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BASELINE CORTICOSTERONE, HEMATOLOGY, AND BIOCHEMISTRY RESULTS AND CORRELATIONS TO REPRODUCTIVE SUCCESS IN NESTING LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*)

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Abstract: Characterizing the health status and reproductive success of wild populations of sea turtles can be difficult; however, obtaining data to do this can provide important insight into the stability and long-term success of a population. This study examined the use of baseline corticosterone to assess reproductive success of a population of nesting loggerhead sea turtles (*Caretta caretta*) on Jekyll Island, Georgia and investigated hematological and biochemical trends in this population. A total of 37 nesting loggerhead sea turtles was sampled for this study. Eleven (29.7%) turtles were sampled in 2013 and 26 (70.3%) were sampled in 2014. A majority of the turtles sampled successfully nested (29/37, 78.4%; false crawls: 8/37, 21.6%). There was no significant correlation between baseline corticosterone concentrations and reproductive success (hatch success or emergence success) in this population. There was a significant positive correlation ($r = 0.461$, $P = 0.02$) between corticosterone concentrations and absolute monocyte counts. There was a significant negative correlation between monocyte count and hatch success ($r = -0.464$, $P = 0.05$) and a positive correlation between phosphorus and hatch success ($r = 0.405$, $P = 0.05$). Calcium concentrations were significantly different ($P = 0.01$) between animals that false crawled and those that nested, with nesting turtles having lower calcium concentrations than those that false crawled. Turtles that false crawled were significantly ($P = 0.008$) more likely to have elevated potassium concentrations than turtles that nested. This study provides the first attempt at characterizing baseline corticosterone, hematology, and biochemistry data and correlations with reproductive success in nesting loggerhead sea turtles. Overall, loggerhead sea turtles capable of nesting were found to be in good health and have good reproductive success while maintaining low levels of corticosterone during reproductive activities.

Key words: *Caretta caretta*, corticosterone, health, reproductive success, sea turtle, stress.

INTRODUCTION

Marine turtle species are considered endangered worldwide, and face many challenges, leading to an increased vulnerability for long-term species survival.[27] Conservation threats to sea turtles include beachfront development, infectious diseases, pollution from chemical toxicants, ingestion of marine debris, and traumatic injuries from entanglement related to recreational and commercial fisheries activities.[12] Determining how these animals cope with environmental stressors is important, and several physiologic responses to a stressor, including adrenocortical, hematologic, and biochemical parameters, are measurable.[1]

Corticosterone evaluation is valuable for many reasons, as it is an indicator of exposure to stressors in vertebrate species.[11,15–17,25,34,37,38] Secretion of this hormone allows the body to adapt to stressors to maintain homeostasis.[1,21,23,24] In wildlife populations, stressors can come in a variety of forms, and the stress response often includes both physiologic and behavioral modifications aimed at adapting to that stressor.[1,20,22,24,37]

Evaluating hormonal responses to stress in reptiles relies on first acquiring baseline corticosterone concentrations for a given physiologic state, which has previously been accomplished with loggerhead sea turtles (*Caretta caretta*) in this Jekyll Island, Georgia nesting population.[13] Although the study of corticosterone during the nesting process has been undertaken in various

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species of sea turtles,[2,32–34] it is currently unknown how the steroid hormone corticosterone may facilitate reproductive commitment and hatch success in nesting loggerhead turtles.

Hematology and biochemistry analyses are important tools that can provide information regarding the physiologic condition of an individual animal as well as a population of animals. It has been suggested that the health of nesting sea turtles affects their reproductive output and that maternal health and energy stores may affect the survival of their offspring.[29] Although a previous study has shown a correlation between maternal hematologic and biochemical results and reproductive success in another species of sea turtle,[29] this has not yet been attempted in nesting loggerhead sea turtles.

The primary goal of this study was to determine whether baseline corticosterone, hematology, and biochemistry data could be used to assess the health status and reproductive success (ie, hatch success and emergence success) of nesting loggerhead sea turtles. The biological hypothesis tested in this study was that corticosterone concentrations and hematologic and biochemical results would not be directly correlated to metrics associated with reproductive success.

MATERIALS AND METHODS

This cross-sectional study was approved by the University of Illinois Institutional Animal Care and Use Committee (protocols #13-072, 13-073). The study subjects represented a population of nesting loggerhead sea turtles being monitored by the Jekyll Island Authority's Georgia Sea Turtle Center (GSTC) on Jekyll Island, Georgia. The GSTC is a state-of-the-art rehabilitation, education, and research center for sea turtles and other local wildlife. From May to early August, the GSTC staff has a nightly monitoring program for loggerhead sea turtles that nest on the beaches of Jekyll Island. Sample size for this study was determined using the following a priori information: an expected difference in corticosterone concentrations between animals that are reproductively successful and those that are not of at least 0.2 ng/ml (SD: 0.25), a power = 0.8, and $\alpha = 0.05$.

Sampling of nesting turtles

Sample collection from nesting loggerhead sea turtles began once a turtle laid a minimum of five eggs, or if a sea turtle was encountered on a false crawl. A false crawl is defined as a crawl resulting from an abandoned nesting attempt (ie, a nonnesting crawl). Confirmation of a false crawl was made by following the tracks of the animal and confirming that no nest was present. The false crawls encountered in this study were turtles identified as already heading back to the ocean after their nesting attempt was previously aborted. These animals were restrained for sampling by standing in front of them to impede their progress. No restraint was required for nesting turtles, although head placement was occasionally adjusted to obtain better positioning for blood sample acquisition. A blood sample was collected from the dorsal cervical sinus using a sodium-heparinized 6-ml syringe fitted to a 20-ga 1.5-inch needle immediately after the encounter. All samples were drawn within 3 min of the initial hands-on time to ensure that baseline corticosterone concentrations were obtained.[13] The venipuncture site was cleaned using 70% ethyl alcohol. Blood samples were stored on frozen gel packs until being transported to the GSTC for processing (6–12 hr).

Once the blood samples were collected, each turtle was identified using flipper tags and a passive integrated transponder (PIT) tag. If the turtle did not have previous identification, a flipper tag was placed on each front flipper and a PIT tag was placed subcutaneously in the right shoulder region. Morphometrics for each turtle were also collected, including notch-to-tip curved carapace length (NTT CCL), NTT straight carapacial length (SCL), notch-to-notch (NTN) CCL, NTN SCL, curved carapace width (CCW), and straight carapace width (SCW).

Nest inventory

Eggs hatch and hatchlings emerge from nests on Jekyll Island, Georgia annually from late July to October. After emergence, nests from the study females were excavated and inventoried to determine hatching success and emergence success. Hatching success was defined as no. of hatched eggs/no. of total eggs (hatched eggs + unhatched eggs). Emergence success was defined as no. of hatched eggs − (no. of live hatchlings in nest + no. of dead hatchlings)/no. of total eggs (hatched eggs + unhatched eggs). Any depredated, inundated, or washed-out nests were noted and not included in statistical analysis.

Hematologic and biochemical testing

Blood smears for the complete blood counts (CBC) were made within 6–12 hr of sample

collection. Packed cell volume was determined using a microhematocrit centrifuge (TRIAC Centrifuge, Clay-Adams, Parsippany, New Jersey 07054, USA) and total solids were determined using a portable refractometer (Jorgenson Labs, Inc., Loveland, Colorado 80538, USA). Air-dried blood-smeared slides were stained with modified Wright–Giemsa stain (HemaTek Stain Pak, Bayer Corporation, Elkhart, Indiana 46514, USA) and placed in dry storage boxes. White blood cell (WBC) estimates and differentials were performed manually by the same individual using standard techniques.[14] Briefly, an estimated WBC count was obtained by counting the number of WBCs in 10 fields at ×400 magnification, dividing that total number by 10, and multiplying the average by 2,000. WBC identification for differential counts was confirmed using previously described cellular morphology for loggerhead sea turtles.[9] Whole blood was centrifuged for 10 min at 1,500 *g*. The plasma was removed and frozen in a cryovial (Nalge International, Rochester, New York 14625, USA) at −80°C until being analyzed for corticosterone and plasma biochemistries.

Plasma samples for biochemistry analysis were transported on dry ice to the University of Illinois (Urbana, Illinois 61801, USA). Plasma biochemistries were processed within 6 mo of collection using a portable chemistry analyzer (VetScan, Abaxis Inc., Union City, California 94587, USA). Previous studies have shown that there can be some variation in stability of plasma samples used for biochemical analysis.[36] To minimize this risk, all samples were run at the same time to limit the likelihood of biasing the results. The following values were measured using an avian/reptile rotor (Abaxis Inc.): glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine kinase (CK), total protein, albumin, globulin, calcium, phosphorus, sodium, potassium, and uric acid. Blood urea nitrogen (BUN) was not analyzed since it was not included on the biochemistry rotor utilized in this study.

Corticosterone analysis

Plasma samples for corticosterone analysis were transported on dry ice to the Department of Biology, Tufts University (Medford, Massachusetts 02155, USA). Corticosterone samples were processed within 6 mo of being collected. Previous studies have shown that corticosterone concentrations in various sample types remain stable for multiple years while frozen at −20°C.[18] The radioimmunoassay used to measure corticosterone has been described in detail previously.[36] Briefly, samples were supplemented with 2,000 counts per minute of tritiated corticosterone for later recovery analysis and allowed to equilibrate. Corticosterone was extracted from the protein component of the plasma using redistilled dichloromethane and dried using nitrogen gas. Samples were then reconstituted in phosphate-buffered saline, with an aliquot used to assess recovery. Samples were assayed in duplicate using tritiated corticosterone and a corticosterone antibody (B3-163, Esoterix, Calabasas Hills, California 91301, USA). Activated charcoal was used to separate the unbound from the bound steroids. The bound-to-unbound ratio was fitted to a standard curve and corrected with the recovery percentage and the original amount of plasma to determine the corticosterone concentration for each sample. All samples were measured in a single assay with an intra-assay variability of 2.0% and a detectability of 0.098 ng/ml. The assay was tested for parallelism and accuracy and validated for this species using standard endocrinology quality-control measures.

Statistical analysis

The distribution of the continuous data was evaluated using the Shapiro–Wilk test, skewness, kurtosis, and *q-q* plots. Normally distributed data are reported by the mean, SD, and minimum–maximum values (min–max), whereas nonnormally distributed data are reported by the median, 10–90 percentiles (%), and min–max. Data that were not normally distributed were log transformed for parametric testing. Independent-sample *t*-tests (univariate analysis) were used to determine if there were any differences in physical measurements or corticosterone, hematology, or biochemistry results between sampling years. Independent-sample *t*-tests were also used to determine if there were any differences in the corticosterone, hematology, or biochemistry results or hatching or emergence success by whether animals false crawled. The Levene test for equality of variances was used to evaluate the variability of the data. Mixed general linear models were used to perform multivariate analysis for the different outcome data (corticosterone, hematology, and biochemistry results) for independent variables that had $P < 0.10$. The mixed models were also used to assess the subset of the population that had multiple samples. If the mixed models were not significant, the univariate results were reported. Pearson's correlation tests

Table 1. Physical measurements of loggerhead sea turtles (*Caretta caretta*) sampled on Jekyll Island ($n = 37$).

Parameter	Mean	SD	Min–max
Notch-to-tip straight carapace length (NTT SCL) (cm)	92.6	5.5	83.7–104.4
NTT curved carapace length (CCL) (cm)	100.2	7.3	82.3–114.0
Notch-to-notch (NTN) SCL (cm)	90.8	5.5	82.5–102.5
NTN CCL (cm)	99.0	7.2	85.2–113.0
Straight carapace width (cm)	70.7	5.8	60.6–86.3
Curved carapace width (cm)	90.6	7.3	67.2–99.5

were used to determine if corticosterone concentrations, physical measurements, hematology and biochemistry results, and reproductive outcomes were correlated. A correction was not applied for these comparisons because the authors were more concerned for a type II error than a type I error because of the limited sample size. A $P \leq 0.05$ was used to determine statistical significance. SPSS 22.0 (IBM Corp., Armonk, New York 10504, USA) was used to analyze the data.

RESULTS

A total of 37 nesting turtles was sampled for this study. There were 11 (29.7%) turtles sampled in 2013 and 26 (70.3%) sampled in 2014. A majority of the turtles successfully nested (29/37, 78.4%; false crawls: 8/37, 21.6%). The majority (35/37, 94.5%) of turtles were sampled when there was no precipitation. Artificial light was an uncommon finding (3/37, 8.1%) on the nesting beach locations where the turtles were sampled.

The physical measurements of the turtles are reported in Table 1. There were no significant differences in the physical measurements between the sampling years (NTT SCL: $t = -1.15$, $P = 0.26$; NTT CCL: $t = 0.36$, $P = 0.72$; SCW: $t = -0.26$, $P = 0.79$; CCW: $t = 0.25$, $P = 0.80$; NTN CCL and NTN SCL were not recorded in 2013). Corticosterone concentrations were not correlated to any of the physical measurements (NTT SCL: $r = -0.10$, $P = 0.58$; NTT CCL: $r = 0.04$, $P = 0.81$; NTN SCL: $r = 0.148$, $P = 0.52$; NTN CCL: $r = 0.07$, $P = 0.74$; SCW: $r = 0.04$, $P = 0.82$; CCW: $r = 0.14$, $P = 0.44$).

Table 2. Reproductive results for loggerhead sea turtles (*Caretta caretta*) on Jekyll Island 2013–2014.

Parameter	Median	10–90%	Min–max
Emergence success[a]	70.0	4.1–94.7	0–97.3
Hatch success[b]	75.8	16.1–96.6	4.4–99.2

[a] No. of hatched eggs – (no. of live hatchlings in nest + no. of dead hatchlings)/no. of total eggs (hatched eggs + unhatched eggs).

[b] No. of hatched eggs/no. of total eggs (hatched eggs + unhatched eggs).

There was no significant correlation between corticosterone concentrations and hatch success ($r = -0.179$, $P = 0.42$), or emergence success ($r = -0.072$, $P = 0.75$). There was a significant positive correlation ($r = 0.461$, $P = 0.02$) between corticosterone concentrations and absolute monocyte counts. Although there was no significant difference in corticosterone concentrations and CK, α did approach significance ($r = 0.35$, $P = 0.068$). The power for that comparison was 0.28. There were no significant correlations found between corticosterone and any other hematology or biochemistry data (all $P > 0.1$).

There was no significant difference ($t = -0.719$, $P = 0.48$) in the corticosterone concentrations between turtles that false crawled ($n = 8$; median: 1.35, 10–90%: 0.41–1.84, min–max: 0.41–2.75) and those that nested ($n = 29$; median: 1.01, 10–90%: 0.16–3.50, min–max: 0.08–4.98). There was no significant difference in the corticosterone concentrations by sampling year ($t = 1.036$, $P = 0.31$) or by presence/absence of artificial light during the nesting process ($t = -0.385$, $P = 0.70$).

Results for hatch success and emergence success are listed in Table 2. There was a significant negative correlation noted between absolute monocyte count and hatch success ($r = -0.464$, $P = 0.05$) and a positive correlation between phosphorus and hatch success ($r = 0.405$, $P = 0.054$). Although there was no significant difference in CK activity and hatch success, α did approach significance ($r = 0.32$, $P = 0.082$). The power for that comparison was 0.36. No other significant correlations were identified between hatching or emergence success and hematology and biochemistry results ($P > 0.20$).

Results for hematology and biochemistry data are found in Tables 3 and 4. Calcium concentrations were significantly different ($t = -2.7$, $P = 0.011$) between animals that false crawled and those that nested, with nesting turtles having lower calcium concentrations (mean: 10.02, SD:

Table 3. Normally distributed hematology and biochemistry results from loggerhead sea turtles (*Caretta caretta*) ($n = 37$).

Parameter	Mean	SD	Min–max
Packed cell volume (%)	33.9	5.3	25.00–44.0
Total solids (g/dl)	5.2	0.7	3.80–6.60
White blood cells ($\times 10^3/\mu l$)	5.1	2.3	2.0–9.50
Heterophils (%)	60.2	8.2	49.0–84.0
Lymphocytes (%)	25.0	8.4	10.0–40.0
Glucose (mg/dl)	93.0	22.4	60.0–137.0
Calcium (mg/dl)			
Nesting ($n = 29$)	10.02[a]	3.97	4.0–20.0
False-crawl ($n = 8$)	13.92[a]	4.45	7.60–20.0
Total protein (g/dl)	4.7	0.9	3.4–7.2
Globulin (g/dl)	3.0	0.7	2.0–4.8
Sodium (mEq/l)	151.5	12.4	128.0–180.0

[a] $P = 0.011$.

3.97, min–max: 4.0–20.0) than those that false crawled (mean: 13.92, SD: 4.45, min–max: 7.60–20.0). Potassium concentrations in turtles that false crawled (median: 5.4, 10–90%: 4.2–6.5, min–max: 4.2–7.9) were significantly higher ($t = -2.8$, $P = 0.008$) than in turtles that nested (median: 4.1, 10–90%: 3.3–5.6, min–max: 3.2–6.1). There were no significant differences in any other hematology or biochemistry parameters on the basis of false crawls or nesting (all $P > 0.12$). There were no significant differences in the hematology or biochemistry parameters or reproductive outcomes on the basis of whether or not the turtles had obvious signs of trauma (all $P > 0.22$).

DISCUSSION

Consistent with our first hypothesis, there was no significant correlation between corticosterone concentrations and reproductive success noted in this population of loggerhead sea turtles. During reproduction, vertebrates exhibit varied behavioral and physiologic strategies to promote reproductive success. Adrenocortical modulation, or the ability to down-regulate the acute stress response, appears to be utilized in some vertebrates during periods of reproductive activity.[31] Previous studies have suggested that the ability of nesting sea turtles to produce corticosterone in response to a stressor is minimized because of their need to utilize all available energy sources for reproductive purposes.[20–22] Studies in other reptilian species, including tuataras (*Sphenodon punctatus*) and New Zealand common geckos (*Hoplodactylus maculatus*), have demonstrated similar findings with gravid females modulating corticosterone secretion during nesting to maintain homeostasis, effectively increasing chances of reproductive success and promoting overall fitness.[4,30] Perrault et al[29] also showed that there was no significant effect of elevated corticosterone on reproductive success in leatherback sea turtles (*Dermochelys coriacea*), or on the morphological measurements, growth, or sprint speed of the offspring. Our study found similar results, in that corticosterone was not a good predictor of reproductive success in the Jekyll Island population of loggerhead sea turtles.

The reported reproductive success (ie, hatch success, emergence success; Table 2) in this sample population of loggerhead sea turtles for the 2013 and 2014 nesting seasons appears consistent with previous trends on Jekyll Island, Georgia (T. Norton, unpubl. data). These results are also consistent with studies in other populations of loggerhead sea turtles where hatch success and emergence success were reportedly

Table 4. Nonnormally distributed hematology and biochemistry results from loggerhead sea turtles (*Caretta caretta*) ($n = 37$).

Parameter	Median	10–90%	Min–max
Monocytes (%)	2.0	0.0–7.8	0–14.0
Eosinophils (%)	9.0	3.4–21.2	3.0–36.0
Basophils (%)	0.1	0.0–1.0	0.0–1.0
Aspartate aminotransferase (IU/L)	141.0	98.0–232.4	86.0–249.0
Creatinine kinase (IU/L)	390.5	171.0–2,380.5	125.0–5,725.0
Uric acid (mg/dl)	0.5	0.3–1.4	0.30–1.70
Phosphorus (mg/dl)	9.7	7.5–14.5	7.0–15.1
Albumin (g/dl)	1.6	1.2–2.5	1.1–3.0
Potassium (mEq/L)	4.0	3.3–5.9	3.2–7.9
Nested	4.1[a]	3.3–4.6	3.2–6.1
False-crawl	5.4[a]	4.2–6.5	4.2–7.9

[a] $P = 0.008$.

similar.[6] Other species of sea turtles, including leatherbacks, have had hatch and emergence successes much lower than loggerhead turtles, with an average global hatch success of approximately 50%.[6] Because of the challenges faced by sea turtle populations, continued monitoring for reproductive trends is an important aspect of conservation and management strategies.

Previous studies have established tissue enzyme activities of biochemical analytes specific to the loggerhead sea turtle[3] and several others have investigated reference values for hematology and biochemistry results.[5,8,10,13,26] Overall, the general trends for hematology and biochemistry results observed in this study were consistent with previous studies focused on adult nesting loggerhead turtles as well as free-living loggerhead turtles.[5,8] However, as expected, phosphorus, calcium, and protein concentrations were higher in this nesting population when compared with a nonnesting, free-living, reference population.[5] In addition, one noteworthy exception is that the mean sodium range in our study was overall higher than concentrations reported in a similar study using the same biochemistry analyzer in free-living loggerhead turtles.[5] This finding is more likely attributable to a few individual turtles that were hypernatremic, as opposed to a general trend toward hypernatremia in the entire sample population. It is also important to note that our study focused on a reproductively active population of nesting turtles, whereas this comparison study population was free living and not actively reproducing at the time of sampling. This difference in study environments (encountered nesting on land vs free living) and physiologic stage (reproductive vs nonreproductive) could have contributed to the difference in sodium ranges observed.

There were few significant correlations found between corticosterone and any other hematology or biochemistry results. This finding was not unexpected, as all samples from this study were obtained from apparently healthy nesting females within 3 min of handling. Sampling of the study population by 3 min allowed for acquisition of baseline corticosterone concentrations as determined in a previous study.[13] This previous study did find a subtle yet significant increase in WBC counts over time, but it occurred as a result of handling stress after the 3-min sampling time.[13] The current study and the previously mentioned study[13] both showed that the sea turtles represented in this nesting population are apparently healthy, and that animals capable of nesting have corticosterone, hematology, and biochemistry results within acceptable ranges.[8,10,12,13] It is important to note that one of the limitations of this study was that sampling only occurred in sea turtles that came to the beach to nest. It is possible that animals that did not approach the beach could have had abnormal corticosterone, hematology, or biochemistry values, and thus were not fit to complete their reproductive cycle and nest. In addition, it is important to note that BUN was not analyzed in this study, but could have provided additional insight into the health status and nesting cycle in this population.

There was a significant positive correlation between corticosterone concentrations and absolute monocyte counts. Monocytes generally occur in low numbers in the blood of healthy reptiles and are thought to be either an indicator of chronic disease or part of a stress leukogram.[7] Although the nesting loggerhead sea turtles sampled in this study were classified as apparently healthy on the basis of physical examination findings and apparently normal CBC and biochemistry results, it is possible that the females exhibiting higher absolute monocyte counts were subclinically affected by a chronic inflammatory process that correspondingly led to elevations in systemic stress (ie, corticosterone concentrations). Since the increase in monocytes in these animals was mild, with seemingly minimal biological significance, further study with an increased sample size would be warranted to further elucidate the correlation between monocytes and corticosterone in this species.

Turtles that false crawled had higher potassium concentrations than turtles that nested. In addition, this study population of nesting loggerhead turtles overall had a higher potassium concentration when compared with two other similar study populations.[5,8] Hyperkalemia can result from a variety of pathologies including decreased renal secretion of potassium, excessive dietary potassium intake, or severe acidosis.[7] In a recent study on foraging, nesting, and stranded loggerhead sea turtles, higher potassium values were found in the foraging turtle population, and this difference was attributed to decreased food intake and physical abnormalities in the other two turtle populations.[12] In the current study, turtles that false crawled aborted the nesting process before ovipositioning. Because of this difference in the phase of the reproductive cycle for false-crawl turtles (ie, individuals may have not nested yet for the year), they may be closer physiologically to their foraging state, and thus may still have higher

potassium as seen during foraging. The cause of a false crawl can be multifactorial (ie, inadequate nesting environment, disorienting artificial light exposure, turtle fatigue), and it is possible that the higher potassium concentrations found in this study population of false-crawl turtles may have contributed the turtle's inability to complete the nesting process during that attempt. Although hyperkalemia is often associated with weakness and lethargy, the turtles in this study may have had reduced ability for muscle recruitment to complete the nesting process. Alternatively, it is possible that the elevated potassium concentrations observed in false-crawl turtles were a direct result of the increased crawling activity or the result of hemolysis or muscle damage due to the increased challenge of venipuncture on a nonnesting turtle. A recent study in leatherback sea turtles described a mild to moderate increase in potassium during ocean capture events[19] and it is possible that the turtles sampled as part of this study were showing similar exertion patterns on biochemical analysis during false-crawl events.

Higher plasma calcium concentrations were observed in female sea turtles that were encountered throughout the nesting season but did not initiate the nesting process (ie, false crawled). In addition, this study population of nesting loggerhead turtles overall had a higher calcium concentration when compared with two other similar study populations.[5,8] During the sampling period for this study, female loggerhead sea turtles were encountered in various phases of their reproductive cycle, with some females having already oviposited one or more nests and others not having nested for the season. In contrast, previous studies in other species of sea turtles have reported minimal changes in maternal calcium concentrations throughout the nesting cycle, or a gradual decline.[29,32] These consistent calcium concentrations support the theory that vitellogenesis is complete before the arrival of the female at the nesting beach.[32] If calcium concentrations are still fluctuating in false-crawl females, this may be a contributing factor to their inability to complete the nesting process.

Higher plasma phosphorus concentrations were associated with an increase in hatch success. Sea turtle hatch success is affected by multiple factors, some of which include environmental factors such as habitat zone, the temperature during incubation, and water inundation. In addition, previous studies have shown that maternal physiologic parameters such as calcium and phosphorus concentrations can play important roles in both embryo development and hatching success in various species.[29,35] Since there is no posthatching parental care in sea turtles, the embryos must survive by relying on the nutritional energy reserves provided by the mother.[29] In poultry operations, breeder diets are focused on energy and nutrients to optimize egg production and maximize chick numbers. The embryonic bone mineralization process requires phosphorus that is derived from the yolk during embryo development.[28,35] Once an embryo enters the growth phase, stores of both calcium and phosphorus are rapidly depleted from the yolk and this decline in yolk phosphorus continues until hatching.[28,35] Although maternal calcium concentrations have been reported to remain relatively consistent throughout the nesting cycle in other sea turtle species,[32] this may not be true for phosphorus. Therefore, it is possible that reproductively active female loggerhead turtles with higher plasma phosphorus concentrations have the potential to produce stronger and more efficient offspring with increased hatch success.

There was a significant negative correlation noted between absolute monocyte count and hatch success. Previous studies in reproductively active sea turtles have shown a correlation between maternal health status and reproductive success.[29] This previously mentioned study[29] also showed that as a nesting season progressed, nesting females become more stressed and fatigued, resulting in a stress leukogram (ie, heterophilia, lymphopenia, and monocytosis). It could be suggested that this chronic stress and fatigue could lead to a decline in reproductive success, including hatch success. It is currently unknown if fatigue, stress, infection, or alterations in maternal immune function affect the nutrient composition of the egg. If these parameters do in fact alter the nutrient quality of the egg (eg, albumin), then the reduced hatch success in this case may be a direct result of the limited nutritional quality provided to the offspring in a stressed or unhealthy female. Alternatively, it is possible that the females exhibiting higher monocyte counts were subclinically affected by a chronic inflammatory process or immune disorder that correspondingly transferred to their offspring, leading to a reduction in reproductive success in those individuals.

CONCLUSIONS

This study provides baseline corticosterone, hematology, and biochemistry data and correlations with reproductive success in nesting logger-

head sea turtles. Overall, loggerhead sea turtles capable of nesting were found to be in good health and have good reproductive success. The sea turtles sampled in this study maintained low levels of corticosterone during reproductive activities, allowing optimal reproductive success, despite any unknown previous stressors. If this population were to face health or reproductive challenges in future years, these results can be used as a comparison for attempting to determine causation and assist in future investigations into health status and reproductive success in this species.

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