

BLOOD BIOCHEMISTRY REFERENCE VALUES FOR WILD JUVENILE LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) FROM MADEIRA ARCHIPELAGO

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ABSTRACT: Standard biochemical parameters were determined in wild juvenile loggerhead sea turtles *Caretta caretta* living offshore Madeira Island, northeast Atlantic. We analyzed the influence of age, sex, sea surface temperature, and body condition index on biochemical parameters including uric acid, total bilirubin, total cholesterol, creatinine kinase (CK), glucose, total protein, urea nitrogen, lactate dehydrogenase, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), albumin, alkaline phosphatase (ALP), sodium (NA), potassium (K), chloride, calcium, phosphorus, and magnesium. Significant positive correlations were found between turtle body size and total cholesterol, total protein, and albumin. Total protein and the enzymes AST and CK were lower than reported levels in adults. Calcium levels were lower than those reported in adult or captive turtles, but similar to wild juveniles from Australian waters, and were interpreted as normal for this age category. These data may be useful to evaluate the health status of stranded or injured animals and to improve veterinary care at rehabilitation centers.

Key words: Blood biochemistry, *Caretta caretta*, juvenile pelagic stage, loggerhead sea turtle.

INTRODUCTION

Marine turtle species are considered endangered worldwide, with six of the seven species listed in threatened categories, the exception being the Australian flatback *Natator depressus*, which is listed as 'Data Deficient' (Polidoro et al., 2008). All species are impacted by human activities at every stage of their life cycle. Loggerheads observed around Madeira and Azores Archipelagos (Portugal) are small juveniles originating mainly from the southeastern nesting beaches in the USA (Bolten et al., 1998); they spend their first 6–12-yr-long pelagic life stage in oceanic foraging habitats (Bjorndal et al., 2000b, 2003) before migrating to neritic foraging habitats back on the western Atlantic. Therefore, they are considered visitors in these archipelagos where they forage and grow during the juvenile pelagic phase (Bolten, 2003; Oliveira et al., 2005).

Although research and conservation efforts have produced a large body of literature on loggerhead blood parameters (Frair and Shah, 1982; Lutz and Dunbar-Cooper, 1987; Bolten et al., 1992, 1994), data on biochemical standard parameters for wild young individuals are scarce and recognized as necessary for different life stages or cohorts within a population (Deem et al., 2009). Two studies have been published on a similar pelagic population in the Canary Islands (Casal et al., 2009) but used rehabilitated turtles. More recently, Flint et al. (2010) performed a complete study in wild juvenile turtles from Australian waters.

The juvenile pelagic loggerhead population has been monitored on Madeira Island since 1994 (Dellinger, 2007), and includes very young turtles, which provides a good framework to build a reference for several health parameters. We provide a population baseline profile

for blood biochemical parameters in juvenile loggerhead turtles from the North Atlantic. Additionally, we tested for correlations between these values and the individual's size (age), body condition index (BCI), and sea surface temperature (SST).

MATERIALS AND METHODS

Twenty-seven juvenile wild loggerhead sea turtles were captured south of Madeira Island, northeast Atlantic (32°32'N, 16°59'W) between May and July 2006 using a scoop net (Dellinger et al., 1997) and brought into land-based facilities. They were clinically examined and acclimatized overnight in tanks filled with seawater (volume 75–1,000 l depending on the turtle's size). The tanks were kept at room temperature in a quiet area. Food was not provided and turtles were released unharmed after sampling, usually within 24 hr.

Blood samples were drawn early in the morning after capture, before any other manipulation, and were performed 12–18 hr after capture to avoid possible effects of acute handling stress (Gregory et al., 1996). Up to 4 ml were collected from the dorsal post-occipital sinus into BD Vacutainers™ (Preanalytical Solutions, Bellerive Industrial Estate, Plymouth, UK). Samples were clotted for 20–30 min and the serum decanted was removed into cryogenic vials and immediately frozen at –20 C. Serum analyses were performed 2–4 wk after blood samplings at the Veterinary and Food Safety Laboratory using an auto-analyzer (Spotchem EZ SP 4430, Arkray Factory Shanghai, Inc., Pudong, Shanghai, China), and the multiparametric analyzer Spotlyte (Menarini Diagnostics, Lisbon, Portugal) was used for ions. Seventeen biochemical parameters were evaluated (Table 1). Standard biometric procedures followed Bolten (1999); straight carapace length until notch SCL(nt), was used as body size indicator. Each turtle was tagged with a passive integrated transponder (PET) tag (Avid Technology, Inc., New York, New York, USA) in the right front flipper. A body condition index (body mass/SCL³; Bjørndal et al., 2000a) was calculated to evaluate the size vs. mass relationship. Sex determination was done through laparoscopy (Delgado et al., 2010). Sea surface temperature data were provided by the Harbor Administration Funchal, Madeira Island, Portugal.

Data were statistically analyzed with the SPSS 15.0 software package (IBM SPSS,

Armonk, New York, USA) and statistical significance accepted at $P < 0.05$. Nonparametric statistical tests (Spearman and Pearson correlation) were used due to nonnormal distribution of data.

RESULTS

The 27 turtles (17 females, eight males, two undetermined) sampled averaged (mean \pm SD) 365.26 \pm 89.46 mm long, SCL(nt), and weighed 9.01 \pm 5.33 kg (Table 1). Median BCI was 0.0158 \pm 0.001 ($n = 27$). None of the turtles presented external signs of fibropapilloma or other indicators of poor health such as emaciation, dehydration, or external parasites. Values and descriptive statistics for all biochemical and biometric parameters are presented in Table 1. Biochemical analyses could not be completed across all parameters and individuals due to limited sample volume. The sex-ratio was female-biased ($\sim 2:1$) and reflects this population's natural sex-ratio (Delgado et al., 2010). Sea surface temperature averaged 20.93 C (range = 17.9–24.3). Only total cholesterol ($P = 0.42$), total protein ($P = 0.72$), and albumin ($P = 0.60$) were positively correlated with SCL.

DISCUSSION

Our results differ from those of Bolten et al. (1994), in which 22 of 26 blood parameters were significantly related to the turtle body size. These discrepancies may be due to the individuals' small size and, consequently, the immature status of the animals sampled in our study compared to those of Bolten et al. (1994) who sampled reproductively active adults. Considering only young turtles, a positive correlation between total protein and turtle size has been documented in juvenile green turtles *Chelonia mydas* (Bolten and Bjørndal, 1992).

Our data may be compared with those of Flint et al. (2010). Despite the different populations and geographic areas, both studies included animals from the same

TABLE 1. Plasma biochemistry values and physical parameters for juvenile loggerhead sea turtles (*Caretta caretta*) captured from Madeira Island waters, May–July 2006.

Variable ^a	Mean value	Range	Median ^b	n
Uric acid mg/dl	1.40	1.00–2.40	1.30	17
Total bilirubin mg/dl	0.44	0.20–1.20	0.35	18
Total cholesterol mg/dl	105.83	60.00–200.00	101.00	24
CK mg/dl	0.40	0.30–0.50	0.40	4
Glucose mg/dl	130.27	71.00–197.00	132.00	26
Total protein g/l	2.93	2.10–4.00	3.00	21
Urea nitrogen mg/dl	183.85	62.06–344.54	201.16	25
AST/SGOT U/l	93.94	13.00–238.00	78.50	18
GGT U/l	33.17	14.00–52.00	31.50	6
Albumin g/l	1.38	1.00–2.00	1.30	14
ALP U/l	74.53	51.00–120.00	68.00	17
Sodium mMol/l	149.62	135.90–166.20	149.80	26
Potassium mMol/l	4.68	3.70–7.30	4.50	26
Chloride mMol/l	116.09	100.00–136.00	116.00	26
Calcium mg/dl	4.90	3.10–7.10	5.10	18
Phosphorus mg/dl	7.24	3.30–13.40	7.40	19
Magnesium mg/dl	4.48	2.90–5.50	4.70	10
SCL(nt) (mm)	365.26	197.00–519.00	NC	27
Weight (kg)	9.01	1.19–20.67	NC	26
BCI	0.02	0.01–0.02	NC	26

^a CK = creatinine kinase; AST/SGOT = aspartate aminotransferase; GGT = gamma glutamyl-transferase; ALP = alkaline phosphatase; SCL(nt) = straight carapace length until notch; BCI = body condition index.

^b NC = not calculated.

species, wild condition, and life stage. A considerable difference was observed in the levels of total protein and albumin (2.93 and 1.38, respectively), which were lower in turtles from the Madeira Archipelago than the lower limit (2.9 and 1.38, respectively) found in turtles from Australian Pacific loggerheads. Due to the positive correlation between protein values and body size detected in the present study, the lower protein values observed in the Madeira population could be justified based on differences in the size of turtles from both areas. In our study, turtles averaged 365.3 mm long whereas those from Australia averaged 838 mm.

According to general previous data, our results fell within the known ranges for total cholesterol (Campbell, 1996; Jacobson et al., 2007), albumin (George, 1997; Whitaker and Krum, 1999; Jacobson et al., 2007; Deem et al., 2009), chloride and phosphorus (Frye, 1991; Campbell, 1996; George, 1997; Whitaker and Krum, 1999;

Jacobson et al., 2007). Levels of uric acid (Campbell, 1996; Whitaker and Krum, 1999; Jacobson et al., 2007), CK (Campbell, 1996), glucose (Frye, 1991; Campbell, 1996; George, 1997; Whitaker and Krum, 1999; Jacobson et al., 2007), GGT (Jacobson et al., 2007; Deem et al., 2009), ALP (Campbell, 1996; George, 1997; Whitaker and Krum, 1999; Jacobson et al., 2007), and K (Frye, 1991; Campbell, 1996; George, 1997; Whitaker and Krum, 1999; Jacobson et al., 2007; Deem et al., 2009) were within the upper ranges referenced in several literature sources.

Values of total bilirubin and urea nitrogen were elevated (Campbell, 1996; Whitaker and Krum, 1999; Flint et al., 2010). Increased urea is usually observed in cases of kidney failure, dehydration, rich protein diet, or excessive protein breakdown. However, the elevated levels of urea nitrogen seem not to be related directly with diet because total protein levels were low, and even captive logger-

heads fed the same diet also showed widely different urea values (Lutz and Dunbar-Cooper, 1987). Dehydration was not observed in the turtles we studied and, even in dehydrated turtles, total protein should be relatively high. As urea is a final product of protein catabolism, a plausible explanation is an increase of protein breakdown during acclimatization when food was not provided.

The enzymes AST and CK were slightly below known levels (Bolten et al., 1994; Campbell, 1996). Although below reference values, Na levels were close to normal ranges (Frye, 1991; Campbell, 1996; George, 1997; Whitaker and Krum, 1999; Jacobson et al., 2007) and between the reference values cited for juvenile turtles from Australian waters.

Some factors may justify the low total protein and calcium levels found in our study in relation to other studies. In turtles, nutrition, season, and environmental factors such as lead pollutants have a significant effect on serum protein (Frair and Shah, 1982). Postpelagic juvenile and adult loggerhead turtles are essentially carnivorous (Bjorndal, 1997), feeding mainly on benthic mollusks, crustaceans, and coelenterates (Dodd, 1988), whereas juveniles feed mainly on jellyfish (Bjorndal, 1997). As loggerheads grow, they appear to feed at progressively higher trophic levels, consuming food higher in nitrogen (Godley et al., 1998). Thus, the relatively low total protein levels found in our study may be attributed to the low energy density and protein content of jellyfish (Doyle et al., 2007) as well as to the lower trophic status (Godley et al., 1998). The significant correlation between carapace length and protein concentration was also previously described for all seven sea turtle species (Frair and Shah, 1982), except *Natator depressus*, which was not included in that study. Apart from the differences due to the very small body size of Madeiran turtles when compared to young turtles from the Pacific (Flint et al., 2010), we could not discard the effect of

chronic exposure to heavy metal contamination on the plasma protein concentrations of the sea turtle population under study. Previous studies showed high levels of mercury and cadmium in the hepatic, kidney, skin, and muscle tissues of marine species from Madeira Archipelago (Afonso et al., 2007). Mercury is usually listed as a general protoplasmic poison, and many of its effects are attributable to the fact that, as a heavy metal, it can act as a protein precipitant. An experiment performed in dogs verified that induced hypoproteinemia protected the animals against lethal mercuric chloride poisoning injuries (Holman and Donnelly, 1942), and this method is still used in human medicine (Koch and Trapp, 2006). As our objective was not focused on this subject, we recommend future studies to verify the relationship between heavy metal contamination and the oscillation on plasma proteins in this population.

Calcium levels were lower than in most previous studies (Campbell, 1996; ISIS, 1999; Whitaker and Krum, 1999) but similar to the values found in wild juveniles turtles from Australia (Flint et al., 2010). We believe the relatively low levels of this ion may be related to the age and the natural condition of these juvenile turtles, as most previous work was done on adult, subadult, or captive juvenile turtles. In the wild, physiologic condition is highly related to the ecologic niche and the probability of finding food. Juvenile turtles live in the open sea, do not dive as deep as the adults do, and feed mainly on jellyfish low in calcium, whereas adult turtles live near the coasts and have mollusks as an important item in their diet. Considering the poor calcium content of the diet and the active growth in this life stage, it is reasonable that the observed values of calcium are physiologically normal for wild juvenile turtles. Moreover, oscillations of serum calcium concentrations related to the individual's sex, or due to reproductive status as known for other reptiles (Des-sauer, 1970; Allender et al., 2006), do not

make sense in our sample. We suggest that the calcium levels presented at the juvenile stage are the baseline levels for this developmental stage, as opposed to much higher levels documented at older stages, and for specific physiologic stages such as reproduction (Lance, 1976).

Parameters such as glucose and chloride, Na, and K were remarkable in their constancy and were within the reference intervals presented for wild young loggerhead turtles (Flint et al., 2010). These ions have a critical physiologic role for enzyme function (Hochachka and Somero, 1984), demonstrating the need for a relatively tight control of their levels in order to maintain physiologic homeostasis, in contrast to other biochemical parameters that can show wider oscillations while remaining within safe a physiologic range. Differences in many parameters, compared to the adult or subadult conspecifics, may also reflect ontogenetic and geographic differences in diet or digestive efficiency (Hamann et al., 2006).

Although the rather small sample size of this study may account for some of the high variance of most biochemical parameters, similar studies using large sample sizes have also reported high variability (Stamper et al., 2005), indicating that our findings reflect natural individual and population variability.

The use of reference values to diagnose or screen for disease implies that health is a relative concept; clinical examination, evaluation of laboratory data, and diagnostic imaging findings all require comparison to a reference standard (Walton, 2001). To our knowledge, this is one of the first studies assessing blood biochemical values in pelagic loggerhead populations and represents a unique dataset for wild, free-ranging oceanic juveniles of *C. caretta* from the North Atlantic Ocean. Additionally, our results point to a few differences that may have physiologic, ontogenetic significance between the juvenile oceanic developmental stage and older life stages.

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