

Changes in Plasma Chemistry and Reproductive Output of Nesting Leatherbacks

Author(s): Shaya Honarvar , Micah C. Brodsky , Daniel B. Fitzgerald , Karen L. Rosenthal , and Gail W. Hearn

Source: *Herpetologica*, 67(3):222-235. 2011.

Published By: The Herpetologists' League

DOI: <http://dx.doi.org/10.1655/HERPETOLOGICA-D-10-00061.1>

URL: <http://www.bioone.org/doi/full/10.1655/HERPETOLOGICA-D-10-00061.1>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

CHANGES IN PLASMA CHEMISTRY AND REPRODUCTIVE OUTPUT OF NESTING LEATHERBACKS

SHAYA HONARVAR^{1,4}, MICAH C. BRODSKY², DANIEL B. FITZGERALD¹, KAREN L. ROSENTHAL³,
AND GAIL W. HEARN¹

¹Drexel University, Department of Biology, 3141 Chestnut Street, Philadelphia, PA 19104, USA

²Dolphins Plus, 31 Corinne Place, Key Largo, FL 33037, USA

³University of Pennsylvania, School of Veterinary Medicine, Department of Clinical Studies, 3900 Delancey Street, Philadelphia, PA 19104, USA

ABSTRACT: Africa's Gulf of Guinea, a major nesting ground for the critically endangered Leatherback Sea Turtle (*Dermochelys coriacea*), is experiencing rapid economic development. This study reports on the plasma biochemistry and packed cell volume (PCV) of turtles (55 samples collected from 23 individuals) nesting on Playa Moaba, Bioko Island, Equatorial Guinea. Because energy reserves and other resources in an individual are finite and turtles may not feed between nesting episodes, decreasing trends are expected in certain plasma biochemical concentrations and PCV values, as well as maternal investment in reproductive output (clutch size and egg mass). Calcium, potassium, sodium, phosphorous, plasma total protein, albumin, and globulin concentrations changed significantly with increasing number of nesting events, but remained within reported intervals in reptiles. Packed cell volume decreased significantly as the number of nesting events per individual increased. Although clutch size did not change, egg mass decreased significantly with increasing number of nesting events. The observed trends may be due to depletion of energy reserves and other resources during reproduction in a possible fasting state, and to the associated physiological stress.

Key words: Bioko Island; *Dermochelys coriacea*; Egg mass; Packed cell volume; Plasma biochemistry; Reproductive output

BECAUSE INTERNAL ENERGY RESERVES are limited in an organism, the life histories of oviparous species often reflect tradeoffs in the use of resources for current and future reproduction (Congdon, 1989; Congdon and Gibbons, 1987). Reproductive investment of most oviparous ectothermic vertebrates, in which there is rarely any posthatching parental care, is limited to resource allocation to the number and size of eggs and clutch frequency (Congdon, 1989). Leatherback Sea Turtles (*Dermochelys coriacea*) are known to have the highest reproductive output in reptiles, with each egg weighing on average 80 g, and with individuals laying an average of 80 eggs per clutch and 7–9 clutches per nesting season (Miller, 1997; Wallace et al., 2007). Individual animals may continue this reproductive investment every 2–4 yr for more than 20 yr (Miller, 1997; Spotila et al., 1996). This reproductive biology, combined with the possibility that leatherbacks may be fasting during the nesting season (Owens, 1980; Wallace et al., 2006a), can be expected to influence plasma biochemistry parameters and hematological values.

Blood health indices have been studied previously in members of the family Cheloniidae. Blood biochemistry parameters and hematological values have been reported in adult and juvenile Green Sea Turtles (*Chelonia mydas*; Aguirre and Balazs, 2000; Bolten and Bjorndal, 1992; Hasbún et al., 1998), adult foraging Green Sea Turtles (Whiting et al., 2007), adult and subadult Loggerhead Sea Turtles (*Caretta caretta*) in the wild and captivity (Day et al., 2007; Kakizoe et al., 2007; Lutz and Dunbar-Cooper, 1987), and in Kemp's Ridley Sea Turtles (*Lepidochelys kempii*) in the wild and captivity (Carminati et al., 1994; Stabenau et al., 1991; Turnbull et al., 2000). Even though there are numerous studies on blood indices in cheloniid turtles, there are few published studies (Deem et al., 2006; Innis et al., 2010) on blood indices of the Leatherback Sea Turtle, which is the sole surviving species in the family Dermochelyidae. In general, to obtain reference intervals for blood indices it is ideal to collect blood samples from a large number of healthy animals of both sexes at different life stages. Due to the challenges associated with capturing and handling Leatherback Sea Turtles, the

⁴CORRESPONDENCE: e-mail, sh333@drexel.edu

most cost-effective approach to obtain and monitor these parameters is to collect blood samples from nesting females on the beach. Such approaches must acknowledge the potential effects of reproduction on blood biochemistry parameters and hematological values. To our knowledge, no studies have tracked biochemical and hematological values within individual sea turtles during the nesting season.

Annual and seasonal variation in clutch size (number of eggs) has been analyzed in a number of sea turtle species (Bjorndal and Carr, 1989; Frazer and Richardson, 1985; Mortimer and Carr, 1987). Loggerhead Sea Turtle and Green Sea Turtle clutches laid during the end of the reproductive season were significantly smaller than earlier clutches (Frazer and Richardson, 1985; Mortimer and Carr, 1987). Conversely, Green Sea Turtles in Tortugero, Costa Rica, laid smaller clutches early in the reproductive season and larger clutches later in the nesting season (Bjorndal and Carr, 1989). However, the increasing trend in clutch size at the population level in Green Sea Turtles nesting at Tortugero was explained by the proportion of recruits (first-time nesters) vs. remigrants, where remigrants produced larger clutches than the recruits (Bjorndal, 1980). In a Leatherback Sea Turtle population on Isla de Culebra, Puerto Rico, clutch size decreased at the population level over 3 yr but did not change at the individual level within a reproductive season (Tucker and Frazier, 1994). Although clutch-size variation within a reproductive season exists for some sea turtle populations (Bjorndal, 1980; Bjorndal and Carr, 1989; Frazer and Richardson, 1985; Mortimer and Carr, 1987; Tucker and Frazier, 1994) and geographically within a taxon (Bjorndal, 1980; Bjorndal and Carr, 1989; Mortimer and Carr, 1987), the potential seasonal variations in egg mass at the population and the individual levels remain poorly understood for sea turtles.

The Gulf of Guinea contains one of the world's largest nesting populations of the critically endangered Leatherback Sea Turtle (Sounguet et al., 2004; Witt et al., 2009). This region has seen rapid economic expansion as the result of more than a decade of offshore petroleum exploration and development (Witherington et al., 2009). Due to the

importance of this region for the future survival of leatherbacks, and the potential threat of an environmental disaster from increasing oil production, it is important to obtain adequate baseline data on these populations as well as to understand how biochemical concentrations change throughout an individual's life history. Proper management of Leatherback Sea Turtles requires health data specific to this species due to their unique physiology. These data can be used in future conservation and management practices, such as comparative studies of clinically normal and diseased turtles (Aguirre and Balazs, 2000; Bolten and Bjorndal 1992; Norton et al., 1990), or as indicators of exposure to contaminants (Lutcavage et al., 1995).

The objectives of this study were threefold: (1) to determine the plasma biochemical and packed cell volume (PCV) reference intervals for Leatherback Sea Turtles nesting on Bioko Island; (2) to assess the variation in plasma biochemical parameters and PCV within individuals during consecutive nesting events throughout the reproductive season; and (3) to assess variation in egg mass and clutch size within individuals during consecutive nesting events throughout the reproductive season.

MATERIALS AND METHODS

Study Site

This study was conducted from November to December 2008, at Playa Moaba, Bioko Island, Equatorial Guinea (3°14'00.48"N, 8°37'51.00"E; datum = WGS84). Playa Moaba is a 2.2-km black sand beach located within the Gran Caldera and Southern Highlands Scientific Reserve. Four species of turtles (Leatherback Sea Turtle; Green Sea Turtle; Olive Ridley Sea Turtle [*Lepidochelys olivacea*]; and Hawksbill Sea Turtle [*Eretmochelys imbricate*]) nest along Bioko's southern coast, with leatherbacks predominantly nesting on Playa Moaba (Butynski, 1996; Rader et al., 2006). The nesting season on Bioko Island corresponds with the dry season, occurring from September to April and peaking in late December (Rader et al., 2006; Tomás et al., 2010).

Physical Examination

The health status of Leatherback Sea Turtles was rated using (1) a visual body

examination, (2) nest-building behavior, and (3) body condition score (BCS) from 1 (emaciated) to 5 (obese). For the visual body examination, a description of lesions or injuries was recorded and the longest dimension was measured. This examination also included the number and location of any epibiotic organisms. Nest-building behavior was described by recording the times and duration of various nesting activities, including construction of the body pit, digging of the egg chamber, oviposition, and covering of the nest. Standard curved carapace length (SCCL) and Standard curved carapace width (SCCW) were measured at least three times and averaged.

Blood Collection and Analysis

Blood samples ($n = 55$) were collected from 23 individual turtles during different nesting events in one reproductive season. Blood collection was initiated prior to any other invasive procedure and immediately after the onset of egg deposition. Venipuncture was performed at the bifurcation of the interdigital vessels (IDV) of a posterior flipper (Deem et al., 2006; Wallace and George, 2007). If that site was unavailable or unproductive, then the dorsal cervical sinus (DCS) was used (Owens and Ruiz, 1980). Prior to venipuncture, the appropriate site was prepared with alternating wipes of povidone-iodine surgical scrub and ethanol. Blood was collected into a 3-mL syringe using a 20-gauge, 3.8-cm needle for the IDV or a 20-gauge, 11.4-cm spinal needle for the DCS. A 3-mL lithium heparin tube (BD-Diagnostics, Pre-Analytical Systems, Franklin Lakes, NJ 07417, USA) was filled directly from the syringe (needle removed from the syringe and cap removed from the vacuum tube) with 2.5 mL of whole blood for PCV and plasma biochemistry analysis. Blood samples were placed on instant cold packs immediately after phlebotomy and transported to the on-site lab for processing.

Blood was analyzed for plasma biochemistry parameters and PCV values within 2 h of collection. Whole blood was placed in untreated hematocrit tubes and centrifuged using the Stat Spin MP Multipurpose centrifuge (IRIS International Inc., Norwood, MA

02062, USA) to obtain PCV. The lithium heparin tubes were centrifuged for 10 min at 1100 g (Medilite centrifuge, Thermo Scientific, Ashville, NC 28806, USA). Plasma total protein (TP) was measured using a handheld refractometer (Atago U.S.A., Inc., Bellevue, WA 98005, USA) calibrated at the site.

Plasma (100 μ L) was decanted from the lithium heparin tube and analyzed in the field using a VetScan VS2 chemistry analyzer (Abaxis[®], Union City, CA 94587, USA). Only samples with no visual signs of hemolysis were analyzed. Comprehensive Diagnostic Profile and Avian and Reptilian Plus Profile rotors were used to measure the following biochemistry parameters: albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), bile acids (BA), blood urea nitrogen, calcium, creatinine, creatine kinase, globulin, glucose, potassium, sodium, phosphorous, total bilirubin (TBIL), and uric acid. The first sample, and every fifth sample thereafter, was analyzed in triplicate to monitor for the precision of the analyzer. Due to a shortage of Comprehensive Diagnostic Profile rotors, the precision study was performed only on the Avian and Reptilian Plus Profile rotors. Human-derived serum with known values was used to test the accuracy of the VetScan VS2 analyzer.

Reproductive Output

All individual Leatherback Sea Turtles were marked with Passive Integrated Transponder tags (AVID Identification Systems, Inc., Norco, CA 92860, USA) during or after oviposition (Dutton and McDonald, 1994), but never before blood collection. During oviposition, the number of eggs was counted and approximately 20 of the eggs were weighed in situ (Ohaus Scout field balance, 200 ± 0.01 g; Ohaus Corp., Pine Brook, NJ 07058, USA). The number and weight of shelled albumin gobs (SAGs) were not included in these measurements.

Statistical Analyses

Mean, SE, and 95% CI were calculated for each plasma biochemistry parameter and PCV value to determine reference intervals for this nesting population. Means and SEs for SCCL and SCCW were also calculated.

To assess whether there were trends in plasma biochemistry parameters and PCV values, nest-building activities (duration of egg chamber construction, oviposition, and covering of the nest) and reproductive output (egg mass and clutch size) during the reproductive season, repeated-measures ANOVAs were conducted on animals for which three or more nesting events were recorded. These data points were not always collected on consecutive nesting events due to logistical issues, but the turtles were observed to nest at each nesting event. Plasma biochemistry parameters and PCV values, egg chamber construction ($n = 7$), oviposition ($n = 11$), covering of the nest ($n = 11$), egg mass ($n = 7$), and clutch size ($n = 11$) were treated as continuous response variables. The time of nesting event was a fixed independent variable and turtle identity was treated as a random factor. There were insufficient data for duration of body pit construction to be included in this analysis.

Temporal effects on biochemical parameters, nest building activity, and reproductive output were tested via full vs. reduced-model comparisons. Five different models were used in these analyses: (1) curvilinear changes between nesting events (full model) with both (a) different slopes for each turtle and (b) similar slopes for each turtle, (2) linear changes between nesting events (reduced model) with both (a) different slopes for each turtle and (b) similar slopes for each turtle, and (3) no change between nesting events. Separate submodel comparisons were performed to test for the significance of each factor, and the resulting chi-square and P -values are reported. All statistical analyses were performed using R 2.11.1 (lme4 package for mixed models; R Development Core Team, 2010).

RESULTS

Physical Examination

All nesting turtles were rated as healthy based on physical examination as well as ability to nest successfully ($n = 23$). No animals received a BCS of 1, 2, or 5. Twenty individuals were given a BCS of 3, and three individuals received a BCS of 4. All turtles maintained their assigned BCS for the duration of this study. Mean SCCL and SCCW were 150.0 ± 2.75 cm and $108.18 \pm$

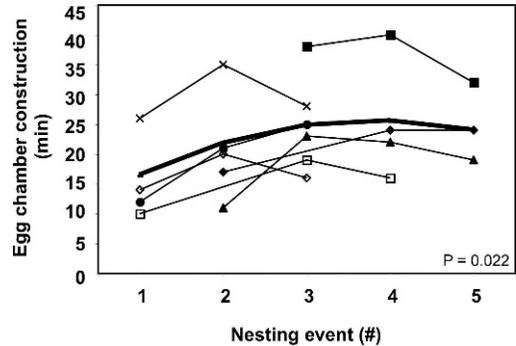


FIG. 1.—Changes in duration of egg chamber construction by Leatherback Sea Turtles (*Dermochelys coriacea*) throughout the nesting season. Nesting event number represents consecutive clutches laid by individual turtles. Each symbol connected with a line represents nesting events for an individual turtle ($n = 7$). P -values for significant variation over nesting events are given in the graph, where duration of egg chamber construction is the fixed independent variable and individual turtles were treated as random factors. The thick black line is the best-fit trend line among individuals.

2.25 cm, respectively. Visual body examinations revealed that 13 individuals had scars located on their shoulders and front flippers, with a mean scar length of 6.45 ± 1.97 cm. Nine individuals had superficial acute to subacute wounds on their necks, heads, or shoulders, with a mean wound length of 4.88 ± 1.48 cm. An additional nine individuals had fully healed small holes in their front and rear flippers, with a mean hole length of 2.33 ± 0.95 cm. Only seven turtles had attached barnacles, with five individuals having fewer than five barnacles and two individuals carrying 20–25 barnacles. Epibiotic loads for all turtles occurred exclusively on the neck and shoulders. One individual's mandibular beak was missing from the rostral notch to the caudoventral aspect of the left orbit. Another individual displayed marked kyphosis of the carapace.

Leatherbacks spent 15.90 ± 1.22 min constructing a body pit and 26.68 ± 2.35 min digging the egg chamber. There was a statistically significant curvilinear relationship in duration between digging the egg chamber and the number of nesting events ($\chi^2 = 5.22$, $df = 1$, $P = 0.02$). The duration of digging increased from the first through the fourth nesting event, and decreased during the fifth nesting event (Fig. 1). There were no significant differences in duration of oviposition (11.13 ± 0.47 min; $\chi^2 = 2.40$, $df = 1$, $P =$

TABLE 1.—Plasma biochemistry concentrations and packed cell volume (mean \pm SE) in free-ranging Leatherback Sea Turtles (*Dermochelys coriacea*) nesting on Bioko Island, Equatorial Guinea. Biochemical concentrations are given for all sampled turtles (55 samples taken from 23 individuals) and for first-time nesters (individual turtle's first clutch of the season). Reference intervals are given as 95% CIs.

Parameters ^a	All sampled leatherback turtles			First-time nesters	
	<i>n</i>	Mean \pm SE	Reference interval	<i>n</i>	Mean \pm SE
Albumin (g/dL)	55	1.77 \pm 0.03	1.30–2.24	16	1.82 \pm 0.08
ALP (U/L)	20	52.35 \pm 1.53	30.10–74.60	3	38.33 \pm 2.19
ALT (U/L)	18	8.83 \pm 0.39	3.20–14.46	2	9.00
Amylase (U/L)	20	345.85 \pm 8.99	215.13–476.57	3	291.33 \pm 11.70
AST (U/L)	55	125.9 \pm 3.27	78.28–173.48	16	128.77 \pm 6.60
BUN (mg/L)	6	2.33 \pm 0.07	1.32–3.34	1	2.00
Calcium (mg/dL)	55	11.16 \pm 0.25	7.50–14.83	16	11.35 \pm 0.55
Creatine kinase (U/L)	52	146.87 \pm 21.08	NA ^b –453.31	16	151.43 \pm 34.64
Globulin (g/dL)	55	2.40 \pm 0.04	1.8–2.99	16	2.40 \pm 0.09
Glucose (mg/dL)	55	86.34 \pm 1.83	59.77–112.91	16	86.44 \pm 3.28
Phosphorous (mg/dL)	55	12.19 \pm 0.16	9.85–14.52	16	12.03 \pm 0.27
Potassium (mmol/L)	55	4.07 \pm 0.05	3.30–4.85	16	4.38 \pm 0.11
Sodium (mmol/L)	55	140.27 \pm 0.37	134.83–145.70	16	140.31 \pm 0.54
TBIL (mg/dL)	20	0.21 \pm 0.003	0.16–0.25	3	0.20
Uric acid (mg/dL)	45	0.39 \pm 0.01	0.23–0.55	16	0.40 \pm 0.02
TP (g/dL)	54	5.08 \pm 0.10	3.60–6.56	16	5.01 \pm 0.23
PCV (%)	55	36.4 \pm 0.59	27.83–44.97	16	37.63 \pm 0.91

^a ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; TBIL, total bilirubin; PCV, packed cell volume; TP, total protein. Only the upper limit is presented for creatine kinase because the lower limits were negative values.

^b NA = not applicable.

0.12) or nest covering (57.74 \pm 3.90 min; $\chi^2 = 0.87$, df = 1, $P = 0.35$) for different nesting events.

Reference Intervals

Mean, SE, and reference intervals for plasma biochemistry parameters and PCV values are presented in Table 1. To exclude potential bias in reporting reference intervals using serial blood samples, mean and SE for

TABLE 2.—Coefficient of variance (CV) for plasma biochemistry parameters from nesting Leatherback Sea Turtles (*Dermochelys coriacea*) obtained from analyzer precision tests. Values are given as percentages and the ranges represent minimum and maximum CV per parameter.

Parameters ^a	CV (%)
Albumin (g/dL)	0.00–5.00
AST (U/L)	0.40–2.75
Calcium (mg/dL)	0.41–3.63
Creatine kinase (U/L)	0.51–45.98
Globulin (g/dL)	0.00–4.56
Glucose (mg/dL)	0.00–1.52
Phosphorous (mg/dL)	0.40–1.87
Potassium (mmol/L)	0.00–5.00
Sodium (mmol/L)	0.40–2.00
Uric acid (mg/dL)	0.00–5.00

^a AST, aspartate aminotransferase.

first-time nesters are also reported in Table 1. A subset of these samples ($n = 20$) was also run on Comprehensive Diagnostic Profile rotors. Every fifth sample was analyzed in triplicate to test for precision, and human-derived serum with known values was used to test the accuracy of the VetScan VS2 analyzer. No significant differences were detected in the accuracy of the analyzer. In the precision study, the coefficient of variance (CV) was calculated only for plasma biochemistry parameters that were present on the Avian and Reptilian Plus Profile rotors. The CV for different plasma biochemical parameters was <5% in all cases, except for creatine kinase, in which the CV values ranged 0.5% to 46% (Table 2). The concentrations for BA ($n = 55$) and creatinine ($n = 20$), which are not reported in Table 1, were lower than the detectable range of the blood chemistry analyzer, and were given as <35 and <0.2, respectively. Plasma TP was measured in 54 samples. None of the samples used in the analysis showed visible signs of hemolysis, icterus, lipemia, or lymph contamination. However, the VetScan VS2 chemistry analyzer

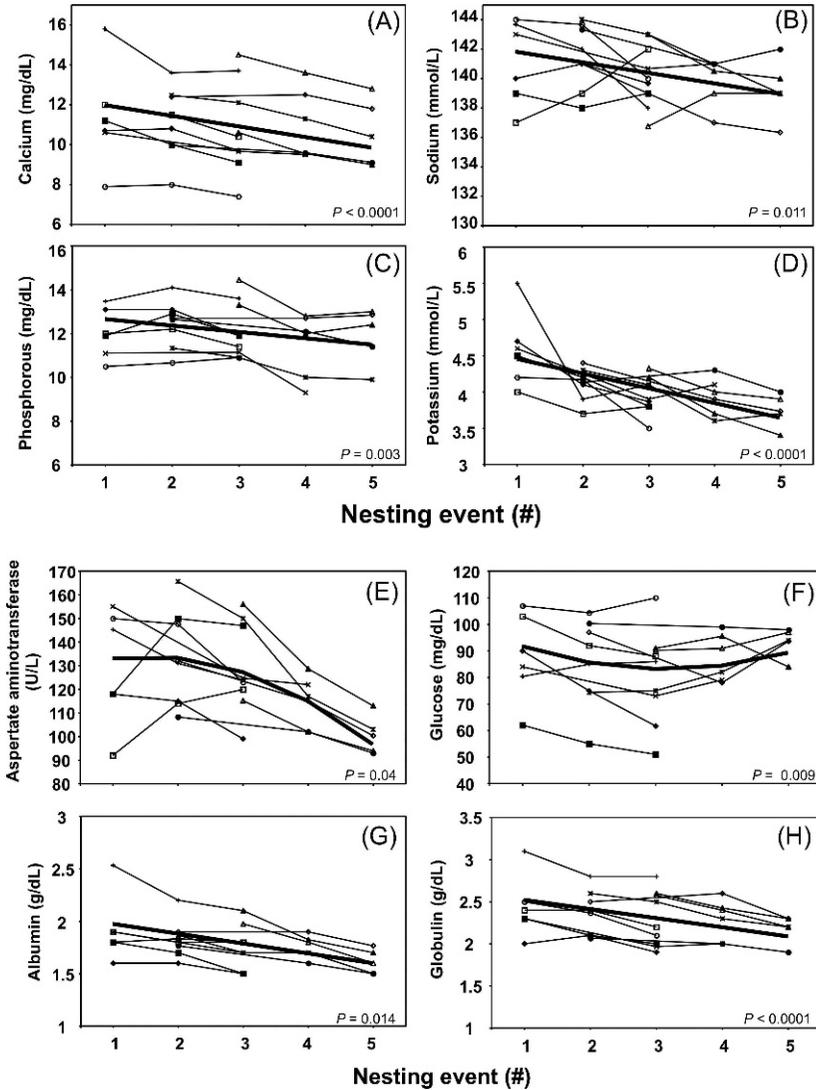


FIG. 2.—Changes in concentrations of biochemical factors in Leatherback Sea Turtles (*Dermochelys coriacea*) throughout the nesting season. The values for (A) calcium, (B) sodium, (C) phosphate, (D) potassium, (E) aspartate aminotransferase, (F) glucose, (G) albumin, and (H) globulin change significantly with increasing nesting event number. Nesting event number represents consecutive clutches laid by individual turtles. Each symbol connected with a line represents nesting events for an individual turtle ($n = 11$). P -values for significant variation over nesting events are given in the graphs, where each biochemical concentration is the fixed independent variable and individual turtles were treated as random factors. Thick black lines are best-fit trend lines among individuals.

rated 27 samples as no hemolysis (score = 0), 23 samples as mild hemolysis (score = 1), 5 samples as hemolysis (score = 2), and a number of samples not included in the analysis as severe hemolysis (score = 3). Two samples taken from the DCS were discarded due to visual lymph contamination. Concentrations of PCV, TP, and potassium

were similar for samples taken from the DCS and IDV, suggesting that significant lymph contamination in DCS samples was not an issue (Crawshaw and Holz, 1996).

Plasma Biochemistry and PCV Trends

There were statistically significant changes in plasma biochemistry parameters and PCV values

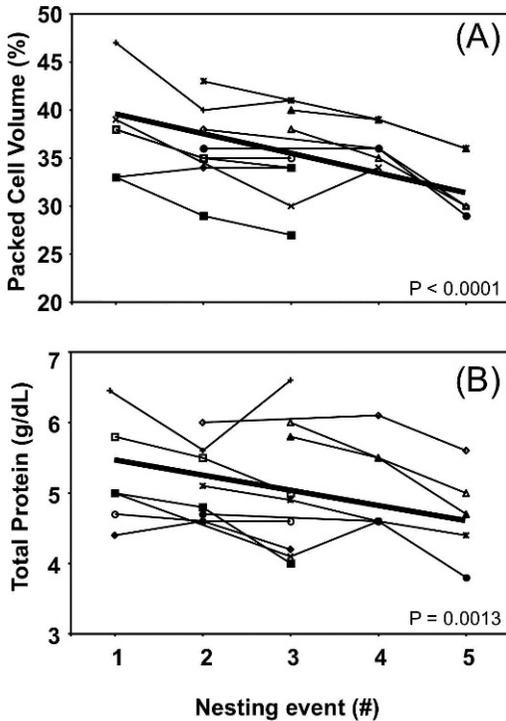


FIG. 3.—Changes in packed cell volume and total protein in Leatherback Sea Turtles (*Dermochelys coriacea*) throughout the nesting season. The values for (A) packed cell volume and (B) total protein decreased significantly with increasing nesting event number. Nesting event number represents consecutive clutches laid by individual turtles. Each symbol connected with a line represents nesting events for an individual turtle ($n = 11$). P -values for significant variation over nesting events are given in the graphs, where packed cell volume and total protein are fixed independent variables and individual turtles were treated as random factors. Thick black lines are best-fit trend lines among individuals.

during a reproductive season ($n = 11$; Figs. 2 and 3). The best-fit model for all analyzed data was linear with a constant slope, unless stated otherwise. All measured electrolytes—calcium ($\chi^2 = 24.89$; $df = 1$; $P < 0.0001$), potassium ($\chi^2 = 16.81$; $df = 1$; $P < 0.0001$), sodium ($\chi^2 = 6.52$; $df = 1$; $P = 0.01$), and phosphate ($\chi^2 = 8.58$; $df = 1$; $P = 0.003$)—decreased significantly with increasing number of nesting events (Fig. 2A–D). Albumin ($\chi^2 = 24.32$; $df = 1$; $P < 0.0001$) and globulin ($\chi^2 = 26.04$; $df = 1$; $P < 0.0001$) also decreased significantly with increasing number of nesting events (Fig. 2G,H). Glucose ($\chi^2 = 6.79$; $df = 1$; $P = 0.009$) and AST ($\chi^2 = 6.43$; $df = 1$; $P = 0.04$) were best fit by a curvilinear model, with AST decreasing and

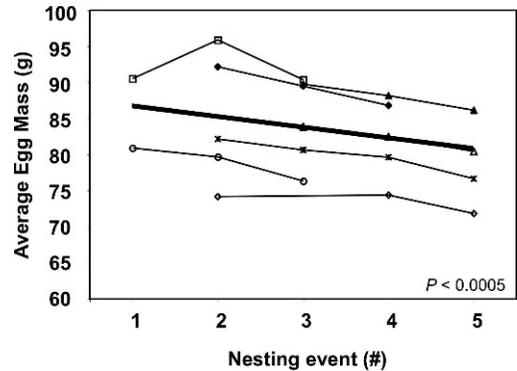


FIG. 4.—Changes in egg mass of Leatherback Sea Turtles (*Dermochelys coriacea*) throughout the nesting season. Average egg mass decreased with increasing nesting event number. Nesting event number represents consecutive clutches laid by individual turtles. Each symbol connected with a line represents nesting events for an individual turtle ($n = 7$). P -values for significant variation over nesting events are given in the graph, where egg mass is a fixed independent variable and individual turtles were treated as random factors. The thick black line is the best-fit trend line among individuals.

glucose first decreasing then later increasing (Fig. 2E,F). The PCV ($\chi^2 = 20.95$; $df = 1$; $P < 0.0001$) and TP ($\chi^2 = 10.34$; $df = 1$; $P = 0.001$) both decreased significantly with increasing number of nesting events (Fig. 3A,B).

Reproductive Output

Egg mass decreased significantly within individuals ($n = 7$) during the reproductive season (Fig. 4). Egg mass also decreased with increasing number of nesting events ($\chi^2 = 12.32$; $df = 1$; $P < 0.0005$), with the mass of each egg decreasing by 1.48 g on average per nesting event. There was no significant difference in clutch size (78.15 ± 2.98) with increasing number of nesting events ($\chi^2 = 0.79$; $df = 1$; $P = 0.38$).

DISCUSSION

Physical Examination

All reasonable efforts were made to exclude turtles with signs of disease. Of the 23 turtles sampled, all received a BCS of 3 or 4 and were in good physical condition. No turtles exhibited dermal lesions of significant size or depth, and none had visible indications of infection. No turtles carried heavy epibiotic loads, and all exhibited biological reproductive parameters

that were similar to those of other populations. Based on physical examination and reproductive parameters, the turtles sampled in this study represented a healthy component of the breeding population (Deem et al., 2006).

The curvilinear relationship between the duration of digging the egg chamber and increasing number of nesting events (Fig. 1) may be explained by precipitation patterns during the study period. The initial increase in duration occurred during 4 wk of periodic rain events, whereas the slight decrease coincided with a 2-wk dry spell. It is possible that the moisture content of the sand during the initial period of rain resulted in longer egg-chamber construction times, although individual energy reserves may also have played a role in these changes.

Reference Intervals

Mean plasma biochemistry concentration for ALT, amylase, AST, and calcium were most dissimilar from published values for leatherbacks (Table 1; Deem et al., 2006; Innis et al., 2010; Rostal et al., 1996). In this study, ALT was higher (8.83 ± 0.39 U/L, SE, $n = 18$) than in nesting leatherbacks in Gabon (4.0 ± 0.5 U/L, SE, $n = 9$; Deem et al., 2006) and lower than in foraging leatherbacks in the northwestern Atlantic (11 ± 2.36 U/L, SE, $n = 18$; Innis et al., 2010). Although ALT values in leatherbacks seem to be lower than the values reported in other reptiles (interval, 10–30 U/L), they are similar to the values reported in Kemp's Ridley Sea Turtles (3.91 ± 0.53 U/L, SE, $n = 60$; Carminati et al., 1994) and are likely indicative of species-specific variation. The difference noted between the leatherbacks in Gabon and those in Playa Moaba could be due to a difference in testing methodology between a dry-slide chemistry analyzer (Kodak 750 X R, Ortho Clinical Diagnostics, Rochester, NY 14626, USA) used at the University of Miami (Deem et al., 2006) and the VetScan VS2 chemistry analyzer used in the field in this study. The mild amounts of hemolysis in this study were unlikely to have contributed to this difference. If hemolysis were significant enough to affect biochemical concentrations in a sample, the VetScan VS2 would have suppressed the results.

Amylase concentrations were lower (345.85 ± 8.99 U/L, SE, $n = 20$) than those for nesting leatherbacks in Gabon (681.0 ± 3.98 U/L, SE, $n = 10$; Deem et al., 2006). Because plasma amylase concentrations increased during digestion, one possible explanation for this discrepancy would be the potential for opportunistic feeding by the animals in Gabon. A decrease in amylase was noted between net-captured Kemp's Ridley Sea Turtles and cold-stunned Kemp's Ridley Sea Turtles, the latter of which would presumably not be foraging (Carminati et al., 1994). In addition, the study in Gabon was conducted later in nesting season relative to the present study and used different testing methodology.

Aspartate aminotransferase was lower in turtles at Playa Moaba (125.9 ± 3.27 U/L, SE, $n = 55$) than those in Gabon (165.0 ± 2.26 U/L, SE, $n = 10$) and the northwestern Atlantic (286 ± 63.4 U/L, SE, $n = 18$), or in comparison with previously reported concentrations in Green Sea Turtles (Bolten and Bjorndal, 1992; Hasbún et al., 1998; Whiting et al., 2007), Kemp's Ridley Sea Turtles, and Loggerhead Sea Turtles (Carminati et al., 1994; Day et al., 2007; George, 1997), but still within the interval of chelonians reported elsewhere (interval, 100–350 U/L; Campbell, 1996). Although little is known about the tissue distribution of AST in sea turtles, AST concentrations are not considered to be organ-specific in reptiles (Campbell, 2004; Day et al., 2007; Evans, 1996). Despite this, significant increases are clinically suggestive of damage to liver or muscle, where the highest AST concentrations are often found (Campbell, 1996). Aspartate aminotransferase has been reported to be elevated in sea turtles with fibropapillomatosis (Aguirre et al., 1995; Norton et al., 1990; Whiting et al., 2007). Significantly elevated AST concentrations were also found in an apparently healthy Green Sea Turtle population that consisted mostly of new recruits (Whiting et al., 2007). Potential explanations for this discrepancy, in addition to methodological differences already described, include interspecific variation, small sample size, and potentially undetected diseases in animals that are reported to be healthy.

Calcium concentrations were higher in nesting leatherbacks at Playa Moaba (11.16 ± 0.25 mg/dL, SE, $n = 55$) than in those in Gabon (8.0 ± 0.71 mg/dL, SE, $n = 10$), the northwestern Atlantic (6.0 ± 0.4 mg/dL, SE, $n = 18$), and in Costa Rica (8.10 ± 0.84 mg/dL, SE, $n = 13$; Deem et al., 2006; Innis et al., 2010; Rostal et al., 1996). These concentrations were also near the upper limit of the published calcium intervals (6–11 mg/dL) in other sea turtles (Bolten and Bjorndal, 1992; Campbell, 1996; Carminati et al., 1994; Day et al., 2007; George, 1997; Hasbún et al., 1998; Whiting et al., 2007). Phosphorous concentrations (12.19 ± 0.16 mg/dL, SE, $n = 55$) were consistent with those found in the Deem et al. (2006) study (11.0 ± 2 mg/dL, SE, $n = 10$), but were elevated compared to those of foraging leatherbacks (9.0 ± 0.4 mg/dL, SE, $n = 18$) and other sea turtles (interval, 6–11 mg/dL; Bolten and Bjorndal, 1992; Campbell, 1996; Carminati et al., 1994; Day et al., 2007; George, 1997; Hasbún et al., 1998; Innis et al., 2010; Kakizoe et al., 2007; Whiting et al., 2007). In this study, the calcium:phosphorous ratio in nesting leatherbacks was 0.92. Both calcium and phosphorous are likely elevated in these turtles due to mobilization associated with egg production, as occurs in other reptiles, including chelonians (Dessauer, 1970; Jacobson, 1993; Marks and Citino, 1990). To our knowledge, a normal calcium:phosphorous ratio has not been established in sea turtles (Aguirre and Balazs, 2000).

Blood urea nitrogen concentrations (2.33 ± 0.07 mg/dL, SE, $n = 6$) were consistent with those found in leatherbacks nesting in Gabon (2.0 ± 0.0 mg/dL, SE, $n = 9$; Deem et al., 2006), but were lower than the values reported in directly captured leatherbacks (128.0 ± 8.74 mg/dL, SE, $n = 11$) and other sea turtles (interval, 20–80 mg/dL; Campbell, 1996; Carminati et al., 1994; Hasbún et al., 1998; Innis et al., 2010). Potential explanations for this disparity include the possible fasting state, normal physiological variation, or an evolutionary adaptation to a low-protein gelatinous-zooplankton diet. Albumin, ALP, creatinine, globulin, glucose, TBIL, potassium, sodium, uric acid, TP, and PCV values were similar to those reported in leatherbacks

nesting in Gabon (Deem et al., 2006) and in other species of sea turtles (Bolten and Bjorndal, 1992; Campbell, 1996; Carminati et al., 1994; Day et al., 2007; George, 1997; Kakizoe et al., 2007; Whiting et al., 2007). However, uric acid concentrations were higher in foraging leatherbacks (1.3 ± 0.24 mg/dL, SE, $n = 11$; Innis et al., 2010), as might be expected if leatherbacks are fasting during the nesting season.

Creatine kinase varied widely in this study, as it has in other published studies of sea turtles. Creatine kinase did not meet the required parameters in the precision study using this testing method, with some of the samples yielding a coefficient of variability as high as 46% (Table 2). Hence, this parameter may not be appropriate for use as a normal value interval nor as an indicator of health in this study.

There are many potential sources of error that could account for the wide range of biochemical concentrations reported among studies (Lutz and Dunbar-Cooper, 1987; Whiting et al., 2007). Biases may arise from sample size, sample collection and handling, analytical testing methodologies, and type of analyzer used, all of which can make comparisons between studies potentially misleading (Bolten and Bjorndal, 1992; Wolf et al., 2008). Differences in subject age, sex, nutritional status, and health status (including disease processes), as well as in environmental conditions (captive vs. free ranging), geographical location, and season, further complicate comparisons between studies. Biochemical intervals reported in this study represent normal concentrations for reproductively active female leatherbacks capable of coming to shore for nesting. Nonetheless, comparing these biochemical concentrations to those of other studies is crucial due to the lack of data on Leatherback Sea Turtles.

Plasma Biochemistry and PCV Trends

Calcium, potassium, sodium, and phosphorous showed significant decreases as the number of nesting events increased (Fig. 2A–D). Calcium is required for egg production and a temporary increase in calcium has been reported during vitellogenesis in reptiles (including chelonians) and birds (Campbell, 1996;

Simkiss, 1961). No seasonal trend for calcium was detected in a population of Loggerhead Sea Turtles that represented a randomly sampled broad selection of age, sex, and reproductive status (Lutz and Dunbar-Cooper, 1987). However, a population of nesting loggerheads showed a similar level of hypercalcemia to that observed in this study (Kakizoe et al., 2007). The decreasing trend in calcium concentration may represent depletion of calcium as the nesting season proceeds, or it may represent an eventual return to normal (nonfolliculogenic) physiological calcium concentrations by the end of the nesting season. Phosphorous metabolism is closely tied to calcium metabolism in vertebrates and may be especially important in reptiles (Simkiss, 1961). Decreasing phosphorus concentrations may be explained by the same mechanisms that potentially affect calcium concentrations.

Sodium and potassium concentrations were similar to reported physiological ranges in sea turtles, and both decreased with increasing number of nesting events (Fig. 2B,D). Food is the primary source of potassium, and a decreasing trend would support extended periods of anorexia during the nesting season (Stockham and Scott, 2008). Hypokalemia causes a commensurate drop in plasma sodium in mammals (Stockham and Scott, 2008).

Plasma total protein significantly decreased with increasing number of nesting events (Fig. 2E-H). Plasma total protein consists primarily of albumin and globulin, with a small component of clotting factors (primarily fibrinogen). A decrease in total protein could be associated with anorexia or malnutrition and explained by either decreased protein production or increased catabolism, commensurate with nutritional stress or disease (Kumar et al., 1972; Stockham and Scott, 2008). Female birds and reptiles have increased total protein concentrations during folliculogenesis (Campbell, 2004), which may be associated with an increased demand for egg production. A potential explanation for the observed decreasing trend is depletion of reserves as the nesting season proceeds, compounded by little or no food intake. Both Green Seaturtles and Olive Ridley Sea Turtles showed significant decreases in protein concentrations when they stopped feeding for

more than 1 mo (Moon, 1992). It could also represent an eventual return to normal (non-folliculogenic) physiological protein concentrations by the end of the nesting season.

A small dip in glucose concentrations was observed in the middle of the nesting season, but these values remained within normal physiological concentrations of glucose. In captive Kemp's Ridley Sea Turtles and Green Sea Turtles that were starved for 14 d, glucose levels decreased to 76% and 29% of initial values, respectively (Moon et al., 1999). A decreasing trend over the course of the season was also observed in AST concentrations.

Packed cell volume decreased with increasing number of nesting events (Fig. 3A). Despite the fact that there was a significant drop in PCV values throughout the nesting season, the recorded values were within the range reported for other nesting leatherbacks (Deem et al., 2006) as well as for other sea turtles (Bolten and Bjørndal, 1992; Campbell, 1996; Carminati et al., 1994; Day et al., 2007; George, 1997; Kakizoe et al., 2007; Whiting et al., 2007). Seasonal changes in reptilian hematologic parameters may be affected by prey availability, temperature changes, and changes in reproductive status (Christopher et al., 1999). In this study, the drop in PCV is likely attributable to seasonal changes, the physiological rigors of folliculogenesis and nesting.

Many of the biochemical parameters and PCV values showed statistically significant decreasing trends as the nesting season progressed. With the exception of ALT, amylase, AST, and calcium, all parameters measured fell within previously reported intervals for chelonians or other reptiles. Furthermore, calcium and PCV followed previously reported changes associated with seasonal or reproductive cycles in chelonians (Christopher et al., 1999). The decreasing trends in plasma biochemical concentrations and PCV values found in this study can probably be attributed to the physiological stress of folliculogenesis and nesting, which is further compounded by a possible period of fasting during the nesting season.

Reproductive Output

If egg production was energy-limited, maternal investment in reproduction would

be expected to decrease as more clutches were laid during the nesting season. Reproductive output is determined by clutch mass, seasonal clutch frequency, internesting interval, and the length of reproductive life (Miller, 1997). Clutch mass, which is a function of both clutch size and egg mass, may serve as one of the measures of reproductive output. Consequently, a decrease in maternal investment in reproduction could result in a decrease in clutch size or egg mass. Because SAGs were not counted or weighed in this study, we are unable to specifically address clutch mass and will limit our discussion to clutch size and egg mass. However, our data for clutch mass (excluding SAGs) suggested a decreasing trend with increasing number of nesting events.

In this study, clutch size did not change significantly with increasing number of nesting events. Because individuals ($n = 11$) were only followed until the fifth nesting event, the exact number of clutches laid for these turtles is unknown. The facts that many individuals went on to nest more than five times and that not all individuals laid the same number of clutches may have obscured any trend that was present. Tucker and Frazer (1994) showed that leatherback clutch size decreased after the fifth nesting event at the population level, regardless of female clutch frequency. It is possible that a similar trend would be seen at the individual level if later nests were considered. In addition, it is thought that such trends may vary within a population and even within individuals among nesting seasons (Tucker and Frazer, 1994).

Clutch size in leatherbacks is probably the result of a number of dynamic, selective pressures (Miller, 1997; Tucker and Frazer, 1994). For instance, an upper limit may be determined by the female's size and the space available in the body cavity for developing eggs (Tucker and Frazer, 1994). The lower limit could be dictated by a minimum number of hatchlings needed to emerge from the nest (Carr and Hirth, 1971; Tucker and Frazer, 1994). The unpredictability of the nest environment could encourage females to distribute their eggs across time and space, limiting the number of eggs placed into any one nest (Eckert, 1987). Energetic and physiological constraints also likely play a role (Tucker and Frazer, 1994).

Average egg mass decreased significantly within individuals as the number of nesting events increased (Fig. 4). Wallace et al. (2006b) found that eggs consisted of 63% albumen and 33% yolk, but variation in egg albumen contributed substantially to variation in egg mass. Hatchling mass increased 2 g for a 10-g increase in egg mass and was roughly 10–20 g greater than yolk mass, suggesting that up to 50% of hatchling mass is derived from albumen and/or water from the nest substrate. In this study, egg mass decreased 1.5 g, or roughly 2% per nesting event. The resulting difference between egg mass of first-time nesters and fifth-time nesters (approximately 6 g or 8% of an egg's weight) represents a significant decrease and may affect hatchling sizes.

One possible explanation for this decrease in egg mass is limited energy reserves available to nesting females and the cost of reproduction. As the season progresses and more clutches are laid, decreasing resources may dictate a need for smaller eggs or clutches. A decrease in egg mass would tend to produce larger hatchlings at the beginning of the season compared to the final clutches. Further studies detailing the variation in clutch mass (including SAGs) in relation to hatchling production and size are needed to begin addressing the mechanisms behind such seasonal declines in egg mass.

Our results for plasma biochemical concentrations and PCV values of free-ranging leatherbacks correspond with many of the values reported in other studies of this species, as well as with values reported for turtles in the family Cheloniidae. Deviations from previously reported values were found in ALT, amylase, AST, and calcium. These differences may be due to testing methodology, sample collection and handling, sample size, analyzers used, time of study, or intraspecific variation. Although the methodologies used in this study present certain logistical difficulties for remote nesting beaches such as Playa Moaba, the advantages of processing samples within minutes to 2 h of collection, not requiring export permits, and limiting the potential effects of freezing samples for international shipment, outweigh the challenges. Reported values for nesting leatherbacks, both in this study and in the study by Deem et al. (2006), are geographically confined

to the Gulf of Guinea region of West/Central Africa. Additional data are needed from geographically distant populations. Further studies including both sexes and various life stages, such as the recent one by Innis et al. (2010), are needed for this species.

This is the first study to look at trends in plasma biochemistry concentrations and PCV values in nesting sea turtles. The decreasing trends we observed were probably due to the physiological stresses of folliculogenesis and nesting, compounded by limited energy reserves or resources and possible fasting. A decreasing trend in egg mass further supports this inference. Although plasma biochemical concentrations and PCV values decreased significantly, these changes would not be considered clinically significant. Individual turtles were only followed until their fifth clutch of the season. However, leatherbacks are known to nest as many as nine or more times per nesting season, and could show clinically significant decreases in all concentrations if these trends continue through the nesting events. Future health assessments conducted on nesting turtles will need to consider the time of sample collection relative to the reproductive season.

Acknowledgments.—We thank S. Falck, J. Howard, K. Kranich, and M. Osipchuk for assistance in the field. We also thank M. Johnson for logistical support in Equatorial Guinea. Comments provided by M. O'Connor greatly improved this manuscript. We are grateful to K. Aron and Abaxis® for the loan of two VetScan VS2 analyzers and for providing us with Comprehensive Diagnostic Profile rotors and Avian and Reptilian Plus Profile rotors. The ExxonMobil Foundation and Drexel University provided funding for this project. This work was conducted under appropriate permits from Universidad Nacional de Guinea Ecuatorial and the Institutional Animal Care and Use Committee at Drexel University, IACUC no. 17243.

LITERATURE CITED

- AGUIRRE, A. A., AND G. H. BALAZS. 2000. Blood biochemistry values of green turtles, *Chelonia mydas*, with and without fibropapillomatosis. *Comparative Haematology International* 10:132–137.
- AGUIRRE, A. A., G. H. BALAZS, T. R. SPRAKER, AND T. S. GROSS. 1995. Adrenal and hematological responses to stress in juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas. *Physiological Zoology* 68:831–854.
- BJORNDAL, K. A. 1980. Demography of the breeding population of the green turtle, *Chelonia mydas*, at Tortuguero, Costa Rica. *Copeia* 3:525–530.
- BJORNDAL, K. A., AND A. CARR. 1989. Variation in clutch size and egg size in the green turtle nesting population at Tortuguero, Costa Rica. *Herpetologica* 45:181–189.
- BOLTEN, A. B., AND K. A. BJORNDAL. 1992. Blood profiles for a wild population of green turtles (*Chelonia mydas*) in the southern Bahamas—Size-specific and sex-specific relationships. *Journal of Wildlife Diseases* 28:407–413.
- BUTYNSKI, T. M. 1996. Marine turtles on Bioko Island, Equatorial Guinea. *Oryx* 30:143–149.
- CAMPBELL, T. W. 1996. Clinical pathology. Pp. 427–435. In D. R. Mader (Ed.), *Reptile Medicine and Surgery*. Saunders, Philadelphia, Pennsylvania, USA.
- CAMPBELL, T. W. 2004. Blood biochemistry of lower vertebrates. Fifty-fifth Annual Meeting of the American College of Veterinary Pathologists (ACVP) & 39th Annual Meeting of the American Society of Clinical Pathology (ASVCP), ACVP and ASVCP (Eds.). American College of Veterinary Pathologists & American Society for Veterinary Clinical Pathology, Madison, Wisconsin, USA.
- CARMINATI, C., E. GERLE, L. L. KIEHN, R. P. PISCIOTTA. 1994. Blood chemistry comparison of healthy vs. hypothermic juvenile Kemp's Ridley sea turtles (*Lepidochelys kempii*) in the New York bight. Pp. 203–207. In K. A. Bjorndal, A. B. Bolten, D. A. Johnson, and P. J. Eliazar (Compilers). *Proceedings of the Fourteenth Annual Symposium on Sea Turtle Biology and Conservation*. National Oceanic and Atmospheric Administration (NOAA) Technical Memorandum NMFS-SEFSC-351. National Oceanic and Atmospheric Administration, Miami, Florida, USA.
- CARR, A., AND H. HIRTH. 1971. Social facilitation in green turtle siblings. *Animal Behavior* 9:68–70.
- CHRISTOPHER, M. M., K. H. BERRY, I. R. WALLIS, K. A. NAGY, B. T. HENEN, AND C. C. PETERSON. 1999. Reference intervals and physiologic alterations in hematologic and biochemical values of free-ranging desert tortoises in the Mojave desert. *Journal of Wildlife Diseases* 35:212–238.
- CONGDON, J. D. 1989. Proximate and evolutionary constraints on energy relations of reptiles. *Physiological Zoology* 62:356–373.
- CONGDON, J. D., AND J. W. GIBBONS. 1987. Morphological constraint on egg size: A challenge to optimal egg size theory? *Proceedings of the National Academy of Sciences* 84:4145–4147.
- CRAWSHAW, G. J., AND P. HOLZ. 1996. Comparison of plasma biochemical values in blood and blood-lymph mixtures from red-eared sliders, *Trachemys scripta elegans*. *Bulletin of the Association of Reptilian and Amphibian Veterinarians* 6:7–9.
- DAY, R. D., A. L. SEGARS, M. D. ARENDT, A. M. LEE, AND M. M. PEDEN-ADAMS. 2007. Relationship of blood mercury levels to health parameters in the loggerhead sea turtle (*Caretta caretta*). *Environmental Health Perspectives* 115:1421–1428.
- DEEM, S. L., E. S. DIERENFELD, G. P. SOUNGUET, A. R. ALLEMAN, C. CRAY, R. H. POPPENG, T. M. NORTON, AND W. B. KARESH. 2006. Blood values in free-ranging nesting leatherback sea turtles (*Dermochelys coriacea*) on the coast of the Republic of Gabon. *Journal of Zoo and Wildlife Medicine* 37:464–471.

- DESSAUER, H. C. 1970. Blood Chemistry of Reptiles: Physiological and Evolutionary Aspects. Academic Press, New Orleans, Louisiana, USA.
- DUTTON, P. H., AND D. L. McDONALD. 1994. Use of PIT tags to identify adult leatherbacks. *Marine Turtle Newsletter* 67:13-14.
- ECKERT, K. L. 1987. Environmental unpredictability and leatherback sea turtle (*Dermochelys coriacea*) nest loss. *Herpetologica* 43:315-323.
- EVANS, G. O. 1996. Animal Clinical Chemistry, A Primer for Toxicologists. Taylor and Francis, Bristol, Pennsylvania, USA.
- FRAZER, N. B., AND J. I. RICHARDSON. 1985. Seasonal variation in clutch size for loggerhead sea turtles, *Caretta caretta*, nesting on little Cumberland Island, Georgia, USA. *Copeia* 1985:1083-1085.
- GEORGE, R. H. 1997. Health problems and diseases of sea turtles. Pp. 363-385. In P. L. Lutz and J. A. Musick (Eds.), *The Biology of Sea Turtles*. CRC Press, Boca Raton, Florida, USA.
- HASBÚN, C. R., A. J. LAWRENCE, J. NALDO, J. H. SAMOUR, AND S. M. AL-GHAIS. 1998. Normal blood chemistry of free-living green sea turtles, *Chelonia mydas*, from the United Arab Emirates. *Comparative Haematology International* 8:174-177.
- INNIS, C., C. MERIGO, K. DODGE, M. TLUSTY, M. DODGE, B. SHARP, A. MYERS, A. MCINTOSH, D. WUNN, C. PERKINS, T. H. HERDT, T. NORTON, AND M. LUCAVAGE. 2010. Health evaluation of leatherback turtles (*Dermochelys coriacea*) in the northwestern Atlantic during direct capture and fisheries gear disentanglement. *Chelonian Conservation and Biology* 9:205-222.
- JACOBSON, E. R. 1993. Blood collection techniques in reptiles: Laboratory investigations. Pp. 144-152. In M. E. Fowler (Ed.), *Zoo and Wild Animal Medicine Current Therapy* 3. W. B. Saunders Company, Philadelphia, Pennsylvania, USA.
- KAKIZOE, Y., K. SAKAOKA, F. KAKIZOE, M. YOSHII, H. NAKAMURA, Y. KANOU, AND I. UCHIDA. 2007. Successive changes of hematologic characteristics and plasma chemistry values of juvenile loggerhead turtles (*Caretta caretta*). *Journal of Zoo and Wildlife Medicine* 38:77-84.
- KUMAR, V., H. P. CHASE, K. HAMMOND, AND D. O'BRIEN. 1972. Alterations in blood biochemical tests in progressive malnutrition. *Pediatrics* 49:736-743.
- LUTCAVAGE, M. E., P. L. LUTZ, G. D. BOSSART, AND D. M. HUDSON. 1995. Physiological and clinicopathological effects of crude-oil on loggerhead sea turtles. *Archives of Environmental Contamination and Toxicology* 28:417-422.
- LUTZ, P. L., AND A. DUNBAR-COOPER. 1987. Variation in the blood chemistry of the loggerhead sea turtle, *Caretta caretta*. *Fishery Bulletin* 85:37-43.
- MARKS, S. K., AND S. B. CITINO. 1990. Hematology and serum chemistry of the radiated tortoise (*Testudo radiata*). *Journal of Zoo and Wildlife Medicine* 21:342-344.
- MILLER, J. D. 1997. Reproduction in sea turtles. Pp. 51-82. In P. L. Lutz and J. A. Musick (Eds.), *The Biology of Sea Turtles*. CRC Press, Boca Raton, Florida, USA.
- MOON, D. Y. 1992. The responses of sea turtles to temperature changes; behavior, metabolism and thyroid hormones. Ph.D. Dissertation, Texas A&M University, College Station, Texas USA.
- MOON, D. Y., D. W. OWENS, AND D. S. MACKENZIE. 1999. The effects of fasting and increased feeding on plasma thyroid hormones, glucose, and total protein in sea turtles. *Zoological Science* 16:579-586.
- MORTIMER, J. A., AND A. CARR. 1987. Reproduction and Migrations of the Ascension Island Green Turtle (*Chelonia mydas*). *Copeia* 1987:103-113.
- NORTON, T. M., E. R. JACOBSON, AND J. P. SUNDBERG. 1990. Cutaneous fibropapillomas and renal myxofibroma in a green turtle, *Chelonia mydas*. *Journal of Wildlife Diseases* 26:265-270.
- OWENS, D. W. 1980. The comparative reproductive physiology of sea turtles. *American Zoologist* 20:549-563.
- OWENS, D. W., AND G. J. RUIZ. 1980. New methods of obtaining blood and cerebrospinal fluid from marine turtles. *Herpetologica* 36:17-20.
- R DEVELOPMENT CORE TEAM. 2010. lme4: Linear mixed-effects models using s4 classes. R Foundation for Statistical Computing, Vienna, Austria.
- RADER, H., M. A. ELA MBA, W. MORRA, AND G. W. HEARN. 2006. Marine turtles on the southern coast of Bioko Island (Gulf of Guinea, Africa), 2001-2005. *Marine Turtle Newsletter* 111:8-10.
- ROSTAL, D. C., F. V. PALADINO, R. M. PATTERSON, AND J. R. SPOTILA. 1996. Reproductive physiology of nesting leatherback turtles (*Dermochelys coriacea*) at Las Baulas National Park, Costa Rica. *Chelonian Conservation and Biology* 2:230-236.
- SIMKISS, K. 1961. Calcium metabolism and avian reproduction. *Biological Reviews* 36:321-359.
- SOUNGUET, G., C. MBINA, AND A. FORMIA. 2004. Sea turtle research and conservation in Gabon by Aventures Sans Frontières, an organizational profile. *Marine Turtle Newsletter* 105:19-21.
- SPOTILA, J. R., A. E. DUNHAM, A. J. LESLIE, A. C. STEYERMARK, P. T. PLOTKIN, AND F. V. PALADINO. 1996. Worldwide population decline of *Dermochelys coriacea*: Are leatherback turtles going extinct? *Chelonian Conservation and Biology* 2:209-222.
- STABENAU, E. K., T. A. HEMING, AND J. F. MITCHELL. 1991. Respiratory, acid-base and ionic status of Kemp's Ridley sea turtles (*Lepidochelys kempii*) subjected to trawling. *Journal of Comparative Biochemistry and Physiology A* 99:107-111.
- STOCKHAM, S. L., AND M. A. SCOTT. 2008. *Fundamentals of Veterinary Clinical Pathology*. Iowa State Press, Ames, Iowa, USA.
- TOMÁS, J., B. J. GODLEY, J. CASTROVIEJO, AND J. A. RAGA. 2010. Bioko: Critically important nesting habitat for sea turtles of West Africa. *Biodiversity and Conservation* 19:2699-2714.
- TUCKER, A. D., AND N. B. FRAZER. 1994. Seasonal variation in clutch size of the turtle, *Dermochelys coriacea*. *Journal of Herpetology* 28:102-109.
- TURNBULL, B. S., C. R. SMITH, AND M. A. STAMPER. 2000. Medical implications of hypothermia in threatened loggerhead (*Caretta caretta*) and endangered Kemp's Ridley (*Lepidochelys kempii*) and green (*Chelonia mydas*) sea turtles. Pp. 31-35. In *Proceedings of the American Association of Zoo Veterinarians/International*

- Association of Aquatic Animal Veterinarians Annual Conference. New Orleans, Louisiana, USA.
- WALLACE, B. P., AND R. H. GEORGE. 2007. Alternative techniques for obtaining blood samples from leatherback turtles. *Chelonian Conservation and Biology* 6:147–149.
- WALLACE, B. P., J. A. SEMINOFF, S. S. KILHAM, J. R. SPOTILA, AND P. H. DUTTON. 2006a. Leatherback turtles as oceanographic indicators: Stable isotope analyses reveal a trophic dichotomy between ocean basins. *Marine Biology* 149:953–960.
- WALLACE, B. P., P. R. SOTHERLAND, P. S. TOMILLO, S. S. BOUCHARD, R. D. REINA, J. R. SPOTILA, AND F. V. PALADINO. 2006b. Egg components, egg size, and hatchling size in leatherback turtles. *Comparative Biochemistry and Physiology Part A* 145:524–532.
- WALLACE, B. P., P. R. SOTHERLAND, P. S. TOMILLO, R. D. REINA, J. R. SPOTILA, AND F. V. PALADINO. 2007. Maternal investment in reproduction and its consequences in leatherback turtles. *Oecologia* 152:37–47.
- WHITING, S., M. GUINEA, C. LIMPUS, AND K. FOMIATTI. 2007. Blood chemistry reference values for two ecologically distinct populations of foraging green turtles, eastern Indian Ocean. *Comparative Clinical Pathology* 16:109–118.
- WITHERINGTON, B., K. PENDOLEY, G. W. HEARN, AND S. HONARVAR. 2009. Ancient mariners, ancient fuels: How sea turtles cope with our modern fossil fuel dependency. SWOT Report 4. State of the World's Sea Turtles, Arlington, Virginia, USA.
- WITT, M. J., B. BAERT, A. C. BRODERICK, A. FORMIA, J. FRETEY, A. GIBUDI, G. A. M. MOUNGUENGUI, C. MOUSSOUNDA, S. NGOUESSONO, R. J. PARNELL, D. ROUMET, G. P. SOUNGUET, B. VERHAGE, A. ZOGO, AND B. J. GODLEY. 2009. Aerial surveying of the world's largest leatherback turtle rookery: A more effective methodology for large-scale monitoring. *Biological Conservation* 142:1719–1727.
- WOLF, K. N., C. A. HARMS, AND J. F. BEASLEY. 2008. Evaluation of five clinical chemistry analyzers for use in health assessment in sea turtles. *Journal of the American Veterinary Medical Association* 233:470–475.

Accepted: 11 May 2011

Associate Editor: Sarah Woodley