

## BLOOD VALUES IN FREE-RANGING NESTING LEATHERBACK SEA TURTLES (*DERMOCHELYS CORIACEA*) ON THE COAST OF THE REPUBLIC OF GABON

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**Abstract:** Leatherback sea turtles (*Dermochelys coriacea*) are the most endangered of the seven species of sea turtles. The health status of leatherbacks is largely unknown, although the number of nesting females recorded throughout the world has decreased precipitously in the last few decades. Central African beaches may provide one of the last strongholds for nesting leatherback females. In the region, oil extraction and incidental capture pose significant threats to the health of the population. Physical examinations, hematology, plasma biochemistry, plasma corticosterone concentration, plasma protein electrophoresis, plasma vitamin concentrations, and toxicological parameters were evaluated in nesting female leatherbacks in the Republic of Gabon. The general clinical condition of the 35 turtles examined in this study was rated as good. The blood value results for a subset of these turtles are presented and compared to published results from other sea turtles. To the authors' knowledge, these are the first published baseline hematology, plasma biochemistry, and plasma protein electrophoresis values from clinically healthy nesting leatherback turtles.

**Key words:** *Dermochelys coriacea*, Gabon, health, hematology, leatherback sea turtle, plasma biochemistry.

### INTRODUCTION

Leatherback sea turtles (*Dermochelys coriacea*) live in oceans throughout the world. The number of female leatherbacks that come to shore to nest has decreased precipitously during the last half century. The global population of female leatherbacks was estimated to be 34,500 in 1996.<sup>35</sup> They are listed as critically endangered and are the most endangered of the seven sea turtle species.<sup>21</sup> Based on

recent data, the leatherback may become extinct in the near future unless protective actions are taken immediately.<sup>36</sup> Threats to the long-term survival of sea turtles include intentional and incidental capture of adults, harvesting of meat, oil, and eggs, beach front development, pollution, and disease.<sup>16,28</sup> The coast of Central Africa has some of the most important nesting sites for leatherbacks in the world.<sup>5,14</sup> In addition to the above threats, logs lost during commercial timber transport clog the beaches and interfere with sea turtle nesting behavior in the region (S. L. Deem, unpubl. data).

In conjunction with a leatherback tagging and monitoring program, the objectives of this study were to establish baseline blood health indices, including hematology, plasma biochemistry, plasma corticosterone concentration, plasma protein electrophoresis, plasma vitamin concentrations, and plasma toxicological parameters in free-ranging, female leatherback sea turtles nesting on the coast of the Republic of Gabon. To the authors' knowledge this is the first published report evaluating these blood health indices in leatherback sea turtles.

### MATERIALS AND METHODS

#### Study period and site description

During January 2001 and February 2002, blood samples were collected from nesting female leatherback sea turtles on Pongara beach, Republic of Gabon (00°21'150"N, 009°21'294"E–00°19'834"N, 009°19'147"E). The study site is a 5-km beach lo-

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cated at the mouth of the Estuary of Gabon near Libreville, the capital of the Republic of Gabon in Central Africa. A turtle protection program led by Aventures Sans Frontières (ASF) and the Wildlife Conservation Society (WCS) has been operating in the region since 1996. Leatherbacks are the primary turtles that nest on this beach although green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), and olive ridley (*Lepidochelys olivacea*) turtles frequent the waters off the coast of Gabon and have been documented to nest at Pongara. The nesting season for leatherbacks at Pongara is November to April with peak nesting occurring December to February (G. P. Sounguet, unpubl. data).

### Sample and data collection and analyses

Nesting females were approached approximately 5 min after nest-building behavior ceased and egg-laying activity had begun. If the female did not appear in an egg-laying trance at the time of the first approach, an additional 5 min wait was employed to avoid disturbance of egg-laying activity. A complete visual physical examination was performed and straight carapace length (SCL) or curved carapace length (CCL) was measured. Health status of turtles was rated based on nest-building behavior and general body condition. Five to 24 ml of blood was collected from the interdigital vein of the hind flipper via a dorsal approach with the use of an 18-gauge, 3.8-cm needle and a 12-ml syringe pre-coated with sodium heparin (heparin sodium injection, USP, Elkins-Sinn, Inc., Cherry Hill, New Jersey 08003, USA) ( $n = 28$ ) or a nonheparin-coated syringe ( $n = 12$ ). Immediately following collection, the heparinized blood was placed in serum separator tubes (Corvac, Sherwood Medical, St. Louis, Missouri 63103, USA) and the blood in plain syringes was placed in lithium heparin tubes (Corvac, Sherwood Medical) and buffered citrate sodium tubes (Becton-Dickinson Diagnostics, Pre-Analytical Systems, Franklin Lakes, New Jersey 07417, USA). Blood tubes were kept on wet ice in a cooler during the remaining time researchers were on the beach collecting samples (range 15 min to 6 hr).

Initial processing of blood samples occurred in the field within 8 hr of blood collection. Whole blood collected in the buffered citrate sodium was placed in cryotubes (Corning Incorporated, Corning, New York 14831, USA). Thin blood smears were fixed with 99% methanol. Packed cell volumes (PCV) were determined using a portable 12-V centrifuge (Mobilespin, Vulcan Technologies, Grandview, Missouri 64040, USA), and plasma total solids were measured with the use of a handheld refractometer (Schulco, Toledo, Ohio 43608, USA) calibrated at

the site. White blood cell (WBC) counts were made using the BD Unopette® Brand Test for Manual Eosinophil Counts (Catalog No. 365877, Becton-Dickinson Diagnostics). Red blood cell (RBC) counts were made using the BD Unopette Brand Test for Manual RBC Counts (Catalog Nos. 365850/365851, Becton-Dickinson Diagnostics). The remaining blood was centrifuged for 10 min and plasma separated and placed in cryotubes. Plasma and whole-blood samples were kept frozen in Gabon in a  $-20^{\circ}\text{C}$  freezer for less than 3 wk. Blood smears, and plasma and whole blood kept frozen on dry ice, were transported to the USA. All appropriate export and import permits accompanied the samples during transport. Plasma and whole blood were kept in a  $-80^{\circ}\text{C}$  freezer in the USA until laboratory tests were performed within 12 mo of collection.

Blood films fixed in methanol were stained with Wright-Giemsa, at the University of Florida, for evaluation of circulating cell morphology, estimation of leukocyte numbers and differential leukocyte counts. A minimum of 200 leukocytes were counted for differential leukocyte determinations. Leukocytes were categorized into one of five groups: monocytes, heterophils, lymphocytes, eosinophils, and basophils. Identification of blood cell types was based on previously described nomenclature.<sup>23</sup> Red blood cells were evaluated for hemoparasite identification. Additionally, total white blood cell counts were estimated from blood films by multiplying the average number of leukocytes observed per microscopic field times the objective power squared.<sup>24</sup> This estimate was used for comparison with the total white blood cell count obtained with the use of the eosinophil Unopette method in the field.

Samples for plasma biochemistry were processed on a dry-slide chemistry analyzer (Kodak 750 X R, Ortho Clinical Diagnostics, Rochester, New York 14626, USA) at the University of Miami. The biochemical panel included alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, cholesterol,  $\text{CO}_2$ , creatine kinase (CK), creatinine, gamma glutamyl transferase (GGT), glucose, lactate dehydrogenase (LD), lipase, phosphorous, potassium, sodium, total protein, triglyceride, and uric acid (UA).

Plasma corticosterone was measured by radioimmunoassay at the University of Miami with the use of a commercially available kit (Diagnostic Products Corporation, Los Angeles, California 90045, USA) that had been used previously for exotic animal samples by the laboratory. Samples were run in duplicate and quantitated from a standard curve.

Plasma electrophoresis was performed at the

University of Miami using SPEP-II agarose gels and the Beckman paragon electrophoresis system (Beckman-Coulter Corporation, Brea, California 92834, USA). The gels were run by manufacturer's instructions. The percent of protein fractions was quantitated by laser densitometry and then fraction values were calculated by multiplying the percentage by the total protein value determined with the use of the dry chemistry analyzer.

Plasma was analyzed at the Wildlife Conservation Society for alpha-tocopherol (vitamin E) and retinol (vitamin A) concentrations with the use of high-performance liquid chromatography (HPLC) with equipment and methodologies as previously described.<sup>3</sup>

Plasma were screened at the University of Pennsylvania for the presence of organochlorine (OC) insecticides (aldrin, alpha-BHC, beta-BHC, alpha-chlordane, pp-DDE, pp-DDD, pp-DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, lindane, methoxychlor), and polychlorinated biphenyl (PCB) (expressed as Arochlor 1260) by gas chromatography with electron capture detection (Agilent GC model 6890, Agilent Technologies, Palo Alto, California 94306, USA). The limits of quantification (LOQ) for all OCs were 20 ppb with the exception of methoxychlor, which had an LOQ of 250 ppb.

Whole blood was analyzed at the University of Pennsylvania for arsenic ( $n = 9$ ), lead ( $n = 9$ ) and mercury ( $n = 6$ ). Arsenic and mercury were determined by atomic absorption spectroscopy (AAS) with the use of hydride generation (AAAnalyst 800 AA, Perkin Elmer, Wellesley, Massachusetts 02481, USA). The LOQs were 25 ppb for mercury and 100 ppb for arsenic. Lead was determined by graphite furnace AAS (AAAnalyst 800, Perkin-Elmer). The lead LOQ was 50 ppb.

Hematologic evaluations ( $n = 28$ ) were performed on blood collected in sodium heparin; plasma biochemistry values were determined for samples collected in both sodium ( $n = 8$ ) and lithium ( $n = 10$ ) heparin, and corticosterone ( $n = 12$ ), plasma electrophoresis ( $n = 12$ ), alpha-tocopherol ( $n = 12$ ), retinol ( $n = 12$ ), and OC and PCB ( $n = 9$ ) levels were all performed on blood collected in lithium heparin. Not all tests were performed on all individuals because the amount of plasma was limited for some of the turtles. Descriptive statistics (mean, standard deviation, minimum, and maximum) were performed with the use of Microsoft Excel (Microsoft Corporation, Redmond, Washington 98052, USA). All values are recorded as mean  $\pm$  SD. Nonparametric statistical tests were used for comparisons because of the non-normal distribution of some data. WBC counts determined in the field by the eosinophil Unopette method

and in the laboratory by microscopic estimation were compared by a nonparametric range test ( $P \leq 0.05$ ). Plasma biochemistry values for samples collected in sodium heparin versus those collected in lithium heparin were analyzed by the Mann-Whitney  $U$ -test ( $P \leq 0.05$ ).

## RESULTS

### Physical examinations and morphometrics

A total of 35 leatherback females (10 in 2001 and 25 in 2002) received physical examinations. Carapace length was measured for 32 turtles. Straight carapace length (SCL) was  $153 \pm 7$  cm with a range of 146–168 cm in 10 turtles and curved carapace length (CCL) was  $150 \pm 6$  cm with a range of 139–169 cm in 22 turtles. Three of 35 turtles had an amputated rear flipper and one had a partial tail amputation. One of the flipper amputations was deemed to be fresh. A bite mark was noted on the flipper of one of these amputees. One turtle had propeller wounds on the carapace and minor to moderate muscle damage was noted on three turtles. Barnacles were recorded on three turtles. No fibropapillomas were observed on any of the 35 turtles and all appeared in good body weight with adequate energy levels based on nesting behavior.

### Hematology

Results of hematologic tests are provided in Table 1. The mean PCV was 0.36 (proportion of 1) with a range of 0.28–0.56. One turtle had a PCV value of 0.56. The next closest PCV value was 0.44. The WBC count based on the eosinophil Unopette method ( $5,900 \pm 2,800$ ) compared to that determined from slide estimation in the laboratory ( $4,500 \pm 1,600$ ) was statistically different ( $P < 0.05$ ). No basophils or hemoparasites were detected in any of the 26 turtles tested.

### Plasma biochemistry and corticosterone concentration

Plasma biochemistry data are provided in Table 2, with values reported for blood collected in sodium and lithium heparin. No statistically significant differences were noted between the two anticoagulants for any of the measures. Of the 12 turtles tested for corticosterone, 11 had concentrations less than the limit of detection (0.1 ng/ml) and one turtle had a concentration of 4.0 ng/ml.

### Plasma protein electrophoresis

Plasma protein electrophoresis results are provided in Table 3. There was no prealbumin band or beta-gamma bridging in any of the 12 turtles tested.

**Table 1.** Hematologic values in free-ranging leatherback turtles (*Dermochelys coriacea*) nesting on Pongara Beach, Republic of Gabon.

Measure <sup>a</sup>	N	Mean	SD	Range
PCV (l/l [%])	28	0.36 [36]	0.054 [5.4]	0.28–0.56 [28–56]
TS (g/l [g/dl])	28	40 [4.0]	7 [0.7]	23–54 [2.3–5.4]
RBC ( $\times 10^3/\mu\text{l}$ )	15	381	198	170–780
WBC ( $\times 10^3/\mu\text{l}$ ) <sup>b,c</sup>	26	5.9	2.8	1.5–14.6
WBC ( $\times 10^3/\mu\text{l}$ ) <sup>c,d</sup>	26	4.5	1.6	2.5–7.5
Heterophils ( $\times 10^3/\mu\text{l}$ )	26	2.4	1.2	0.0–5.1
Lymphocytes ( $\times 10^3/\mu\text{l}$ )	26	1.6	0.9	0.0–3.3
Monocytes ( $\times 10^3/\mu\text{l}$ )	26	0.2	0.2	0.0–0.8
Eosinophils ( $\times 10^3/\mu\text{l}$ )	26	0.1	0.1	0–0.5

<sup>a</sup> PCV, packed cell volume; TS, total solids; RBC, red blood cells; WBC, white blood cells. No basophils were detected in any of the 26 leatherback turtles evaluated. Samples were collected in sodium heparin.

<sup>b</sup> Estimated WBC count performed in the field with the use of the eosinophil Unopette method.

<sup>c</sup> Statistically different at ( $P < 0.05$ ).

<sup>d</sup> Estimated WBC count performed in the laboratory by blood smear evaluation.

**Table 2.** Plasma biochemistry values from blood collected in sodium and lithium heparin from free-ranging leatherback turtles (*Dermochelys coriacea*) nesting on Pongara Beach, Republic of Gabon.

Measure <sup>a</sup>	Sodium heparin			Lithium heparin		
	N	Mean $\pm$ SD	Range	N	Mean $\pm$ SD	Range
Glucose (mmol/L [mg/dl])	8	4.33 $\pm$ 0.72 [78 $\pm$ 13]	3.05–5.11 [55–92]	10	4.66 $\pm$ 0.50 [84 $\pm$ 9]	3.55–5.27 [64–95]
Sodium (mmol/L)	8	138 $\pm$ 6	127–144	9	136 $\pm$ 9	124–148
Potassium (mmol/L)	8	4.0 $\pm$ 0.9	2.8–5.1	9	4.0 $\pm$ 1.0	3.0–5.0
CO <sub>2</sub> (mmol/L)	8	22 $\pm$ 2	18–25	9	23 $\pm$ 2	21–25
BUN (mmol/L [mg/dl])	8	1.07 $\pm$ 1.43 [3 $\pm$ 4]	0.71–4.64 [2–13]	9	0.71 $\pm$ 0 [2 $\pm$ 0]	0.71 [2]
Creatinine ( $\mu\text{mol/L}$ [mg/dl])	8	26.52 $\pm$ 8.84 [0.3 $\pm$ 0.1]	17.68–44.2 [0.2 $\pm$ 0.5]	9	17.68 $\pm$ 8.84 [0.2 $\pm$ 0.1]	8.84–44.2 [0.1–0.5]
TP (g/L [g/dl])	8	46 $\pm$ 10 [4.6 $\pm$ 1.0]	32–60 [3.2–6.0]	10	40 $\pm$ 10 [4.0 $\pm$ 1.0]	30–50 [3.0–5.0]
Cholesterol (mmol/L [mg/dl])	8	8.96 $\pm$ 2.49 [346 $\pm$ 96]	6.58–12.87 [254–497]	10	7.58 $\pm$ 1.89 [293 $\pm$ 73]	3.52–10.10 [136–390]
Triglyceride (mmol/L [mg/dl])	8	3.89 $\pm$ 0.84 [344 $\pm$ 74]	2.62–4.63 [232–410]	10	4.65 $\pm$ 0.40 [412 $\pm$ 35]	4.17–5.34 [369–473]
Calcium (mmol/L [mg/dl])	8	1.78 $\pm$ 0.45 [7.1 $\pm$ 1.8]	1.1–2.4 [4.4–9.6]	10	2.0 $\pm$ 0.5 [8 $\pm$ 2]	1.25–2.5 [5–10]
Phosphorous (mmol/L [mg/dl])	8	3.55 $\pm$ 0.48 [11.0 $\pm$ 1.5]	2.87–4.36 [8.9–13.5]	10	3.55 $\pm$ 0.65 [11 $\pm$ 2]	2.91–4.50 [9–14]
Uric acid ( $\mu\text{mol/L}$ [mg/dl])	8	11.9 $\pm$ 0.0 [0.2 $\pm$ 0.0]	11.9 [0.2]	10	11.9 $\pm$ 0.0 [0.2 $\pm$ 0.0]	11.9 [0.2]
ALT (U/L)	8	4 $\pm$ 2	3–10	9	4 $\pm$ 1	3–5
AST (U/L)	8	159 $\pm$ 49	94–234	10	165 $\pm$ 29	126–221
LD (U/L)	8	1,716 $\pm$ 852	1,041–3,564	10	1,502 $\pm$ 528	793–2,531
CK (U/L)	8	1,228 $\pm$ 2,390	20–7,086	10	287 $\pm$ 359	20–884
Amylase (U/L)	8	628 $\pm$ 71	538–779	10	681 $\pm$ 104	495–895
Lipase (U/L)	8	2 $\pm$ 2	1–6	10	1 $\pm$ 0	1
GGT (U/L)	8	12 $\pm$ 2	10–14	10	11 $\pm$ 1	10–11

<sup>a</sup> BUN, blood urea nitrogen; TP, total protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LD, lactate dehydrogenase; CK, creatine kinase; GGT, gamma glutamyl transferase. No measures were statistically different between samples collected in lithium versus sodium heparin.

**Table 3.** Plasma protein fractions in free-ranging leatherback turtles (*Dermochelys coriacea*) nesting on Pongara Beach, Republic of Gabon.

Measure <sup>a</sup>	Prealbumin (g/L [g/dl])	Albumin (g/L [g/dl])	Alpha-1 (g/L [g/dl])	Alpha-2 (g/L [g/dl])	Beta (g/L [g/dl])	Gamma (g/L [g/dl])
Mean ± SD	0 ± 0 [0 ± 0]	18.1 ± 3.7 [1.18 ± 0.37]	1.6 ± 0.7 [0.16 ± 0.07]	8.2 ± 2.0 [0.82 ± 0.20]	8.0 ± 1.1 [0.80 ± 0.11]	8.1 ± 2.1 [0.81 ± 0.21]
Minimum	0 [0]	10.7 [1.07]	0.7 [0.07]	5.1 [0.51]	6.0 [0.60]	5.4 [0.54]
Maximum	0 [0]	23.9 [2.39]	3.3 [0.33]	12.4 [1.24]	9.1 [0.91]	12.8 [1.28]
N	12	12	12	12	12	12

<sup>a</sup> Samples were collected in lithium heparin.

Albumin, alpha-1, alpha-2, beta, and gamma globulins were detected in all 12 turtles.

### Vitamin E and A

Plasma alpha-tocopherol concentrations in the 12 turtles tested was  $8.0 \pm 3.7$   $\mu\text{g/ml}$  with a range of 0.6–12.7  $\mu\text{g/ml}$ . Plasma retinol was evaluated in 12 turtles and the concentration was  $0.5 \pm 0.1$   $\mu\text{g/ml}$  with a range of 0.1–0.7  $\mu\text{g/ml}$ .

### Toxins

Plasma OCs and PCBs were below their LOQs in the nine turtles tested. The whole-blood lead concentration was  $87.2 \pm 30.9$  ppb (range of 42–151 ppb). The mercury concentration for the six turtles tested was  $200 \pm 200$  ppb (range of 100–400 ppb). Arsenic was not detected above the LOQ of 100 ppb in eight of nine turtles tested. One turtle had a detectable arsenic concentration of 100 ppb.

## DISCUSSION

The general health of the turtles in this study was rated as good based on nest-building activity, energy level, and body condition. Although 3 of the 35 turtles had amputated flippers (one classified as recent), and one had evidence of propeller wounds, all these turtles were able to successfully come ashore to nest. Therefore, the sample population was most likely biased toward the more healthy turtles in the population. A previous study on the causes of mortality for 41 leatherback turtles in Gabon found that 5% died of uncertain cause, 29% were slaughtered by humans, 44% had evidence of capture in nets, 20% died from boat strike, and 2% had evidence of oil-related death.<sup>5</sup> It is probable that the health of the population is impacted by a number of anthropogenic factors (i.e., boats, trawling, oil exploitation) that our sample population may not adequately reflect.

The turtle with the PCV of 0.56 (proportion of 1) had no other blood parameters that appeared to be outliers and the turtle had no evidence of de-

hydration or other possible causes of a high PCV. The differential count varied slightly from those reported in other sea turtle species.<sup>33,37,39</sup> The leatherbacks in our study had lower eosinophils than those reported in green and loggerhead turtles.<sup>2,39</sup> High circulating eosinophils in green and loggerhead turtles may be related to spirorchids and other parasites commonly found in those species.<sup>18,19</sup> Few helminth species have been found in leatherback turtles, possibly associated with their strictly pelagic existence and dietary preference of gelatinous species.<sup>29</sup>

The eosinophil Unopette method has been the most practical for determining WBC counts in a number of reptilian and avian species in remote field locations (S. L. Deem, unpubl. data). However, in this study WBC counts determined by the eosinophil Unopette method were significantly higher than those estimated from blood slides. It has been previously noted that WBC counts vary greatly in sea turtles based on the method employed.<sup>2</sup> This finding should be taken into consideration when one is comparing the results between studies.

Although anticoagulants have been suggested as influencing plasma biochemistry values for reptilian species,<sup>30</sup> values for the turtles in this study were not statistically different for any of the measures. Although in this study samples were not paired from individual turtles for the two anticoagulants, in a previous study comparing plasma biochemistry values of paired samples for loggerhead turtle blood collected in sodium or lithium heparin, no differences were detected.<sup>7</sup>

Cholesterol (8.96 mmol/L Na heparin; 7.58 mmol/L lithium heparin) and triglyceride (3.89 mmol/L Na heparin; 4.65 mmol/L lithium heparin) values were high in the nesting leatherback turtles, when compared to cholesterol and triglyceride findings in juvenile wild green turtles (5.62 mmol/L and 1.94 mmol/L, respectively)<sup>6</sup> and free-ranging loggerheads (2.75 mmol/L and 0.98 mmol/L, respectively).<sup>7</sup> Cholesterol and triglyceride values are

often elevated during vitellogenesis; however, it has been demonstrated that vitellogenesis is complete in nesting leatherbacks prior to their first arrival on the beach during a nesting season.<sup>31</sup>

The mean calcium values (1.78 mmol/L Na heparin; 2.0 mmol/L lithium heparin) were in the low to middle range (1.63–2.43 mmol/L) reported in nesting leatherback turtles in Costa Rica.<sup>32</sup> In Gabon, leatherback turtles had a calcium:phosphorus ratio <1, with a mean phosphorus value of 3.55 mmol/L for both Na and lithium heparin samples. The mean phosphorus value appeared elevated in comparison with findings from other sea turtles. Mean values ranged from 1.91–2.58 mmol/L in healthy sea turtles<sup>6,7,16</sup> and 0.32–1.62 mmol/L in most reptiles.<sup>8</sup> In one study of gravid green iguanas (*Iguana iguana*) ( $n = 3$ ), the mean phosphorus value was 3.88 mmol/L.<sup>22</sup> Unlike the leatherback turtles, the calcium:phosphorus ratio was >1 in these iguanas.<sup>22</sup> It is possible that the phosphorus values were elevated in the leatherback turtles as a result of phosphorus mobilization in association with egg production. However, the calcium values in the leatherback turtles were low, even though calcium should also be mobilized during this period.<sup>8</sup> Alternatively, phosphorus values may have been elevated as a result of phosphorus release from the erythrocytes, because samples were not processed immediately.<sup>8</sup> An inverse calcium:phosphorus ratio may be normal for nesting leatherback turtles, or for the species in general, similar to that determined for juvenile loggerhead turtles.<sup>4</sup> Phosphorus values were not determined for the nesting leatherback turtles in Costa Rica.<sup>32</sup> Further studies are necessary to determine the calcium:phosphorus ratio in nesting and nonnesting leatherback turtles.

Both the BUN and UA values were low (1.07 mmol/L Na heparin; 0.71 mmol/L lithium heparin and 11.9  $\mu$ mol/L Na and lithium heparin, respectively) which was consistent with those found in reptiles.<sup>8</sup> However, low values for BUN and UA in chelonians can also be associated with low protein diets or hepatic insufficiency,<sup>8</sup> and values in these leatherback turtles were lower than those previously reported for loggerhead turtles (BUN 17.5–32.8 mmol/L; UA 41  $\mu$ mol/L), green turtles (BUN 2.5 mmol/L; UA 89.22  $\mu$ mol/L), and Kemp's ridley (*Lepidochelys kempi*) (BUN 26.42 mmol/L).<sup>6,7,9,16,37,38</sup>

The CK values varied widely from a low of 20 U/L to a high, in one turtle, of 7,086 U/L. The next highest CK value was 884 U/L. The turtle with the high CK value was missing half a flipper and had evidence of muscle damage, which is consistent

with a high CK, an enzyme used as an indicator of striated muscle injury.

The one turtle with a corticosterone value of 4.0 ng/ml had no clinical or laboratory findings indicative of stress. This value, although much higher than the other study turtles was similar to those found in nesting leatherback turtles in Costa Rica, in which the range was 1.4–3.9 ng/ml.<sup>32</sup>

Plasma protein electrophoresis, an increasingly important diagnostic modality for nondomestic species, can provide information about chronic or acute inflammatory processes.<sup>10,40</sup> However, baseline values must be determined for each species. Table 3 reports the first known values for nesting leatherback turtles. The leatherback turtles had higher albumin values and lower gamma globulin values when compared to free-ranging loggerhead turtles in Florida.<sup>17</sup> Additionally, whereas none of the leatherback turtles had beta-gamma bridging, some Florida loggerhead turtles had this bridging, which may be associated with chronic disease or parasites.<sup>17</sup>

Plasma vitamin E (alpha-tocopherol) concentrations in the leatherbacks were similar to those reported for the nesting herbivorous green sea turtle ( $6.08 \pm 0.40$   $\mu$ g/ml, range 0.90–25.31)<sup>15</sup> but not as high as reported for the more omnivorous loggerhead turtle in the same study ( $12.98 \pm 0.65$   $\mu$ g/ml, range 0.01–48.6),<sup>15</sup> despite the fact that leatherbacks specialize on eating jellyfish. In studies that have been conducted on sea turtles,<sup>15</sup> there was no effect of vitamin E concentration on either clutch size or hatching success, and concentrations remained stable throughout the nesting season.

The retinol concentrations, as a measure of vitamin A activity, in the leatherback turtles were similar to those reported in nesting green turtles ( $0.52 \pm 0.04$   $\mu$ g/ml) and loggerhead turtles ( $0.43 \pm 0.02$   $\mu$ g/ml).<sup>15</sup> There appears to be some seasonality in circulating retinol concentrations, particularly for the herbivorous species, which may link with vegetation variability. Omnivorous sea turtles, however, showed a decline in retinol concentration over the nesting period,<sup>15</sup> because turtles tend not to feed during that time and may be depleting stored resources.

The negative results for the OCs and PCBs may reflect the dietary habits (e.g., jellyfish) of the leatherback turtles, because these toxicants are known to bioaccumulate. However, whole-blood OC concentrations are greatly affected by the mobilization of fat stores as might occur during long migrations, breeding, ovipositioning, or disease events. This makes assessment of spatial and temporal trends more diffi-

cult,<sup>25</sup> but also suggests that OC concentration would tend to increase during nest-laying activity.

The significance of the detected lead ( $87.2 \pm 30.9$  ppb) and total mercury ( $200 \pm 200$  ppb) concentrations is unknown. Based upon clinically relevant whole-blood lead concentrations for many avian and mammalian species, the detected concentrations were low and unlikely to be associated with adverse health consequences. Lead and mercury have also been detected in other sea turtle species. The mean whole-blood lead and total mercury concentration from 106 Kemp's ridley sea turtles were 11 and 18 ppb, respectively.<sup>26</sup> The mean whole-blood mercury concentrations for 34 live captured and 6 stranded loggerhead turtles was 29 ppb and 99 ppb, respectively.<sup>11</sup> It is interesting to note that the mercury detected in the study leatherback turtles was higher than in both the Kemp's ridley and loggerhead sea turtles, as one would expect a higher concentration in the latter two species due to their dietary preferences (e.g., crabs and molluscs).

There is little published information regarding blood or tissue arsenic concentrations in sea turtles. Only a single leatherback in the study turtles had a minimally detectable level of blood arsenic. Arsenic has been detected in the livers of two leatherback turtles at 0.58 ppm (dry weight) and 1.2 ppm (wet weight)<sup>20</sup> and hepatic concentrations in loggerhead turtles are among the highest detected in sea turtles ( $11.2 \pm 3.0$  ppm dry weight).<sup>27</sup> However, it is uncertain how blood levels may compare with these tissue levels, and further assessments are necessary.

Health data such as those presented in this article are imperative to collect, together with ecologic studies, to amass much needed baseline health data on free-ranging wildlife species, and to fully understand the effects of anthropogenic changes on species conservation.<sup>12,13</sup> Additionally, leatherbacks, and other sea turtles, may serve as indicator species for marine ecosystem health.<sup>1</sup> Although the general good condition of the turtles in this study is promising, the study was biased to those females healthy enough to come to shore to lay eggs. The leatherback turtle population nesting on the Central African coast is probably one of the largest remaining populations of this species.<sup>14,34</sup> It will be beneficial to expand health monitoring of this population to assess the impacts of oil extraction and ecotourism as they increase in the region.

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