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Health condition of juvenile *Chelonia mydas* related to fibropapillomatosis in southeast Brazil

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ABSTRACT: Packed cell volume (PCV), plasma biochemistry, visual body condition (BC), and calculated body condition index (BCI) were evaluated in 170 wild juvenile green sea turtles *Chelonia mydas* from an aggregation in the effluent canal of a steel mill in Brazil. Occurrence of cutaneous fibropapillomatosis (FP) was observed in 44.1% of the animals examined. BCI alone did not differ significantly between healthy animals and those afflicted with FP. However, all turtles with low BCI were severely afflicted and were uremic, hypoglycemic, and anemic in relation to healthy animals. Severe FP was not always reflected by a poor health condition of the individual. Clinical evaluation and plasma biochemistry indicated that most animals afflicted with FP were in good health condition. Differences in FP manifestations and associated health conditions in different geographic regions must be assessed by long-term health monitoring programs to help define priorities for conservation efforts.

KEY WORDS: Green sea turtle · Clinical biochemistry · Body condition index · Fibropapillomatosis · Health monitoring · Packed cell volume

INTRODUCTION

Diseases are becoming a major threat to sea turtles around the world, including in Brazil, where the 5 species found are considered Threatened with extinction by the International Union for the Conservation of Nature and the Brazilian Institute for Environment and Natural Resources (IBAMA). The green sea turtle *Chelonia mydas* (Linnaeus, 1758) is most abundant along the coast of Espírito Santo State in southeastern Brazil. It nests on oceanic islands, feeds in Brazilian coastal waters, and is threatened by fisheries interactions and boat strikes (Baptistotte 2007). Recently, disease has become more prominent and is considered among the top 20 priorities for studies on sea turtle conservation (Hamann et al. 2010). Understanding the impact of disease on sea turtle populations depends on extensive knowledge of the etiology, epidemiology, climate, pollution, and influences of seasonality. Long-term monitoring programs are an important part of sea turtle conservation projects, but health evaluation is not always included because of limited resources and difficulty in relating health to environmental drivers of disease. Traditionally, monitoring the health of sea turtles has been based prima-

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rily on the evaluation of animals found dead or stranded on beaches or recovered in nets or as part of in-water studies of specific agglomerations (Aguirre & Lutz 2004). Studies of live turtle health involve physical assessment, including visual inspection and evaluation of behavior (Walsh 1999) or laboratory analyses of biochemical and hematological parameters and serology (Work et al. 2003, Ene et al. 2005, Casal et al. 2009, Santos et al. 2009, Santoro et al. 2010, Labrada-Martagón et al. 2010a).

Comparative studies of unhealthy and healthy animals can generate important information for management and conservation. However, the information produced may have limited applicability if normal reference values for commonly measured parameters such as hematology and blood chemistry for each population are not established (Flint et al. 2010).

In Brazil, fibropapillomatosis (FP) is considered the most important disease threatening sea turtles (Matushima et al. 2001, Baptistotte 2007, Santos et al. 2010). It is a cosmopolitan transmissible neoplasia, probably of herpesviral etiology, that results in external and internal tumors which affect the function and survival of the turtles (Herbst 1994, Matushima et al. 2001, Aguirre & Lutz 2004, Rodenbusch et al. 2012, 2014). Biochemical and hematological parameters may be affected by the chronic debilitating condition of the disease and the presence of internal tumors (Aguirre et al. 1995, Work & Balazs 1999, Aguirre & Balazs 2000, Swimmer 2000). Polluted areas have a higher prevalence, suggesting that FP can be an indicator of environmental damage, and that sea turtles can be used as 'sentinel species' (Aguirre & Lutz 2004). This pollution versus FP occurrence aspect has been recently examined by associating nutrient pollution of coastal areas with high prevalence of FP in Hawaii, USA, proposing that nitrogen input to the local food web promotes higher FP prevalence by favoring alphaherpesvirus-induced tumor formation (Van Houtan et al. 2010, 2014). As novel or controversial as this hypothesis may appear, given that nutrient intake is strictly related to dietary conditions, agglomerations around more eutrophic and productive feeding areas are prone to occur (Santos et al. 2010, Torezani et al. 2010). Turtles also show differences in biochemical parameters related to diet (Anderson et al. 2011). The metropolitan region of Vitória, located on the southeastern coast of Brazil, is a major industrial site for steel production. One of these steel mills uses seawater to cool its plant. The cooling water is carried back to the sea via a discharge canal where a large number of juvenile *C. mydas* aggregate. In 2000, the TAMAR/IBAMA Project, together with the mill, started a monitoring program for this aggregation of turtles (Torezani et al. 2010).

The objective of the current study was to compare health parameters of green turtles with and without FP to provide basic health information for conservation actions in the region. Body condition index (BCI) was calculated, plasma biochemical analyses were performed, and packed cell volume (PCV) was estimated in order to establish reference ranges and to distinguish between healthy and unhealthy turtles.

MATERIALS AND METHODS

Study area

This study took place in the terminal part of the effluent canal of a steel mill located in the greater Vitória region, Espírito Santo (ES), on the southeastern coast of Brazil (20° 16′ 06″ S, 40° 3′ 35″ W). The canal is 500 m by 33 m wide, with an average depth of 2 m. The effluent is largely from seawater, collected about 1 km north from the site of the study, with a flux of approximately 28 000 to 32 000 m^3 h^{-1}, and it is used for cooling the factory, after which it is returned to the sea at a temperature of 8.75 ± 1.15°C above the seawater temperature, according to the mill records. About 5% of the water is collected from rivers, which also receive treated sewage water from domestic and industrial sources (Torezani et al. 2010). The high temperature of the water and the abundance of organic material possibly favor the growth of algae, prompting turtles to come into the canal in great numbers to feed, thereby facilitating their capture and study.

Capture

Animals were captured weekly from June 2001 to July 2004. Initially, the turtles were caught using a round cast net (5 m in diameter, 6 cm mesh), deployed from the shore or a boat. Because of the difficulty of successfully using the cast net, in October 2003 a gill net (70 m × 6 m with 7 cm mesh) was deployed across the canal to entangle turtles as they entered or left the canal. The gill net was monitored every 10 to 15 min to ensure that entangled turtles were not injured. No turtle was harmed during the study. The study was performed under the license

SISBIO/IBAMA 14122 of the Brazilian Program of Protection, Management and Research of Sea Turtles. (Projeto TAMAR/ICMBIO).

Biometry and determination of BCI

Following capture, curved carapace length (CCL) and curved carapace width were measured (cm) with a flexible measuring tape, and each animal was weighed (kg) to the nearest 200 g (Bjorndal & Bolten 1992). Animals were marked with Inconel tags (National Band and Tag, model 681) on the cranial flippers (Marcovaldi & dei Marcovaldi 1999). Fulton's BCI was calculated, according to the formula BCI = weight/CCL^3 (Christopher et al. 1999, Bjorndal et al. 2000, Labrada-Martagón et al. 2010b). The sex of the animals was not determined because of the absence of external sexual dimorphism at age of capture.

Determination of body condition and tumor score

Following measuring and tagging, animals underwent a physical examination to determine the body condition (BC) involving a visual evaluation of the concavity of the plastron (Thomson et al. 2009), the depression of chest musculature, and presence of fat in the neck and cervical musculature. Animals were also examined for tumors, wounds, mutilation, epibionts, sunken eyes, and behavior (e.g. capture avoidance, palpebral reflex, lethargy, or responsiveness). Based on these criteria, BC was scored as either good, average, or poor (Walsh 1999). Animals with FP were assigned a tumor score (TS) that takes into account the number and size of tumors, according to Work & Balazs (1999).To avoid inter-observer variation, the same person examined the turtles over the entire period.

Blood sampling

Following capture, blood samples were collected from the occipital venous sinus and dispensed in tubes containing heparin as anticoagulant. The volume of the collected blood did not surpass 1% of the live weight. The samples were divided, with 1 portion analyzed for PCV whereas the other, protected from light, was centrifuged and the plasma was harvested and then stored frozen (–20°C). All samples with visible hemolysis were not analyzed.

Biochemical analyses

All samples were analyzed at the Marcos Daniel Laboratory, in Vitória, ES, using an automatic biochemical analyzer Dimension AR (Dade Behring) according to the manufacturer's instructions.

The following parameters were determined: glucose, total protein, albumin, blood urea nitrogen (BUN), creatinine, uric acid, cholesterol, triglycerides, calcium, phosphorus, iron, chlorine, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl transpeptidase, creatine kinase, lipase, and amylase. The values of globulins, the albumin:globulin ratio, and the calcium:phosphorus ratio were calculated, and PCV was determined by capillary microcentrifugation.

Statistics

Data were summarized using means, medians, and standard deviation and evaluated for normality using the Kolmogorov-Smirnov test and for equal variance with Levene's test. Comparisons of BCI with visual BC categories, TS values, and biochemical values were done using ANOVA with post hoc Tukey pairwise comparisons for data meeting assumptions of equal variance and normality. Non-parametric Kruskal-Wallis ANOVA and post hoc Bonferroni pairwise testing was done for data not meeting assumptions of normality and equal variance. Spearman correlations were tested between BCI and biochemical values. Stepwise multiple linear regression was conducted to evaluate the influence of biochemical parameters of healthy and FP-afflicted turtles on BCI. Reference intervals for healthy animals were expressed as mean ± 2SD or 2.5–97.5th percentiles for data that were not normally distributed (Labrada-Martagón et al. 2013). The level of significance for all statistical tests was $\alpha = 0.05$, and all statistics tests were performed using SPSS 20.0.

RESULTS

BC and BCI evaluation

We captured 170 animals with CCL ranging from 29.5 to 77.5 cm (mean ± SD: 42.8 ± 7.3 cm). Based on this length interval, all animals captured were considered post-pelagic juveniles (Torezani et al. 2010). Of 155 turtles visually examined, 133 (85.8%) were

classed as being in good BC, 16 (10.3%) in average BC, and 6 (3.9%) in poor BC. Fifteen turtles had to be released before physical evaluation to avoid long handling time and excessive stress. Turtles in poor BC had lower BCI values than turtles with average BC, which in turn had lower BCI than the turtles in good BC. Therefore, the visual evaluation of BC was consistent with the calculated BCI (Table 1).

Characterization of FP occurrence

Of the 170 captured turtles, 95 (55.9%) were FP free, whereas 75 (44.1%) had FP of varying degrees of severity. Of these, 33 turtles were classified as TS-1 (19.4%), 35 (20.6%) as TS-2, and 7 (4.1%) as TS-3. FP-afflicted turtles were found in all BC categories; however, all turtles in poor BC had FP. No significant difference in mean BCI among TS categories was observed (see Table 3) The majority (74%) of turtles with FP had tumors in the cranial flippers, neck (67%), eyes (58%), and caudal flippers and cloacae (58%). No turtles had tumors that were visible inside the mouth or throat. Two turtles showed typical signs of tumor regression, such as skin pigmentation at the tumor sites, but without total remission of the disease.

Biochemical and PCV analyses

Relative to healthy animals, turtles with TS-3 had significantly lower PCV and glucose and elevated BUN levels (Tables 2 & 3). In healthy animals, we found a significant positive correlation between BCI and total protein ($r = 0.221$, $p = 0.036$) and between BCI and cholesterol ($r = 0.256$, $p = 0.014$), while there was a significant negative correlation between BCI and urea ($r = -0.234$, $p = 0.028$) and between BCI and the urea:creatinine ratio ($r = -0.262$, $p = 0.02$; Table 4).

FP-afflicted animals showed a significant positive correlation ($p < 0.001$) between total protein and BCI ($r = 0.389$, $p = 0.001$) and between globulins and BCI ($r = 0.441$, $p = 0.000$), but not between cholesterol and BCI. These animals also had a significant positive correlation between BCI and PCV ($r = 0.478$, $p = 0.000$; Table 4).

Table 1. Comparison of frequency and averages of body condition index (BCI) and visual evaluations of body condition of green sea turtles *Chelonia mydas* at Espírito Santo, Brazil, between 2001 and 2004. Asterisk (*) indicates significant differences in relation to the good body condition

Condition	N	%	BCI Mean (SD)	p	% of certainty
Good	133	85.8	1.162 (0.102)	–	97.65
Average	16	10.3	1.099 (0.072)	0.046*	93.75
Bad	6	3.9	0.875 (0.114)	0.000*	83.33
Total	155	100.0	–		

Table 2. Mean and SD of curved carapace length, weight, and reference interval for packed cell volume (PCV) and plasma biochemical values of healthy juvenile green sea turtles *Chelonia mydas* at Espírito Santo, Brazil, between 2001 and 2004. Reference range is mean ± 2SD for normal distributions, or 2.5–97.5th percentiles for non-normal distributions (see footnotes)

Parameter	n	Mean	SD	Reference range
Curved carapace length (cm)	95	42.5	8.5	–
Weight (kg)	93	9.5	5.6	–
Body condition index	93	1.2	0.1	1.0–1.3[a]
PCV (%)	91	32.7	7.3	19.3–45.1[a]
Glucose (mg dl^{-1})	91	89.3	18.5	59.6–120.2[a]
Triglycerides (mg dl^{-1})	89	89.6	87.5	11.3–209.8
Cholesterol (mg dl^{-1})	93	113.6	71.8	15.0–212.7
Total protein (g dl^{-1})	92	3.7	1.1	1.3–5.5[a]
Albumin (g dl^{-1})	86	1.0	0.5	0.1–1.7
Globulin (g dl^{-1})	84	2.8	1.2	0.4–4.6[a]
Albumin:globulin ratio	84	0.5	0.6	0.1–1.1
Blood urea nitrogen (BUN; mg dl^{-1})	90	52.6	49.2	5.0–156.2
Creatinine (mg dl^{-1})	88	0.3	0.2	0.0–0.5
BUN:creatinine ratio	80	295.8	378.8	11.7–789.6
Uric acid (mg dl^{-1})	94	1.1	0.9	0.0–2.5
Calcium (mg dl^{-1})	85	9.4	2.3	5.4–12.0[a]
Phosphorus (mg dl^{-1})	87	6.2	1.7	2.7–8.7[a]
Calcium:phosphorus ratio	82	1.7	0.9	0.6–2.6
Sodium (mmol l^{-1})	93	158.0	8.0	145.7–165.0[a]
Potassium (mmol l^{-1})	93	4.7	0.7	3.3–5.8[a]
Chlorine (mmol l^{-1})	89	120.2	7.8	105.5–127.3[a]
Iron (µg dl^{-1})	93	65.4	47.0	9.4–147.8
Magnesium (mg dl^{-1})	88	12.1	2.8	8.0–16.4[a]
Total bilirubin (mg dl^{-1})	95	0.1	0.1	0.0–0.3
Direct bilirubin (mg dl^{-1})	94	0.0	0.1	0.0–0.1
Alkaline phosphatase (UI l^{-1})	76	46.4	24.6	14.5–91.2
Aspartate aminotransferase (UI l^{-1})	94	254.2	135.8	91.8–442.3
Alanine aminotransferase (UI l^{-1})	92	18.0	24.0	0.0–31.1
Lactate dehydrogenase (UI l^{-1})	93	168.1	115.8	3.7–351.2
Gamma glutamyl transpeptidase (UI l^{-1})	91	3.4	7.8	0.0–6.1
Amylase (UI l^{-1})	86	541.4	267.0	92.3–1020.1[a]
Lipase (UI l^{-1})	85	78.2	60.1	0.0–164.0
Creatine kinase (UI l^{-1})	83	1423.9	2671.7	234.9–3429.0

[a]Normal distribution

Table 3. Mean and SD for curved carapace length, weight, packed cell volume (PCV), and plasma biochemical values of juvenile green sea turtles *Chelonia mydas* afflicted with cutaneous fibropapillomatosis, according to tumor score (TS), at Espírito Santo, Brazil, between 2001 and 2004. N (%) = no. of animals captured in each TS group; n = no. of animals measured; asterisks (*) show parameters with significant statistical differences between TS-3 and healthy animals ($p < 0.05$)

Parameter	Low (TS-1) N (%) = 33 (19.4%)			Moderate (TS-2) N (%) = 35 (20.6%)			High (TS-3) N (%) = 7 (4.1%)		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
Curved carapace length (cm)	33	42.2	4.8	34	44.1	5.5	7	44.4	7.7
Weight (kg)	32	8.7	3.0	33	9.7	3.8	6	7.4	2.6
Body condition index	32	1.2	0.1	33	1.1	0.1	6	1.0	0.2
*PCV(%)	32	30.6	7.1	34	28.3	11.1	7	**20.6**	**11.2**
*Glucose (mg dl^{-1})	33	96.8	17.2	34	88.1	21.7	7	**70.3**	**31.8**
Triglycerides (mg dl^{-1})	31	104.0	73.6	31	86.9	75.0	7	82.0	57.2
Cholesterol (mg dl^{-1})	33	120.2	70.5	33	113.8	86.9	7	83.6	28.9
Total protein (g dl^{-1})	33	4.1	0.7	34	3.7	1.1	7	3.3	1.7
Albumin (g dl^{-1})	30	1.2	0.5	31	1.0	0.6	6	1.3	0.2
Globulin (g dl^{-1})	30	2.9	0.8	31	2.7	1.2	6	2.2	1.7
Albumin:globulin ratio	30	0.5	0.3	31	0.6	1.1	6	1.0	0.8
*Blood urea nitrogen (BUN; mg dl^{-1})	33	30.5	18.2	34	40.1	34.5	7	**113.7**	**56.0**
Creatinine (mg dl^{-1})	32	0.2	0.1	34	0.2	0.1	7	0.6	1.1
BUN:creatinine ratio	28	129.3	97.4	30	208.7	229.9	5	481.2	365.6
Uric acid (mg dl^{-1})	33	1.4	1.5	35	1.1	0.9	7	2.7	2.6
Calcium (mg dl^{-1})	32	9.7	1.8	30	8.7	2.0	7	9.0	1.5
Phosphorus (mg dl^{-1})	31	5.7	1.6	28	6.1	1.7	7	5.6	1.2
Calcium:phosphorus ratio	30	1.9	0.8	28	1.6	0.7	7	1.7	0.5
Sodium (mmol l^{-1})	31	157.9	4.8	35	159.7	6.6	6	158	6
Potassium (mmol l^{-1})	31	4.9	0.8	34	4.7	0.7	6	4.9	0.6
Chlorine (mmol l^{-1})	31	120.2	4.8	27	120	6.8	6	121.3	3
Iron (µg dl^{-1})	32	85.6	69.2	34	57.8	39	7	46.7	19.7
Magnesium (mg dl^{-1})	28	12.6	3.4	28	11.2	2.6	7	12.1	2.7
Total bilirubin (mg dl^{-1})	33	0.1	0.1	33	0.1	0.1	7	0.1	0.1
Direct bilirubin (mg dl^{-1})	33	0	0.1	33	0	0.1	7	0.1	0.1
Alkaline phosphatase (UI l^{-1})	25	70.4	64.3	25	39	26.8	6	40.3	24.1
Aspartate aminotransferase (UI l^{-1})	33	251.8	106.9	34	296.2	393	7	202.3	39.2
Alanine aminotransferase (UI l^{-1})	33	16.3	10.4	32	25.3	67.1	7	14.6	2.4
Lactate dehydrogenase (UI l^{-1})	31	165.3	78.6	33	169.6	152	7	157.7	55.3
Gamma glutamyl transpeptidase (UI l^{-1})	33	3.2	4.4	33	6.4	16	7	4.6	6.8
Amylase (UI l^{-1})	32	546.2	262.5	27	516.9	221.7	7	488	182.7
Lipase (UI l^{-1})	29	122.3	130.9	27	58.5	42.9	6	62.3	18.4
Creatine kinase (UI l^{-1})	27	1229	1501.6	26	1903.4	2163.7	7	754.6	442.9

Table 4. Correlations between body condition index and biochemical parameters and packed cell volume of green sea turtles *Chelonia mydas* with and without cutaneous fibropapillomas (FP) at Espírito Santo, Brazil, between 2001 and 2004

Parameter	r	p
Healthy ($p < 0.05$)		
Blood urea nitrogen	−0.234	0.028
Urea:creatinine ratio	−0.262	0.020
Total protein	0.221	0.036
Cholesterol	0.256	0.014
FP afflicted ($p < 0.01$)		
Packed cell volume	0.478	0.000
Creatinine	0.390	0.01
Total protein	0.389	0.001
Globulins	0.441	0.000
Urea:creatinine ratio	−0.327	0.012

Based on multiple regression, BCI was only predicted at a low percent ($R^2 = 0.138$, $p = 0.025$) by ALT. All other parameters did not have any significant correlation. Multiple regression revealed that for FP-afflicted turtles, the urea:creatinine ratio was responsible for 51% of BCI variation and together with magnesium, AST, and direct bilirubin for almost 75% ($p < 0.05$; Table 5).

DISCUSSION

BC and BCI evaluation

The classification of BC based on visual inspection of the animals agreed with the quantitative values of

Table 5. Summary of multiple regression of green sea turtles *Chelonia mydas* with fibropapillomatosis considering body condition index as the dependent variable and biochemical parameters as predictors ($p < 0.05$). AST: aspartate aminotransferase

Predictor model	R	R^2	Adjusted R^2	SE of estimate	ANOVA *F*	p
Urea:creatinine ratio	0.718	0.515	0.493	0.073	23.369	0.000
Urea:creatinine ratio/magnesium	0.777	0.604	0.566	0.067	16.018	0.000
Urea:creatinine ratio/magnesium/AST	0.825	0.681	0.634	0.062	14.256	0.000
Urea:creatinine ratio/magnesium/AST/direct bilirubin	0.866	0.749	0.697	0.056	14.198	0.000

BCI (Table 1). While it is subjective, visual evaluation can be a good complementary assessment of BC, and this agrees with previous evaluationa of adult green turtles (Thomson et al. 2009). Using a single observer, we were able to achieve a good relationship between calculated BCI and subjective visual evaluation, although this technique would need to be done my multiple observers to determine its reproducibility.

Characterization of FP occurrence

The distribution of tumors in the animals predominantly in the cranial region was seen in turtles from our study site and resembled the distribution also observed in a nearby area in Espírito Santo Bay, less than 10 km away (Santos et al. 2010), but with fewer and smaller tumors at our study site. The preponderance of anterior tumors was similar to findings of others (Adnyana et al. 1997, Work et al. 2004), whereas in turtles in Indonesia, the number of posterior FP tumors was higher (Adnyana et al. 1997). The absence of tumors inside the mouth, which would impair the ability to feed, in our study was different from the high occurrence of oral tumors in Hawaii (Aguirre et al. 2002, Work et al. 2004). Brazilian green turtles with FP may carry 6 variants of the Atlantic and the western Atlantic/eastern Caribbean phylogenetic groups of the chelonid herpesvirus 5 (CHV-5), and this may partly explain the different manifestations of FP. Also, the tumor size, aspect, and TS vary according to the viral variants (Rodenbusch et al. 2012, 2014). These differences in FP manifestations between geographical areas reinforce the need for regional long-term health monitoring plans to determine the occurrence and distinct features of FP, to evaluate the role of the disease as a threat to sea turtles, and to inform future conservation efforts.

Two animals showed signs of tumor regression. Unfortunately, recapture data were not within the scope of this study. Therefore, it was not possible to estimate a regression rate for the agglomeration. Torezani et al. (2010) reported 1 complete regression of a TS-1 turtle 6 mo after first capture in the same location. Regression was photographically documented in Hawaii, where it is estimated that about 30% of turtles recover from the disease (Bennett et al. 1999) perhaps due to genetic resistance or other factors. Elucidating the factors that allow some animals to recover and others not would help us understand the pathogenesis of this disease. In Espírito Santo, a beach-stranding monitoring program was established in 2011 as a compensatory measure for oil and gas activities. As part of this program, tumors are surgically excised and turtles are released without monitoring for reoccurrence. The possibility of genetic resistance to FP suggests that rehabilitation of FP-afflicted turtles may be inappropriate, as such activities may maintain a greater level of individuals that are genetically susceptible to FP in the population, and thus alter the natural auto-limitation of the disease within the larger green turtle population. The tumor-excised turtles also continue carrying the latent herpesvirus and potentially transmit it to others (Herbst et al. 1999).

The main problem that cutaneous FP brings to turtles is the impeded ability to see, move, and feed (Adnyana et al. 1997). Thus, it is expected that severely afflicted animals have a lower BCI. Only 3 animals from the current study were classified as both low BCI and TS-3. Overall, there was no significant difference in BCI values among healthy and FP-afflicted turtles, in contrast to Hawaii, where the disease appears to be more severe for turtles. Also, turtles with severe FP are immunosuppressed (Work et al. 2001) and bacteremic (Work et al. 2003) in Hawaii, again suggesting that the disease is more severe in that region than in Brazil.

Our results indicate that the TS, as applied, does not necessarily reflect the physical condition of an afflicted animal. Adnyana et al. (1997) found a negative correlation of body weight:CCL ratio with number of tumors, and more specifically, with the number of tumors around the eyes. A previous study with a

larger sample from the same area as our study (Torezani et al. 2010) and another in Hawaii (Balazs et al. 1998) found smaller growth rates in animals severely afflicted with FP, indicating that growth rate may be a better index than BCI alone for evaluating the debilitation associated with FP.

Biochemistry and PCV analyses

The positive correlation between BCI and the levels of total protein and cholesterol in healthy animals can be explained by the fact that both parameters are positively affected by nutritional condition (Aguirre & Balazs 2000). Thus, total protein and cholesterol can be good proxies for nutritional health (Whiting et al. 2007) and can be useful as health indicators and should be included in basic biochemical analyses of green turtle population health monitoring. Urea and the urea:creatinine ratio decrease as the BCI increases. This is likely because animals in poor BC catabolize proteins, as reflected in higher blood urea levels, and may also indicate dehydration, considering that BUN is not a reliable indicator of renal dysfunction in turtles (Keller et al. 2004, Campbell 2006), as noted in the 3 turtles with poor BC and TS-3. Aguirre & Balazs (2000) also found TS-3 turtles to be uremic, but suggested that this condition was due to internal tumors that are frequent in Hawaii. In Brazil, internal tumors are not frequent (4%) but cannot be excluded as a cause (Baptistotte 2007). It seems more plausible to attribute internal organ illness to parasitic chronic granulomatosis caused by *Learedius learedi* (Spirochidae), as these parasites seem to be very common (Werneck et al. 2006). Bolten & Bjorndal (1992) suggested that the transition from an omnivorous to an herbivorous diet due to the size and age of the turtles might be responsible for the decrease in the biochemical values of urea in juvenile wild *C. mydas*.

The positive correlation of protein and globulins with the BCI may reflect better overall condition of TS-1 and TS-2 turtles, given that advanced FP can impair the physical ability to swim, dive, and search for food (Anderson et al. 2011).

The correlations of the urea:creatinine ratio, magnesium, AST, and direct bilirubin, as shown in the multiple regression analysis, are other nonspecific biochemistry alterations in FP-afflicted turtles that are secondary to chronic debilitation associated with the disease. Given the variability of these parameters, these changes should not be considered pathognomonic for the disease.

The PCV values of healthy animals in this study were lower than those obtained by Work & Balazs (1999) in Hawaii but were similar to those found in the Bahamas, Arabian sea, Australia, and Galapagos (Bolten & Bjorndal 1992, Alkindi & Mahmoud 2002, Flint et al. 2010, Lewbart et al. 2014) and other places in Brazil (Rossi et al. 2009, Santos et al. 2009). Hematocrit is simple and cheap to measure, and the test has a low coefficient of variation, making it a useful tool in the evaluation of the health of sea turtles. Anemia may be a feature in chronic debilitating diseases such as neoplasias (Aguirre et al. 1995), severe parasitism, and prolonged anorexia (Saggese 2009), as is evidenced by the positive correlation of the PCV with the BCI and the low PCV in TS-3 turtles.

Future studies should characterize environmental and seasonal variability of biochemical and hematological parameters of sea turtles. There is also a need to better understand cofactors associated with FP such as parasites, secondary infections, starvation, and the presence of internal tumors to better understand the status of FP. Many questions also remain regarding the role of the virus in disease causation and disease pathogenesis. Addressing these questions can contribute to better understanding of how to address and manage sea turtle health in Brazil.

CONCLUSION

The BCI reflects the subjective assessment of body condition of green turtles in Brazil and correlates with alterations in the biochemical counts related to the general health condition of the animals (not necessarily with FP). Thus, it is advisable to use BCI in field protocols, when possible, along with visual inspection as parameters for the evaluation of the health of sea turtles. Any visual subjective measurement of BC should be done by trained personnel to minimize inter-observer variability. FP tumor scores alone do not always reflect the general health condition of individuals, and should be used together with other indicators such as BCI, PCV, and biochemical profiles of sea turtle health monitoring programs, as they are sensitive indicators of the physical condition of the animals. Biochemical data and PCV counts are indirectly related to FP, since they are related to variable conditions of individual animals. Thus, the interpretation of such information must always be accompanied by a general evaluation of the health of the afflicted animals and the diseases concomitant to FP. The relevance and application of hematological and biochemical tests in sea turtle health monitoring pro-

grams in Brazil rely on the development of basic studies of the occurrence and effects of other natural and anthropogenic diseases other than FP in the area.

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