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COMPARISON OF BLOOD VALUES IN FORAGING, NESTING, AND STRANDED LOGGERHEAD TURTLES (*CARETTA CARETTA*) ALONG THE COAST OF GEORGIA, USA

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ABSTRACT: The health status of 83 loggerhead sea turtles (*Caretta caretta*; 39 foraging, 31 nesting, and 13 stranded turtles) was analyzed using physical examinations, hematology, plasma biochemistry, plasma protein electrophoresis, and toxicologic parameters. Significant differences were noted in a number of health parameters between turtles exhibiting each of these behaviors. On physical examinations, stranded turtles had the highest prevalence of heavy carapace epibiont loads, miscellaneous abnormalities, emaciation, and weakness. Differences in hematologic values included a lower packed cell volume, higher number of lymphocytes, and lower number of monocytes in stranded turtles; lower white blood cell counts in foraging turtles; and significant differences in total solid values among turtles exhibiting all behaviors with the lowest values in stranded turtles and the highest values in nesting turtles. Differences in plasma biochemistry values included the highest uric acid, creatine kinase, and CO_2 values in stranded turtles; the highest glucose and potassium values in foraging turtles; and the highest cholesterol and triglyceride values, and lowest alanine aminotransferase, in nesting turtles. Differences in total protein, albumin, and globulin were found using plasma biochemistry values, with lowest values in stranded turtles and highest values in nesting females, whereas differences in blood urea nitrogen between turtles included the lowest values in nesting turtles and the highest in foraging turtles. Plasma organochlorine and polychlorinated biphenyl levels were below their limits of quantification in the 39 foraging, 11 nesting, and three stranded turtles tested. A statistically significant difference was noted in the level of whole blood mercury between the 23 foraging and 12 nesting turtles tested. There was no difference in arsenic or lead levels between turtles exhibiting any of the three behaviors. Although a few limitations exist with the present study and include unknown ambient temperatures, turtle handling times that varied from 15 min to 53 min per turtle, and the use of a different laboratory for processing complete blood counts and plasma biochemistries in stranded versus foraging and nesting turtles, we provide baseline blood values for two cohorts (foraging and nesting) of loggerhead sea turtles on the coast of Georgia. Additionally, we demonstrate significant differences in clinical findings and blood parameters between foraging, nesting, and stranded loggerhead turtles in the region.

Key words: *Caretta caretta*, hematology, loggerhead turtles, plasma biochemistry, plasma protein electrophoresis, toxicants.

INTRODUCTION

Loggerhead sea turtles (*Caretta caretta*) are the most common sea turtle species nesting on the coast of Georgia (Norton, 2005). In addition to loggerheads, four other sea turtle species including green (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), hawksbill (*Eretmochelys imbricata*), and Kemp's ridley (*Lepidochelys kempi*) use coastal Georgia waters for food, shelter, occasional nesting, and as a travel corridor (Norton, 2005). Conservation threats to sea turtles in Georgia are primarily associated with beachfront development, pollution from chemical toxins, ingestion of marine debris and drowning and traumatic injuries from incidental entanglement related to recreational and commercial fisheries activities (Norton, 2005). Additionally, diseases, including the debilitated loggerhead sea turtle syndrome, are direct threats to loggerhead conservation (George, 1997; Norton et al., 2005; Jacobson et al., 2006).

In addition to monitoring population demographics and causes of mortality, baseline blood values can be used as one indicator of population health. Life stages (e.g., foraging, nesting), age, sex, nutritional status, pathogen, parasite and toxin exposure, and environmental conditions all impact physiologic status and influence baseline blood parameters in sea turtles (Whitaker and Krum, 1999; Herbst and Jacobson, 2003). Therefore, the establishment of baseline values for different cohorts within a population must be obtained. These baseline values are invaluable for providing an indication of the overall health of a wild population, for measuring the health of a population over time, for comparing the health of populations, and for use as prognostic indicators for individual health assessments of stranded turtles.

The objective of this study was to compare health indices, including physical examination findings, hematology, plasma biochemistry values, plasma protein electrophoresis, and toxicologic parameters of foraging, nesting, and stranded loggerhead sea turtles on the coast of Georgia.

MATERIALS AND METHODS

Study period and site

This study was carried out between May and September during the years 2000 to 2004. Foraging turtles ($n=39$) were captured by trawl in waters off the coast of northeast Florida and Georgia in conjunction with ongoing studies of sea turtle population biology. Foraging turtles were collected by fishery independent trawlers operating in near-shore waters between Savannah, Georgia, USA (32°5′N, 81°5′W) and St. Augustine, Florida, USA (29°50′N, 81°15′W) in 4.5–12.2-m-deep water. (Maier et al., 2004). Trawlers used 20-m four-seam nets with 20-cm mesh, without turtle excluder devices and trawl duration was limited to 30 min, as previously described (Maier et al., 2004). Nesting females were approached on the beach after they completed egg laying and were returning to the ocean. Nesting females were sampled on Blackbeard Island, Georgia, USA (31°30′N, 81°25′W) ($n=27$); Cumberland Island, Georgia, USA (30°51′N, 81°26′W) ($n=1$); and Jekyll Island, Georgia, USA (31°4′N, 81°25′W) ($n=3$). Live stranded turtles ($n=13$) were found all along the Georgia coast and transported to the Georgia Department of Natural Resources for their initial evaluation.

Sample and data collection and analyses

A complete physical examination was performed and abnormalities categorized into heavy epibiont load, flipper amputations, miscellaneous abnormalities (e.g., propeller wound, shark bite, fishing hook, neurologic signs), emaciation, and weakness. Epibiont loads were based on epibiont presence on the carapace categorized using the ordinal scale of 1 to 3 (1=mild [<20 epibionts]; 2=moderate [20–50 epibionts]; 3=heavy [>50 epibionts]). Curved carapace length (CCL), notch to tip, was measured using a nylon tape measure. Foraging and stranded turtles were weighed using hanging spring scales. Nesting females were not weighed. A turtle was classified as emaciated based on a body weight that was less than expected for known carapace length (in those turtles in which both parameters were collected), and a sunken appearance to axillae, flank, and/or plastron regions. A turtle was classified as weak if it displayed minimal response to human handling with minimal to

no head and flipper movements. Age (adult versus subadult) of foraging and stranded turtles was determined based on CCL; with adults ≥87 cm and subadults <87 cm. Age categories were established using the smallest nesting adult female in our study. Sex was determined using tail length (e.g., long tail in adult males) in adult stranded turtles that survived and visualization of the gonad at necropsy in stranded turtles that did not survive. In foraging animals, plasma testosterone levels were measured for sex determination using radioimmunoassay as previously described (Owens et al., 1978). Complete postmortem examinations (gross and microscopic examination) were performed on all stranded turtles that died ($n=6$).

Approximately 35 ml of blood was collected from the dorsal cervical sinus using a 20 gauge or 22 gauge 3.8-cm needle and dispensed directly into large (10-ml) vacutainer tubes containing either lithium heparin (Corvac, Sherwood Medical, Saint Louis, Missouri, USA), for hematology and plasma biochemistry values, or buffered citrate sodium (Becton-Dickinson Diagnostics, Pre-Analytical Systems, Franklin Lakes, New Jersey, USA) for heavy metal testing (Owens and Ruiz, 1980). Tubes were kept in a cooler with ice packs until processed.

Initial processing of blood samples occurred in the field within 4 hr (range 15 min to 4 hr) of blood collection for trawl-caught turtles, nesting females, and stranded turtles. Thin blood smears were made within 30 min of collection and then fixed in the field with 99% methanol. Packed cell volumes (PCV) were determined using a portable 12-V centrifuge (Mobilespin, Vulcan Technologies, Grandview, Missouri, USA) and plasma total solids were measured using a handheld refractometer (Schulco, Toledo, Ohio, USA) temperature-calibrated at the site. White blood cell (WBC) counts were performed using the BD Unopette® brand test for manual eosinophil counts (Catalog 365877, Becton-Dickinson Diagnostics, Pre-analytical Systems,). Red blood cell (RBC) counts were made using the BD Unopette® brand test for manual rbc counts (Catalog 365850/365851, Becton-Dickinson Diagnostics, Pre-analytical Systems). The remaining heparinized blood was centrifuged for 10 min and plasma was then pipetted into cryotubes (Corning Incorporated, Corning, New York, USA). Plasma was kept frozen (−70 C) and transported on dry ice ($n=70$) to laboratories for diagnostic testing, or shipped immediately on wet ice for stranded turtles ($n=13$). Whole blood collected in buffered citrate sodium tubes was placed in cryotubes (Corning Incorporated) and kept frozen (−70 C) and transported on dry ice to the laboratory. All laboratory diagnostics were performed within 6 mo of blood collection.

For foraging and nesting loggerheads, 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 six groups: monocytes, heterophils, lymphocytes, eosinophils, basophils, and azurophils. Identification of blood cell types was based on previously described nomenclature (Hawkey and Dennett, 1989). 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 (Harvey, 2001). White blood cell estimates and differential cell counts were performed on blood films from stranded turtles by a commercial laboratory (Antech Diagnostic Laboratories, Memphis, Tennessee, USA) as part of their complete blood count (CBC) (Antech's Comprehensive Reptile Profile-AE 160). For the stranded turtles, the blood smear was reviewed for the WBC estimate using a 40× lens with the average of the WBCs in 10 consecutive fields multiplied by 1,000 to obtain the WBC estimate.

For foraging and nesting loggerheads, samples for plasma biochemistry were processed on a dry slide chemistry analyzer (Kodak 750 X R, Ortho Clinical Diagnostics, Rochester, New York, USA) at the University of Miami. The biochemical panel included albumin, alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, chloride, cholesterol, CO_2, creatine kinase (CK), creatinine, gamma glutamyl transferase (GGT), globulin, glucose, lactate dehydrogenase, lipase, phosphorous, potassium, sodium, total protein, triglyceride, and uric acid (UA). Plasma biochemistries for stranded turtles were performed by a commercial laboratory (Antech Diagnostic Laboratories) as part of their chemistry profiles using a Hitachi 747-100.

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, USA). The gels were run according to manufacturer's instructions

as described previously (Cray and Tatum, 1998). The percentage of protein fractions was quantitated by laser densitometry and then each fraction value was calculated by multiplying the percentage of the fraction by the total protein value determined using the dry chemistry analyzer.

Plasma was 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, USA). The limits of quantification (LOQ) for all OCs were 20 parts per billion (ppb) with the exception of methoxychlor, which had an LOQ of 250 ppb.

Whole blood, collected in buffered citrate sodium tubes, was analyzed for detectable arsenic, lead, and mercury at the University of Pennsylvania. Arsenic and mercury were determined by atomic absorption spectroscopy (AAS) using hydride generation (AAnalyst 800 AA, Perkin Elmer, Wellesley, Massachusetts, USA). The LOQs were 25 ppb for mercury and 100 ppb for arsenic. Lead was determined by graphite furnace AAS (AAnalyst 800, Perkin-Elmer). The lead LOQ was 50 ppb. All toxicant results were expressed on a wet weight basis.

The distribution of each physical measurement, hematologic, plasma biochemistry, and plasma electrophoresis variable was evaluated separately for the entire sample population, age, gender, and behavior. The mean, standard deviation, median, and range were determined for each parameter. The Shapiro-Wilk statistic, kurtosis, and skewness were used to evaluate the distribution of the data. The X±SD are reported for normally distributed data; the median, 10%, and 90% quartiles are reported for nonnormally distributed data. Levene's test for equality of variances was used to determine if the data were homogeneous. Comparisons were made between age groups, gender, and behavior. A one-way analysis of variance (ANOVA) was used to assess between group differences for normally distributed data. Specific between-group differences were evaluated using a Tukey's test. For data that were not normally distributed, a Kruskal-Wallis one-way ANOVA and Dunn's test were used to assess differences between and within groups, respectively. After completion of the crude analysis, a univariate general linear model was used to evaluate behavior and gender while controlling for age. Nonnormally distributed data were log transformed for this analysis. A 4×2 exact test was done to determine if physical exam findings significantly differed between the nesting, foraging, and stranded turtles. When a difference was found at this level, a Fisher exact test was used to further classify differences between groups. Historically, agreement between different methods of measuring the same biologic parameter has been determined on the basis of correlation; however, this is not considered optimal for agreement analysis (Bland and Altman, 1999). In this study, agreement between the two techniques for evaluating the WBC counts was determined by the Bland-Altman method (Bland and Altman, 1986, 1999). For this analysis, bias was defined as the mean difference between the two methods and limits of agreement were calculated as the bias ±1.96 SD (Med-Calc Software, Mariakerke, Belgium). Values of $P<0.05$ were considered to reflect statistical differences. Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Physical examinations

Based on testosterone levels in the 39 foraging turtles, there were 30 females, eight males, and one turtle of unknown sex. Curved carapace length (mean±SD) was 70.2 cm±9.1 with a range of 55.6–93.9 cm, and ages were determined as 35 subadults and four adults for the foraging turtles. Thirty-one nesting adult females received physical examinations and a CCL of 99.6 cm±5.8 and range of 87.0–108.4 cm was recorded for 29 of these females. Thirteen stranded turtles-seven female, one male, five of unknown sex-received physical examinations and were classified as subadult ($n=9$), adult ($n=3$), and unknown ($n=1$). Curved carapace length in 12 of these stranded turtles was 72.5 cm±14.2 with a range of 56.0–100.2 cm. Body weights were recorded for all 39 foraging turtles (44.7 kg±17; range 20–105 kg) and 12 stranded turtles (45.8 kg±27.5; range 21.2–91.0 kg).

A number of clinical abnormalities were detected in the turtles in each of the three

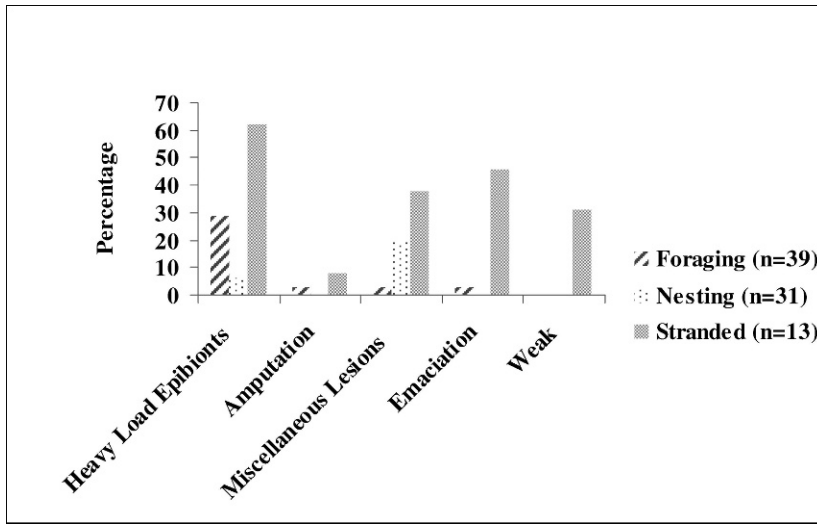

FIGURE 1. Clinical lesions noted in foraging, nesting, and stranded loggerhead sea turtles (*Caretta caretta*) along the coast of Georgia.

(39 foraging, 31 nesting, 13 stranded) behaviors (Fig 1). There was a significant difference in the number of heavy epibonts on stranded turtles (62%) compared with both nesting (6%, $P=0.0003$) and foraging turtles (29%, $P=0.03$). There was also a significant difference in the number of epibonts on the foraging turtles compared to the nesting turtles ($P=0.02$). Both stranded and nesting turtles were significantly more likely to have miscellaneous abnormalities (stranded: 38%, $P=0.002$; nesting: 19%, $P=0.03$) compared with foraging turtles (3%). There were significant differences in the number of emaciated turtles in the stranded group (46%) compared with both the nesting (0%) ($P=0.0002$) and foraging turtles (3%; $P=0.0005$), and weak turtles in the stranded group (31%) compared with other behaviors (0%, $P<0.010$). There was no difference in the number of flipper amputations between the three groups ($P=0.4$). In the miscellaneous abnormalities, one stranded turtle had a fish hook lodged in its oral cavity; one had severe neurologic clinical signs from a suspected spirorchid trematode infestation based on clinical signs, response to treatment, and similar conditions affecting loggerheads in Florida during this time period (Jacobson et al., 2006); and one had a spindle cell sarcoma within the cloacal region diagnosed by surgical excision and histopathologic examination after 6 mo in rehabilitation post-stranding. No fibropapillomatosis-like tumors were observed on any of the 83 loggerheads examined.

Of the 10 stranded turtles evaluated for epibiont loads, one had mild, one had moderate, and eight had heavy infestations. Epibiont loads in foraging turtles included seven mild, 15 moderate, and 10 heavy infestations, with seven loads not recorded; nesting turtles had 13 mild, 11 moderate, and two heavy infestations, with five loads not recorded.

Necropsies

Of the six stranded turtles that were euthanized or died, necropsies revealed a number of pathologic findings including two turtles with heavy external epibiont loads; two turtles with severe spirorchid trematode infestations; two turtles with significant carapace defects, one with associated pneumonia and the other with many barnacles observed on the coelomic lining and thought to have entered via the carapace defect; one turtle with hepatitis;

and one turtle with osteomyelitis associated with a flipper lesion.

Hematology

Results of hematologic tests are provided in Table 1. The PCV was statistically lower ($P<0.005$) in the stranded (0.19 l/l, 0.09–0.35) turtles than in the foraging (0.32 l/l±0.05) or nesting (0.30 l/l±0.04) turtles. Total solids were statistically different ($P<0.05$) between turtles exhibiting all three behaviors, with nesting (48±10 g/L) turtles having the highest values and stranded (20±8 g/l) turtles the lowest values. The foraging turtles had statistically lower WBC counts (9,005±3,536) than the nesting ($P=0.05$) and stranded ($P=0.009$) turtles using the eosinophil Unopette method, but not by WBC estimate using blood smears. Stranded turtles had statistically higher ($P<0.005$) lymphocyte counts (4,380, 912–11,700) and lower monocyte counts (0, 0–150) than foraging ($P<0.005$) and nesting ($P=0.003$) turtles. No hemoparasites were detected in any of the 68 turtles tested. There was a low degree of agreement between the two WBC count measures (bias: −1018.9, limits of agreement: −10282.7433 to 8244.8123; Fig. 2). Because of the potential for disagreement, the sampling techniques were evaluated individually.

Plasma biochemistry

Plasma biochemistry values are provided in Table 2. Foraging turtles had higher glucose (5.88 mmol/l±1.11) than the stranded ($P=0.002$) and nesting ($P=0.001$) turtles and higher potassium (5.1 mmol/l±2.0) values than turtles exhibiting the other two behaviors ($P<0.001$). Carbon dioxide values were statistically higher ($P<0.005$) in stranded (31 mmol/l, 28–36.5) versus foraging (15 mmol/l± 4) and nesting (14.7 mmol/l±8.6) turtles. Nesting females had higher cholesterol (6.94 mmol/l, 4.35–8.60) and triglyceride values (4.54 mmol/l, 1.71–9.75) and lower ALT values (4 U/l, 3–127) than turtles exhibiting the other two behaviors ($P<0.005$). Stranded turtles had higher CK (1,366 U/l, 136–2,223) and UA (77.32 μmol/l, 47.59–279.56) values than turtles exhibiting the other two behaviors ($P<0.005$). Significant differences in BUN were found between turtles exhibiting the various behaviors ($P<0.005$) with the lowest values in nesting turtles (2.86 mmol/l, 2.00–4.43) and the highest in foraging turtles (29.63 mmol/l, 14.28–42.48). Total protein was statistically different ($P<0.005$) between turtles exhibiting various behaviors, with lowest values (25 g/l±9) in stranded turtles and highest (52 g/l±5) in nesting females. Albumin values were statistically different ($P<0.005$) between turtles exhibiting various behaviors, with lowest values (7 g/l±3) in stranded turtles and highest (17 g/l±2) in nesting females. Difference in globulin values were also present with values from foraging turtles (29 g/l±9) different from nesting (40 g/l±7; $P=0.02$) and stranded (17 g/l±7; $P=0.003$) turtles; nesting and stranded turtles were also statistically different at $P<0.005$.

Plasma protein electrophoresis

Plasma protein electrophoresis values are provided in Table 3. Nesting females had significantly higher albumin values (11.5 g/l±2.9) than turtles exhibiting the other two behaviors. There was no beta-gamma bridging in any of the 71 turtles evaluated.

Toxicants

Plasma OCs and PCBs were below their LOQs in the 39 foraging, 11 nesting, and three stranded turtles tested. There was no statistical difference in arsenic levels between the 23 foraging (7.917±2.682 ppb), 12 nesting (1.823±1.313 ppb), and three stranded (3.833±2.182 ppb) turtles or for the lead levels between these same 23 foraging (0.085±0.167 ppb), 12 nesting (0.096± 0.041 ppb), and three stranded (0.050±0.000 ppb) turtles. There was a significant difference ($P<0.005$) in the

mercury levels between the different groups of turtles, with the nesting turtles having higher mercury levels (0.3 ppb, 0.25–0.8) than foraging turtles (0.25 ppb, 0.25–0.40). There was no difference in the mercury levels between the stranded turtles (0.25 ppb, 0.25–10.0) and turtles exhibiting either of the other two behaviors. There was a single stranded turtle with an exponentially higher mercury level (10 ppm) than that found in any other turtle.

DISCUSSION

In this study a number of differences in health parameters between foraging, nesting, and stranded loggerhead turtles along the coast of Georgia were noted. The overall health of the foraging and nesting turtles was rated as good based on physical exam findings and blood values obtained, although significant differences were noted between turtles exhibiting the various behaviors for many of these parameters. A number of clinically significant physical abnormalities were noted in the stranded turtles, as well as significant differences in blood parameters between the stranded turtles and those exhibiting the other two behaviors. However, because of study constraints a different laboratory was used to determine CBC and plasma biochemistry values in the stranded turtles. Other limitations of this study were the nature in which samples were collected (e.g., on a boat and beach, and at a rehabilitation facility) resulting in not all tests being performed on all turtles because of poor sample quantity and/or quality (e.g., hemolysis) for some of these turtles. Blood samples were processed within 4 hr (range 15 min–4 hr) for turtles exhibiting all the behaviors and therefore any potential influence of time on blood parameters (e.g., glucose and potassium) would be similar across behaviors.

The WBC for foraging turtles was statistically lower than for the nesting and stranded turtles using the eosinophil Unopette method, but not based on laboratory blood smear estimation. In this study, values determined by the eosinophil Unopette method and the blood smear evaluations were statistically significantly different for the stranded turtles (data not shown) and may account for the differences between turtles exhibiting different behaviors using the eosinophil Unopette method, but not the blood smear estimations.

Significant differences in the differential count between turtles exhibiting different behaviors included the increased lymphocyte count in the stranded turtles, which may indicate antigenic stimulation of turtles within this behavior. Stranded turtles also had lower monocyte counts, but the significance of this finding is unknown.

The low mean PCV for the stranded turtles supports that anemia was present in most of these turtles and likely associated with a chronic condition such as poor nutrition, a chronic infectious or parasitic disease, an immune deficiency related to their debilitated state, or a combination of some or all of these causes. Additionally, the significantly lower total protein levels in these stranded turtles also supports a debilitated state associated with malnutrition, parasitism, a protein-losing disease, or a multifactorial condition. In contrast, the nesting females had high protein levels, likely a reflection of their egg laying activity as seen in a number of reptile species (Harr et al., 2001; Campbell, 2004).

Although epibiont load in this study was determined on an ordinal scale, we did observe a significant difference between the behaviors, with >50% of the stranded turtles having a heavy epibiont load on their carapace; 26% of foraging turtles and only 7% of nesting turtles had heavy epibiont loads. The stranded turtles also had significantly lower PCV and total proteins as compared to the turtles exhibiting other behaviors although we can not make a direct association of these

TABLE 1. Hematologic values in foraging, nesting, and stranded loggerhead sea turtles (*Caretta caretta*) along the coast of Georgia.

Parameter (SI units)	Foraging[a]	Nesting[a]	Stranded[b]
Packed cell volume (l/l)			
(Mean±SD) or (Median, 10–90% quartiles)	0.32±0.05	0.30±0.04	0.19[c], 0.09–0.35
Range	0.18–0.40	0.23–0.40	0.09–0.36
(*n*)	(39)	(34)	(11)
Total solids (g/l)			
(Mean±SD)	37±12[c]	48±10[c]	20±8[c]
Range	10–60	10–66	06–29
(*n*)	(39)	(33)	(6)
Red blood cells ($\times 10^3/\mu l$)			
(Median, 10–90% quartiles)	520,000, 300,000–820,000	450,000, 298,000–704,000	130,000
Range	220,000–1,220,000	250,000–1,110,000	
(*n*)	(39)	(30)	(1)
White blood cells ($\times 10^3/\mu l$)[d]			
(Mean±SD) or (median, 10–90% quartiles)	9,005±3,536[c]	10,050, 6,690–18,620	17,900, 7,650–17,900
Range	4,000–17,400	4,000–23,950	7,650–24,450
(*n*)	(39)	(33)	(3)
White blood cells ($\times 10^3/\mu l$)[e]			
(Mean±SD)	9,022±2,081	9,658±2,881	11,042±4,505
Range	5,000–12,500	5,000–14,500	1,000–19,000
(*n*)	(39)	(18)	(12)
Heterophils ($\times 10^3/\mu l$)			
(Mean±SD)	3,677±1,365	6,629±2,830	4,766±3,795
Range	345–7,164	2,400–14,220	200–14,060
(*n*)	(39)	(18)	(11)
Lymphocytes ($\times 10^3/\mu l$)			
(Mean±SD) or (median, 10–90% quartiles)	2,725±1,150	2,298±732	4,380,[c] 912–11,700
Range	299–4,830	900–3,300	780–33,430
(*n*)	(39)	(18)	(11)
Monocytes ($\times 10^3/\mu l$)			
(Median, 10–90% quartiles)	960, 224–1,840	615, 261–1,860	0,[c] 0–150
Range	64–2,750	0–1,950	0–150
(*n*)	(39)	(18)	(11)

TABLE 1. Continued.

Parameter (SI units)	Foraging[a]	Nesting[a]	Stranded[b]
Eosinophils ($\times 10^3/\mu l$)			
(Median, 10–90% quartiles)	1,152, 448–2,100	973, 298–2,521	0, 0–360
Range	192–3,584	105–2,530	0–380
(*n*)	(39)	(18)	(11)
Basophils ($\times 10^3/\mu l$)			
(Median, 10–90% quartiles)	0	0, 0–18	0, 0–18
Range		0–180	6–70
(*n*)	(39)	(18)	(11)
Azurophils ($\times 10^3/\mu l$)			
(Median, 10–90% quartiles)	0	0, 0–18	0, 0–523
Range		0–180	0–570
(*n*)	(39)	(18)	(11)

[a] Values obtained at the University of Florida Clinical Pathology Laboratory.

[b] Values obtained at Antech Diagnostic Laboratories, Memphis, Tennessee, USA.

[c] Significant difference, $P<0.05$.

[d] White blood cell count performed in field using eosinophil Unopette system.

[e] White blood cell count performed in laboratory by absolute count.

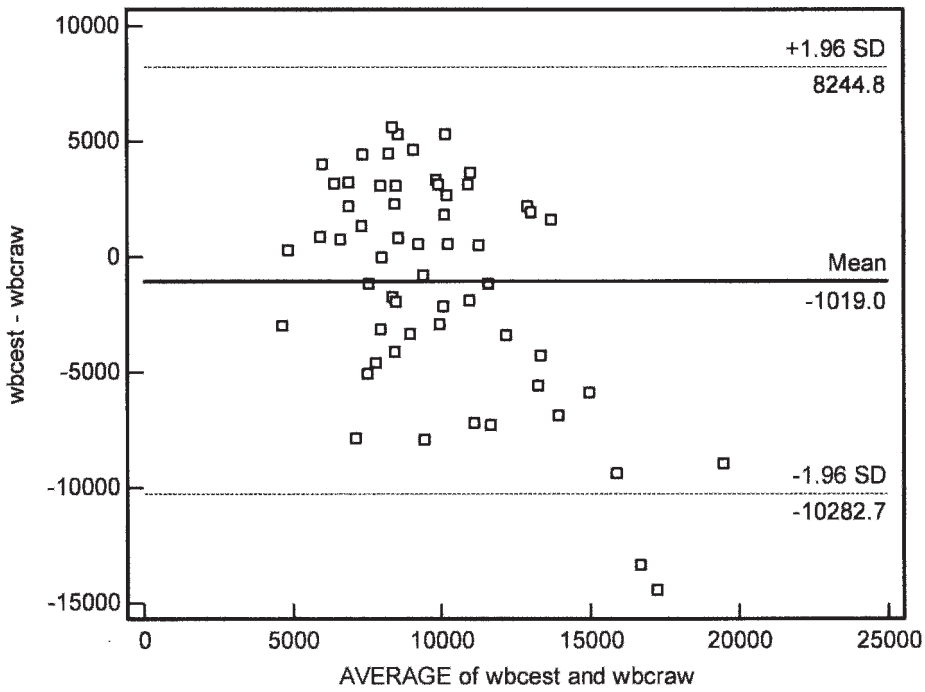

FIGURE 2. A Bland-Altman plot measuring the level of agreement between two different methods for evaluating white blood cell counts in loggerhead sea turtles (*Caretta caretta*) along the coast of Georgia.

blood parameters and epibiont loads based on our data. This finding differs from a study by Stamper et al. (2005) in which no correlation was noted between epibiont load and any blood parameters evaluated in loggerhead turtles. However, the turtles in that study were all captured free-ranging in water and thus not debilitated and live-stranded as were turtles in the stranded behavior in the present study.

Foraging turtles had the highest glucose, which is expected based on their foraging state as compared to the fasting state of most nesting females and the debilitated condition of stranded turtles. Additionally, higher potassium values were found in the foraging turtles, which may be due to decreased food intake in nesting and debilitated turtles and possible physical abnormalities (e.g., diarrhea) in stranded turtles.

Nesting turtles had significantly higher cholesterol and triglyceride values than turtles exhibiting the other two behaviors, a finding compatible with vitellogenesis and their egg-laying condition (Hamann et al., 2003). Values from this study are similar to values in nesting leatherback turtles, cholesterol (7.58 mmol/l± 1.89) and triglyceride (4.65 mmol/l± 0.40; Deem et al., 2006), but differs from previously published cholesterol (2.74 mmol/l) and triglyceride (0.98 mmol/l) values for free-ranging foraging loggerhead juvenile and adult turtles (Bolten et al., 1992), values similar to the foraging loggerheads in this study. The higher total protein, albumin, and globulin values for nesting females is also compatible with the reproductive state of turtles in this behavior (Harr et al., 2001; Campbell, 2004).

Blood urea nitrogen values differed among turtles exhibiting the three behaviors with nesting turtles having the lowest values; most likely a reflection of their fasting state. The statistically higher UA of the stranded turtles may be associated with the debilitated state of these turtles and dehydration. The CK value was

TABLE 2. Plasma biochemistry values in foraging, nesting, and stranded loggerhead sea turtles (*Caretta caretta*) along the coast of Georgia.

Parameter (SI units)	Foraging[a]	Nesting[a]	Stranded[b]
Glucose (mmol/l)			
(Mean±SD)	5.88±1.11[c]	5.33±0.83	4.50±2.1
Range	3.9–7.6	4.1–6.3	1.72–7.66
(*n*)	(38)	(25)	(13)
Sodium (mmol/l)			
(Mean±SD)	156±11	148±6	155±8
Range	135–175	139–162	146–174
(*n*)	(38)	(25)	(13)
Potassium (mmol/l)			
(Mean±SD)	5.1±2.0[c]	4.0±1.0	3.7±0.3
Range	3.3–13.9	3–5	3.3–4.4
(*n*)	(38)	(25)	(13)
Chloride (mmol/l)			
(Mean±SD)	130±11	115±4	121±6
Range	107–158	110–123	113–134
(*n*)	(16)	(8)	(9)
CO_2 (mmol/l)			
(Mean±SD) or (median, 10–90% quartiles)	15±4	14.7±8.6	31,[c] 28–36.5
Range	10–24	5.0–34.0	25–38
(*n*)	(39)	(25)	(5)
Blood Urea Nitrogen (mmol/l)			
(Median, 10–90% quartiles)	29.63,[c] 14.28–42.48	2.86,[c] 2.00–4.43	24.63,[c] 9.35–33.24
Range	0.357–38.2	1.79–4.64	9.28–33.92
(*n*)	(39)	(25)	(12)
Creatinine (μmol/l)			
(Median, 10–90% quartiles)	26.52, 17.68–53.04	8.84, 8.84–30.06	35.36, 8.84–57.46
Range	8.84–44.2	8.84–53.04	8.84–70.72
(*n*)	(39)	(25)	(6)
Total protein (g/l)			
(Mean±SD)	37±11[c]	52±5 [c]	25±9 [c]
Range	16–56	46–61	4–39
(*n*)	(39)	(25)	(13)

TABLE 2. Continued.

Parameter (SI units)	Foraging[a]	Nesting[a]	Stranded[b]
Albumin (g/l)			
(Mean±SD)	13±3[c]	17±2[c]	7±3[c]
Range	8–16	14–19	3–12
(*n*)	(12)	(8)	(13)
Globulin (g/l)			
(Mean±SD)	29±9[c]	40±7[c]	17±7[c]
Range	10–40	27–51	1–24
(*n*)	(12)	(8)	(13)
Cholesterol (mmol/l)			
(Median, 10–90% quartiles)	1.94, 1.17–4.51	6.94,[c] 4.35–8.60	2.03, 0.13–2.25
Range	1.17–5.18	4.90–8.78	0.13–5.05
(*n*)	(39)	(25)	(9)
Triglyceride (mmol/l)			
(Median, 10–90% quartiles)	0.62, 0.25–1.38	4.54,[c] 1.71–9.75	0.11, 0.11–0.25
Range	0.17–1.38	4.41–4.89	0.11–0.29
(*n*)	(39)	(25)	(5)
Calcium (mmol/l)			
(Mean±SD) or (Median, 10–90% quartiles)	1.85, 1.48–2.35	2.03±1.05	1.45±0.25
Range	1.4–2.08	0.65–3.18	1.08–1.9
(*n*)	(39)	(25)	(13)
Phosphorus (mmol/l)			
(Mean±SD)	2.07±0.36	2.23±0.58	2.33±0.39
Range	1.32–2.55	2.03–3.59	1.74–3.10
(*n*)	(39)	(25)	(13)
Uric acid (μmol/l)			
(Median, 10–90% quartiles)	41.64, 23.80–89.22	23.79, 11.90–47.58	77.32,[c] 47.58–279.56
Range	11.90–71.38	11.90–53.53	11.09–297.4
(*n*)	(39)	(25)	(13)
Alanine aminotransferase (U/l)			
(Mean±SD) or (median, 10–90% quartiles)	16±6	4.0,[c] 3–127	22±13
Range	0–29	3–30	10–44
(*n*)	(39)	(25)	(6)

TABLE 2. Continued.

Parameter (SI units)	Foraging[a]	Nesting[a]	Stranded[b]
Aspartate aminotransferase (U/l)			
(Median, 10–90% quartiles)	165, 118–256	157, 129–260	199, 138–941
Range	2–255	116–190	113–1,199
(*n*)	(39)	(25)	(13)
Lactate dehydrogenase (U/l)			
(Median, 10–90% quartiles)	572, 229–1,401	592, 255–1,141	429, 244–3,057
Range	6–1,376	22–1,172	244–3,876
(*n*)	(39)	(25)	(5)
Creatine kinase (U/l)			
(Median, 10–90% quartiles)	534, 243–1,075	359, 147–1,392	1,366,[c] 136–2,223
Range	3–1,899	81–1,627	100–26,070
(*n*)	(39)	(25)	(13)
Amylase (U/l)			
(Mean±SD)	263±99	407±131	180±106
Range	2–417	176–593	50–307
(*n*)	(39)	(25)	(5)
Lipase (U/l)			
(Median, 10–90% quartiles)	1.0, 1–9	20.0, 1–42	5.5, 1–50
Range	1–14	13–49	1–63
(*n*)	(39)	(25)	(5)
Gamma glutamyl transferase (U/l)			
(Median, 10–90% quartiles)	9.0, 5–10	8.0, 5–10	7.5, 6–9
Range	5–15	5–14	6–9
(*n*)	(39)	(25)	(5)

[a] Values obtained at the University of Miami, Department of Pathology.

[b] Values obtained at Antech Diagnostic Laboratories, Memphis, Tennessee, USA.

[c] Significant difference, $P<0.05$.

TABLE 3. Plasma protein fractions identified in foraging, nesting, and stranded loggerhead sea turtles (*Caretta caretta*) along the coast of Georgia, USA.

Parameter (SI units)	Foraging	Nesting	Stranded
Pre-albumin (g/L)			
(Median, 10–90% quartiles)	0.0, 0–0.1	0.8, 0–0.11	0
Range	0.00–1.0	0.00–1.2	
(n)	(39)	(24)	(8)
Albumin (g/L)			
(Mean ± SD)	7.9±2.6	11.5±2.9[a]	6.3±3.7
Range	3.1–12.4	10.4–18.3	2.3–12.0
(n)	(39)	(24)	(8)
Alpha-1 (g/L)			
(Mean ± SD)	1.4±0.6	1.5±0.6	0.9±0.3
Range	0.5–2.5	0.5–2.7	0.3–1.3
(n)	(39)	(24)	(8)
Alpha-2 (g/L)			
(Median, 10–90% quartiles)	1.2, 0.5–2.5	2.3, 1.5–6.5	1.5, 0.9–2.7
Range	0.5–3.1	1.1–10.0	0.9–3.3
(n)	(39)	(24)	(8)
Beta (g/L)			
(Mean ± SD)	9.9±2.8	17.1±5.2	7.4±4.5
Range	4.5–14.6	8.7–25.9	2.2–15.8
(n)	(39)	(24)	(8)
Gamma (g/L)			
(Mean ± SD)	15.7±7.4	11.8±3.7	9.5±3.6
Range	5.1–28.8	7.4–21.6	4.7–15.7
(n)	(39)	(24)	(8)

[a] Significant difference $P<0.05$.

significantly higher in the stranded turtles as compared to the other turtles, consistent with muscle injury and wasting noted in a number of these debilitated turtles. Additionally, the higher CO_2 value may be a reflection of decreased respiratory ventilation in the stranded, debilitated turtles.

The only significant difference in plasma protein electrophoresis between the turtles was a higher albumin value for nesting females. There was no beta-gamma bridging in any of the loggerhead turtles in our study, which differs from a study in which bridging was found in 28% of 41 clinically normal loggerhead sea turtles tested from Florida waters (Gicking et al., 2004). Beta-gamma bridging is often associated with chronic disease or parasites (Gicking et al., 2004) so it is interesting to note the absence of this finding in all of the turtles in this study, especially those in the stranded behavior. Additionally, we noted a discrepancy within behaviors in protein values obtained using the three methods-refractometer and chemistry analyzer (total proteins), and chemisty analyzer and SPEP-II agarose gels (albumin)-which is consistent with findings for other species (Lumeij et al., 1990). This discrepancy should be taken into consideration when one is comparing the results between studies.

A number of studies have examined loggerhead turtles for PCBs, OC insecticides, and metals (Gordon et al., 1998; Sakai et al., 2000; Day et al., 2005; Gardner et al., 2003; Franzellitti et al., 2004; Keller et al., 2004a, b; Storelli et al., 2005). These studies have measured either PCBs and OCs or metals, but not both. Additionally, most studies have involved assessment of tissues from dead animals. Thus, there are a limited number of studies in which direct comparisons to the data from this study can be made.

There is little information regarding whole blood mercury, lead, and arsenic concentrations in sea turtles generally and loggerhead turtles specifically. The mean whole blood lead and total mercury concentrations from 106 Kemp's ridley sea turtles were 11 ppb and 18 ppb, respectively (Kenyon et al., 2001). The mean whole blood mercury concentrations for 34 live captured and 6 stranded loggerhead turtles was 29 ppb and 99 ppb, respectively (Day et al., 2005). The detected concentrations in the current study are below these levels, with nesting turtles having higher mercury concentrations than turtles exhibiting the other two behaviors.

The mean concentration of total PCBs in the plasma of 12 juvenile loggerhead sea turtles off the coast of North Carolina was 7.13 ppb (±4.94 ppb), below the LOQ in our study (Keller et al., 2004a, b). Although such concentrations are low, a variety of adverse health effects have been postulated (Keller et al., 2004a, b) and thus a lower LOQ than that used in the current study is recommended for future studies.

Although a few limitations exist with the present study, we provide baseline blood values for two cohorts (foraging and nesting) of loggerhead sea turtles on the coast of Georgia. Additionally, we demonstrate significant differences in clinical findings and blood parameters between foraging, nesting, and stranded loggerhead turtles in the region.

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