela, as well as in the Caribbean and the Atlantic Ocean.

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