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EFFECTS OF "SWIM WITH THE TURTLES" TOURIST ATTRACTIONS ON GREEN SEA TURTLE (*CHELONIA MYDAS*) HEALTH IN BARBADOS, WEST INDIES

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ABSTRACT: Along the West Coast of Barbados a unique relationship has developed between endangered green sea turtles (*Chelonia mydas*) and humans. Fishermen began inadvertently provisioning these foraging turtles with fish offal discarded from their boats. Although initially an indirect supplementation, this activity became a popular attraction for visitors. Subsequently, demand for this activity increased, and direct supplementation or provisioning with food began. Food items offered included raw whole fish (typically a mixture of false herring [*Harengula clupeola*] and pilchard [*Harengula humeralis*]), filleted fish, and lesser amounts of processed food such as hot dogs, chicken, bread, or various other leftovers. Alterations in behavior and growth rates as a result of the provisioning have been documented in this population. The purpose of this study was to determine how tourism-based human interactions are affecting the overall health of this foraging population and to determine what potential health risks these interactions may create for sea turtles. Juvenile green sea turtles ($n=29$) were captured from four sites off the coast of Barbados, West Indies, and categorized into a group that received supplemental feeding as part of a tour ($n=11$) or an unsupplemented group ($n=18$) that consisted of individuals that were captured at sites that did not provide supplemental feeding. Following capture, a general health assessment of each animal was conducted. This included weight and morphometric measurements, a systematic physical examination, determination of body condition score and body condition index, epibiota assessment and quantification, and clinical pathology including hematologic and biochemical testing and nutritional assessments. The supplemented group was found to have changes to body condition, vitamin, mineral, hematologic, and biochemical values. Based on these results, recommendations were made to decrease negative behaviors and health impacts for turtles as a result of this provisioning.

Key words: Animal welfare, Barbados, biochemistry, *Chelonia mydas*, green sea turtle, health, hematology.

INTRODUCTION

Green sea turtles (*Chelonia mydas*), the largest of the hard-shelled species of sea turtles, have a circumglobal distribution and are found in the waters of over 140 countries worldwide (Groombridge and Luxmoore 1989; Seminoff 2004). Populations have suffered a decline as a result of anthropogenic effects including direct harvest, fisheries bycatch, habitat degradation, injury, and disease. Analysis of globally distributed index sites has led to estimates that nesting green sea turtle populations have decreased by up to 68% over the past three generations, which has resulted in a designation of endangered for the species (IUCN 2014).

Green sea turtles exhibit a unique dietary shift with age. As post hatchings while occupying pelagic waters, they are omnivorous. Once juveniles reach a curved carapace length (CCL; notch to tip) of about 20–25 cm, they migrate to and begin to occupy coastal zones and shift to an herbivorous diet of sea grass and algae (Bjorndal 1985, 1997). There can be some variance in the time this dietary shift occurs depending on geographic location (Cardona et al.

2009). In the Caribbean, the food item of choice for these and larger age classes has been shown to be turtle grass, *Thalassia testudinum* (Mortimer 1976; Eckert and Abreu Grobois 2001).

The foraging aggregations of green turtles in Barbados have been characterized as juvenile to subadult, with a CCL ranging from 31 to 70 cm (Bjorndal and Carr 1989; Aguirre and Balazs 2000; Luke et al. 2004; Labrada-Martagón et al. 2011). At this stage of their life cycle they should have transitioned from the omnivorous feeding habits of pelagic post hatchings to those of the coastal herbivorous life stage. However, in the 1990s a unique relationship between green sea turtles and humans developed along the West Coast of Barbados at Mt. Standfast, St. James. Fishermen began inadvertently provisioning foraging turtles with fish offal discarded from their boats as they cleaned their catch. This unintentional provisioning of sea turtles is a widespread occurrence in other parts of the world as the result of discarding fisheries bycatch (Thomas et al. 2001; Seney and Musick 2007). This activity in Barbados, although initially having arisen as an indirect supplementation, quickly became a popular attraction for visitors. Subsequently, tours to "Swim with Turtles" became advertised, and demand for this activity markedly increased. Direct supplementation or provisioning with food began, and additional feeding sites have been developed. It was estimated that during the peak tourist season at the most popular feeding site, Paynes Bay, over 900 people participated in tours each day. Food items offered included raw whole fish (typically a mixture of false herring [*Harengula clupeola*] and pilchard [*Harengula humeralis*]) and filleted fish, as well as lesser amounts of processed food such as hot dogs, chicken, bread, and various other leftovers (Horrocks et al. 2007).

Alterations in behavior and growth rates as a result of the provisioning have been documented in this population. Supplemented turtles have been shown to spend more time at the surface, predisposing them to watercraft strike (Horrocks et al. 2007). Growth rates of juveniles exceed those found in foraging populations throughout the Caribbean and Pacific, as well as the captive population at the Cayman Turtle Farm (Limpus and Walter 1980; Mendonca 1981; Wood and Wood 1981; Balazs 1982; Bjorndal and Bolten 1988; Collazo et al. 1992; Horrocks et al. 2007).

The purpose of this study was to determine how tourism-based human interactions are affecting the overall health of this foraging population and to determine what potential health risks these interactions may create for sea turtles. The biological hypothesis being tested in this study was that the subset of the green turtle population being provisioned would exhibit changes to their body condition, vitamin, mineral, hematologic, and biochemical values as a result of the supplementation.

MATERIALS AND METHODS

Juvenile green sea turtles ($n=29$) were hand captured while snorkeling from four sites off the coast of Barbados, West Indies, in August 2009 at Oistins Bay (13°03′31.24″N, 59°32′53.47″W), Carlisle Bay (13°05′18.14″N, 59°36′52.20″W), Cobbler's Cove (13°14′33.5394″N, 59°38′44.952″W), and Paynes Bay (13°09′56.28″N, 59°38′17.45″W). The study animals were categorized into a group that received supplemental feeding as part of a tour (SUPPL; $n=11$; Cobbler's Cove and Paynes Bay) or an unsupplemented group (UNSUPPL; $n=18$) that consisted of individuals that were captured at sites that did not provide supplemental feeding (Carlisle Bay and Oistins Bay).

Following capture, a general health assessment of each animal was conducted. This included recording the body weight and morphometric measurements, a thorough systematic physical examination, determination of body condition score (BCS; numeric scale 1–5 assigned), epibiota assessment and quantification, and clinical pathology. Morphometric measurements obtained included curved carapace length notch to notch (CCL - NN), CCL notch to tip (CCL - NT), curved carapace width (CCW), straight carapace length notch to notch (SCL - NN), SCL notch to tip (SCL - NT), straight carapace width (SCW), straight plastron

length (SPL), body depth, and head width. Following measurement, body condition index (BCI) scores were determined for each animal using the equation (weight [kg]/SCL [cm]^3) $\times$10,000 (Bjorndal et al. 2000).

A 22-gauge, 1.5-inch needle fastened to a 20-mL syringe precoated with sodium (Na) heparin was used to collect a blood sample from the dorsal cervical sinus (Owens and Ruiz 1980) of each animal. The venipuncture site was disinfected using Betadine© (VEDCO INC, Stamford, CT, USA) scrub and 70% isopropyl alcohol prior to collection. Twenty mls of blood were drawn from the site and used to perform hematologic and biochemical testing, as well as nutritional analyses.

Samples were immediately stored on wet ice until being delivered to the University of the West Indies (UWI) laboratory for final processing. Because of the remote location of the field site, samples could not be immediately processed in the field. Rather, processing occurred following transport to the UWI laboratory within 6–12 h of sample acquisition. Once in the laboratory, centrifugation was performed to collect plasma. Whole blood and plasma samples were stored in 1–2-mL aliquots in cryovials (VWR International, Radnor, Pennsylvania, USA) at −62°C.

Once at the laboratory, four blood smears were made and the packed cell volume (PCV) was determined using a microhematocrit centrifuge (Zipocrit™, LW Scientific Inc., Lawrenceville, Georgia, USA). Total solids were determined using a portable refractometer (Vital Sine™, Aquatic Eco-Systems, Apopca, Florida, USA). After being air dried, the blood slides were stained (Modified Wright's stain, Hema Tek Stain Pak, Bayer Corporation, Elkhart, Indiana, USA) and placed in dry storage boxes. Total white blood cell counts were estimated from the stained blood slides by totaling the white blood cells counted from 10 fields at 40× and multiplying by 200.

Following CITES permit acquisition, plasma aliquots were sent to the University of Miami, where they were processed on an Ortho Vitros 250 dry slide chemistry analyzer (Ortho, Rochester, New York, USA). Values measured included creatinine (CREA), blood urea nitrogen (BUN), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), calcium (Ca), gamma-glutamyl transpeptidase (GGT), glucose, phosphorus (P), total protein (TP), albumin, globulin, sodium (Na), potassium (K), uric acid (UA), carbon dioxide (CO_2), amylase, lipase, cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL).

Plasma electrophoresis was performed on all samples using SPEP-11 agarose gels and the Beckman paragon electrophoresis system (Beckman-Coulter Corporation, Brea, California, USA) at the University of Miami. Values measured included albumin:globulin ratio, prealbumin, alpha-1 globulins, alpha-2 globulins, beta globulins, and gamma globulins.

All-*trans* retinol (as a measure of vitamin A activity), α-tocopherol (as a measure of vitamin E activity), and carotenoids were measured at the University of Arizona following the protocol outline by McGraw et al. (2008). Vitamin D (25-hydroxycholecalciferol; n552) was analyzed at Boston University School of Medicine using a 25(OH) D3 protein binding assay (Chen et al. 1990).

Trace mineral analyses were performed at the New Bolton Center Toxicology Laboratory, University of Pennsylvania, School of Veterinary Medicine in Kennett Square, Pennsylvania. The samples were analyzed for Ca, copper (Cu), iron, magnesium (Mg), total P, K, Na, and zinc using a NexION ICP-MS (PerkinElmer, Shelton, Connecticut, USA), and the analysis of selenium (Se) was performed on an atomic absorption spectrometer AA800 (PerkinElmer) equipped with a graphite furnace (PerkinElmer).

The mean, median, and standard deviation of all continuous variables and the proportions of all categoric variables were determined. Bivariate associations with the variable "group" (UNSUPPL vs. SUPPL) were determined using the Mann-Whitney *U*-test for continuous variables and the Fisher's exact test for categoric variables. All tests for significance were performed at $\alpha=0.05$ level.

RESULTS

Physical examination and morphometrics

The UNSUPPL turtles weighed less and were smaller for all morphometric measurements (Table 1). The median BCS for UNSUPPL turtles (2.5) was significantly lower ($P=0.001$) than the BCS for SUPPL turtles (3.8). The median BCI for UNSUPPL turtles (1.20) was significantly lower ($P=0.002$) than the median BCI for SUPPL turtles (1.4). Fibropapillomas were not observed in either the SUPPL or UNSUPPL groups. Epibiota, including algae and barnacles, were found on turtles from both the UNSUPPL and SUPPL groups without a statistically significant

TABLE 1. Comparison of the body weight and morphometric measurements of groups of free-ranging green sea turtles (*Chelonia mydas*) in Barbados that were either unsupplemented or supplemented.

	Unsupplemented ($n=18$)		Supplemented ($n=11$)		
	Median	SD	Median	SD	*P* value
Weight (kg)	7.8	3.9	23.3	24.4	**<0.001**
Body condition score	2.5	0.4	3.8	0.8	**0.001**
Body condition index	1.2	0.1	1.4	0.1	**0.002**
CCL, notch to notch (cm)	41.7	6.4	58.3	13.7	**0.002**
CCL, notch to tip (cm)	41.6	6.0	58.7	13.6	**<0.001**
Curved carapace width (cm)	35.3	5.9	51.4	12.8	**<0.001**
SCL, notch to notch (cm)	39.1	5.6	54.4	12.7	**0.002**
SCL, notch to tip (cm)	40.2	6.3	55.6	11.9	**<0.001**
Straight carapace width (cm)	32.0	5.6	43.3	8.9	**<0.001**
Body depth (cm)	15.0	4.3	22.4	5.1	**<0.001**
Straight plastron length (cm)	33.4	5.5	47.3	9.0	**<0.001**
Head width (cm)	6.3	0.6	8.9	1.5	**<0.001**

The bold type indicates values for which $P<0.01$ and are therefore statistically significant.

SD = standard deviation.

difference in amount or type of epibionts between groups.

Hematology

The median total white blood cell count was $10.2\times10^3/\mu L$ for the SUPPL population and $9.8\times10^3/\mu L$ for the UNSUPPL population, with no significant difference seen between the UNSUPPL and SUPPL groups for total white blood cell count. The UNSUPPL population exhibited a significantly higher percentage of eosinophils than the SUPPL population ($P=0.049$; Table 2). No hemoparasites or basophils were observed in any of the sampled population.

The median PCV was significantly higher (32.5%) for the UNSUPPL than the SUPPL group (28%; $P=0.002$). In contrast, the median total solids were significantly higher for the SUPPL population (4.8 g/dL) when compared to the UNSUPPL population (2.9 g/dL; $P=0.001$; Table 2).

Plasma biochemistry and lipid panel

With the exception of CK, K^+, creatinine, CO_2, amylase, LDL, LDH, and CK, all values measured on the Ortho Vitros 250 dry slide chemistry analyzer were significantly higher in the SUPPL group except lipase, which was higher in the UNSUPPL population ($P<0.05$; Table 3). A significant difference was found in lipemic index ($P=0.041$) between the UNSUPPL population and the SUPPL population, with three members of the SUPPL population demonstrating lipemia in contrast to none of the UNSUPPL group (Table 4). The median and standard deviation for the Ca:P ratio in UNSUPPL and SUPPL turtles were 1.59 (0.49) and 1.27 (0.51) respectively, but this difference was not significant. Triglycerides ($P<0.001$), HDL ($P=0.004$), and VLDL ($P<0.001$) were all significantly higher in the SUPPL animals (Table 3).

Plasma protein electrophoresis

Values for total protein ($P<0.001$), albumin:globulin ratio ($P=0.013$), albumin ($P=0.009$), alpha-2 globulins ($P<0.001$), and beta globulins ($P<0.001$), were all found to be significantly higher in the SUPPL populations (Table 5). Seven of the individuals tested had prealbumin bands, five from the UNSUPPL group and two from the SUPPL group.

Vitamin panels

The SUPPL group was found to have significantly higher levels of all-*trans* retinol ($P=0.001$), α-tocopherol ($P=0.008$), and Vitamin D ($P=0.047$), whereas the UNSUPPL population was found to have higher levels of lutein and zeaxanthin ($P=0.001$ and $P=0.004$ respectively; Table 6).

TABLE 2. Comparison of the complete blood cell counts of groups of free-ranging green sea turtles (*Chelonia mydas*) in Barbados that were either unsupplemented or supplemented.

	Unsupplemented ($n=18$)		Supplemented ($n=11$)		
	Median	SD	Median	SD	*P* value
Packed cell volume (%)	32.5	4.5	28.0	4.6	**0.002**
Total solids (g/dL)	2.9	0.5	4.8	0.9	**0.001**
Estimated total white blood cell count	9,800.0	3,260.8	10,200.0	5,281.5	0.529
Lymphocytes	12.5	9.4	12.0	6.1	0.982
Absolute lymphocytes	1,254.0	1,323.1	1,314.0	1,391.8	0.753
Heterophils	23.0	11.5	28.0	21.3	0.311
Absolute heterophils	2,582.0	1,635.9	2,244.0	4,615.9	0.418
Eosinophils	3.0	4.7	1.0	1.9	**0.049**
Absolute eosinophils	242.0	714.0	76.0	343.4	0.104
Monocytes	5.5	6.0	6.0	4.3	0.526
Absolute monocytes	444.0	829.2	690.0	664.5	0.432
Basophils	0.0	0.0	0.0	0.0	—
Absolute basophils	0.0	0.0	0.0	0.0	—

The bold type indicates values for which $P<0.05$ and are therefore statistically significant.
SD = standard deviation.

Mineral panels

Significant differences between the UNSUPPL and SUPPL groups were found in plasma Cu ($P=0.010$), Mg ($P<0.001$), total P ($P=0.011$), and Se ($P=0.002$) values (Table 7). Copper values ranged from 0.163 to 1.60 ppm, with the median for the UNSUPPL group being 0.54 ppm compared to 0.86 ppm (SUPPL). Magnesium levels ranged from 62.8 to 133.0 ppm (117.1 ppm

TABLE 3. Comparison of Ortho Vitros 250 dry slide chemistry analyzer biochemistry panel and lipid panel of groups of free-ranging green sea turtles (*Chelonia mydas*) in Barbados that were either unsupplemented or supplemented.

	Unsupplemented ($n=18$)		Supplemented ($n=11$)		
Analyte	Median	SD	Median	SD	*P* value
Glucose (mg/dL)	62.0	7.8	89.5	16.2	**0.002***
Blood urea nitrogen (mg/dL)	6.0	6.2	90.5	33.5	**<0.001***
Creatinine (mg/dL)	0.3	0.1	0.4	0.1	0.068
Blood urea nitrogen/creatinine ratio	23.3	32.8	222.5	103.8	**<0.001***
Sodium (meq/L)	153.0	5.1	163.5	10.4	**0.042***
Potassium (meq/L)	4.4	0.5	4.2	0.4	0.960
Carbon dioxide (mmol/L)	34.0	4.4	29.0	6.3	0.056
Amylase (U/L)	290.0	108.8	275.5	172.9	0.651
Lipase (U/L)	13.0	10.7	7.0	8.6	**0.008***
Calcium (mg/dL)	8.6	1.2	10.2	1.3	**0.011***
Phosphorus (mg/dL)	5.4	1.0	7.6	2.0	**0.006***
Cholesterol (mg/dL)	96.0	26.1	127.0	38.9	**0.006***
Triglycerides (mg/dL)	72.0	36.3	170.5	125.1	**<0.001***
High-density lipoproteins (mg/dL)	10.0	5.8	18.0	7.7	**0.004****
Very-low-density lipoproteins (mg/dL)	14.0	7.2	34.0	25.1	**<0.001****
Low-density lipoproteins (mg/dL)	69.0	18.8	63.5	15.6	0.547
Uric acid (mg/dL)	0.8	0.1	1.6	0.7	**<0.001***
Total protein (g/dL)	3.8	0.6	5.0	1.0	**0.001***
Aspartate aminotransferase (U/L)	133.0	108.2	333.0	379.5	**0.016***
Alanine aminotransferase (U/L)	9.0	4.8	21.5	32.0	**0.015***
LDH (U/L)	725.0	258.7	837.0	210.9	0.192
Creatine kinase (U/L)	365.0	347.4	395.5	454.8	0.514

Values denoted with * indicate values for which $P<0.05$. Values denoted with ** indicate values for which ** $P<0.01$. Bold faced type indicates that the values are statistically significant.
SD = standard deviation.

TABLE 4. Comparison of hematologic and lipemic indices of groups of free-ranging green sea turtles (*Chelonia mydas*) in Barbados that were either unsupplemented or supplemented. The number contained within () indicates degree of lipemia and/or hemolysis graded on a 0–4 scale where 4 is considered severe.

	Unsupplemented ($n=18$)	Supplemented ($n=11$)	P value
Hemolysis index (1); No. (%)	8 (47.1)	2 (20.0)	0.161
Lipemic index (1); No. (%)	0 (0.0)	3 (30.0)	**0.041**

The bold type indicates values for which $P<0.05$ and are therefore statistically significant.

vs. 80.9 ppm for UNSUPPL and SUPPL, respectively). Total P levels ranged from 73.6 to 243.0 ppm, with the median for the UNSUPPL group being 108 ppm and the mean for the SUPPL group being 161.50 ppm. Total Se levels ranged from 0.040 to 0.346 ppm and the median for the UNSUPPL group was lower (0.08 ppm) when compared to the median for the SUPPL group (0.20 ppm).

DISCUSSION

Morphometrics

Luke et al. (2004) reported a difference in size between UNSUPPL green turtles and green turtles that were fed at the "Swim with the Turtle" sites in Barbados. They also showed that growth rates of the latter exceeded ranges established in other regions whereas the ranges for the former did not (Schmidt 1916; Limpus and Walter 1980; Mendonca 1981; Balazs 1982; Bjorndal and Bolten 1988; Collazo et al. 1992; Luke et al. 2004). Both the SUPPL and UNSUPPL groups in our study contained animals that measured below the minimum size in Luke et al. (2004). However, the turtles in the SUPPL group were still significantly larger, with significantly higher BCIs ($P<0.001$) than the turtles in the UNSUPPL group, most likely because of the provisioned diet they received. Data are unavailable to confirm that the UNSUPPL and SUPPL turtles are of the same age class. Further studies using skeletochronology of mortalities, for example, are recommended to determine if this is the case.

Effects of higher dietary loads of protein on growth rates in juvenile green turtles has been documented through studies at the Cayman Turtle Farm, where it was found that 5-kg animals fed pelleted diets consisting of 25–35% protein had nitrogen digestibility of 82–88% compared to free-ranging turtles weighing 8-kg on a natural diet, which had nitrogen

TABLE 5. Comparison of plasma protein electrophoresis profiles of groups of free-ranging green sea turtles (*Chelonia mydas*) in Barbados that were either unsupplemented or supplemented.

	Unsupplemented ($n=18$)		Supplemented ($n=11$)		
Analyte	Median	SD	Median	SD	P value
Total protein (g/dL)	3.80	0.62	5.00	0.96	**<0.001****
Prealbumin (g/dL)	0.02	0.03	0.08	0.11	**0.024***
Albumin (g/dL)	0.89	0.22	1.71	0.64	**0.009****
Alpha-1 globulins (g/dL)	0.20	0.12	0.24	0.08	0.646
Alpha-2 globulins (g/dL)	0.40	0.11	0.60	0.13	**<0.001****
Beta globulins (g/dL)	1.24	0.24	1.86	0.29	**<0.001****
Gamma globulins (g/dL)	0.56	0.13	0.42	0.10	**0.035***
Albumin:globulin ratio	0.40	0.08	0.63	0.17	**0.013***

Values denoted with * indicate values for which $P<0.05$. Values denoted with ** indicate values for which ** $P<0.01$. Bold faced type indicates that the values are statistically significant.
SD = standard deviation.

TABLE 6. Comparison of vitamin panels of groups of free-ranging green sea turtles (*Chelonia mydas*) in Barbados that were either unsupplemented or supplemented.

Analyte	Unsupplemented ($n=18$)		Supplemented ($n=11$)		P value
	Median	SD	Median	SD	
All-*trans* retinol (μg/mL)	0.234	0.37	1.706	0.91	**<0.001**
α-tocopherol (μg/mL)	0.029	0.04	0.457	0.50	**<0.001**
Vitamin D (μg/mL)	10.000	3.46	12.500	5.91	0.125
Lutein (μg/mL)	0.351	0.39	0.001	0.12	**<0.001**
Zeaxanthin (μg/mL)	0.141	0.13	0.014	0.07	**0.008**

Bold faced type indicates values for which $P<0.01$ and are therefore statistically significant.
SD = standard deviation.

digestibility of 15%. As the turtles grew, digestibility for turtles in both environments increased (86–89% for 23-kg farmed turtles versus 39% for 30-kg free-ranging animals) but they were still markedly higher for the turtles fed high-protein pellets versus those feeding on the natural food source, *T. testudinum*. A number of factors contribute to this increased digestibility. For example, natural diets of *T. testudinum* contain more fiber (44%) and less nitrogen (3.4%) than pelleted diets (4% fiber and 4.8–6.6% nitrogen; Bjorndal 1985). Additionally, because the nutritional contents of *T. testudinum* are contained within cell walls, those nutrients are not available until they reach the cecum, where microbial fermentation digests cell walls; pellets, on the other hand, can be immediately absorbed in the stomach and small intestine (Wood and Wood 1981; Brown and Brown 1982; Bjorndal 1985; Bjorndal and Bolten 1988; Wood 1991). The higher digestibility of the higher-protein diets leads to more rapid growth rates and earlier reproductive maturity. Although animals in the SUPPL group were not fed pelleted food, they were typically fed raw fish, which is a high protein source with likely a high digestibility.

Hematology

Although total white blood cell count ranges for both the UNSUPPL and SUPPL groups in this study fell outside previously established reference ranges for green sea turtles (Anderson et al. 2011; Jacobson et al. 2008), the means for both groups were

TABLE 7. Comparison of a mineral panel of groups of free-ranging green sea turtles (*Chelonia mydas*) in Barbados that were either unsupplemented or supplemented.

Analyte	Unsupplemented ($n=18$)		Supplemented ($n=11$)		P value
	Median	SD	Median	SD	
Calcium (ppm)	106.00	15.85	108.50	17.36	0.269
Copper (ppm)	0.54	0.16	0.86	0.41	**0.010***
Iron (ppm)	3.42	0.59	3.41	0.87	0.744
Magnesium (ppm)	119.00	11.92	82.55	12.05	**<0.001****
Total phosphorus (ppm)	108.00	22.69	161.50	58.85	**0.011***
Potassium (ppm)	187.00	22.52	178.00	17.53	0.160
Sodium (ppm)	3,080.00	161.43	3,150.00	118.93	0.339
Zinc (ppm)	1.57	0.31	1.50	0.31	0.880
Selenium (ppm)	0.08	0.05	0.20	0.09	**0.002****

Values denoted with * indicate values for which $P<0.05$. Values denoted with ** indicate values for which ** $P<0.01$. Bold faced type indicates that the values are statistically significant.
SD = standard deviation.

within previously established ranges. Thus, the difference in values was not considered clinically significant.

Total protein values for both the UNSUPPL and SUPPL groups fell within previously reported ranges for green sea turtles (Bolton and Bjorndal 1992; Mader 1996, 2006; Labrada-Martagón et al. 2011) with some of the mean values for the SUPPL group being just above the means recorded for greens sampled in Florida (Jacobson et al. 2008). Although for the most part these values fell within previously reported ranges, the SUPPL population had higher values for this analyte, which is to be expected because this group was consuming a provisioned diet higher in protein than the UNSUPPL group, which was likely foraging on mainly plant food sources.

Plasma biochemistry and lipid panel

The AST and ALT levels for the UNSUPPL population fell within previously established reference ranges (Bjorndal and Bolten 1992; Mader 1996, 2006; Labrada-Martagón et al. 2011), but the means for both analytes in the SUPPL population were found to be higher than previously reported means (Bjorndal and Bolten 1992; Labrada-Martagón et al. 2011; Jacobson et al. 2008). AST is not considered to be a liver-specific enzyme, as it is found in highest concentrations in both hepatocytes and muscle cells, with a wide distribution throughout the reptilian body (Thrall 2006); for that reason elevated AST levels in the plasma of reptiles are not considered indicative of liver disease (Anderson et al. 2013). However, because no elevations were noted in CK levels in either the UNSUPPL or SUPPL group, the significant elevation in AST levels within the SUPPL group is most likely of liver rather than muscle origin, and may indicate that these animals are in the early stages of development of liver disease. To confirm liver disease, a liver biopsy would be necessary. One disease process of liver origin commonly diagnosed in captive reptiles is hepatic lipidosis. One of the main factors leading to development of hepatic lipidosis in reptiles is inappropriate diet. Other factors include obesity, lack of exercise, anorexia, and vitellogenesis (Divers and Cooper 2000; Mader 2006; Ahlstrom et al. 2012). Hepatic lipidosis has been confirmed in a wide range of reptilian species, including in clinical cases diagnosed by liver biopsy by the authors in greens undergoing rehabilitation that were maintained on a primarily seafood diet. Additionally, among sea turtles, it has also been confirmed in free-ranging greens sampled from acute fisheries mortality events in Argentina (Rees et al. 2008) and in a Kemp's ridley (*Lepidochelys kempii*) sea turtle in a rehabilitation setting (Ahlstrom et al. 2012). The high-protein diet and readily available sources of calories being offered to these animals and resulting obesity, combined with the decreased activity required for foraging, places them at a high risk for the development of hepatic lipidosis. As chelonians tend to have a higher tolerance for lipidosis than other reptiles, clinical signs may not manifest until the disease has progressed extensively (Silvestre 2013) or until the animals undergo a stress event such as migration. Therefore, it is imperative that these values be monitored in a regular health assessment program, and efforts be implemented to adjust supplements offered to a lower-fat and lower-protein product to lessen the chances of obesity and predisposition to hepatic lipidosis.

Uric acid concentrations did not fall outside previously established reference ranges (Jacobson et al. 2008), but the SUPPL group had a significantly higher concentration of uric acid than did the UNSUPPL group. Uric acid is the end product of protein catabolism, and because the SUPPL group within this study is regularly provisioned with fish and other animal protein sources, this difference is most likely due to the increased dietary intake of purine-based proteins. If fed long enough, a high-protein diet may predispose these turtles to the development of

gout (Frye 1991; Mader 2006). Long-term health monitoring will be important to see if these elevations are biologically significant. Elevations in uric acid are also seen secondary to increased dietary intake, a postprandial blood draw, and renal compromise (Mader 2006; Anderson et al. 2011).

Glucose values for our study populations were within normal ranges (Bolten and Bjorndal 1992; Mader 1996, 2006). Although statistically significant, the mild elevation is too slight to be attributed to stress or diabetes mellitus (Frye 1991; Campbell 1996; Mader 2000). These elevated levels relative to the UNSUPPL group are most likely the result of increased caloric intake due to the provisioning.

Mean Ca and P levels for both the UNSUPPL and SUPPL populations in this study did not fall outside of previously reported references ranges for green sea turtles in the Bahamas, but values for the UNSUPPL group did fall below established means for green sea turtles in Florida (Bolten and Bjorndal 1992; Jacobson et al. 2008). The Ca:P ratios were normal for both groups. The higher Ca and P levels in the SUPPL group versus the UNSUPPL may simply reflect a higher adjusted homeorhesis in the SUPPL turtles, because these analytes are so tightly regulated.

The Na and K levels for both study populations fell within previously established reference ranges for green sea turtles; however, the SUPPL group had higher values than the UNSUPPL group. Elevations in Na levels are a result of dehydration, increased dietary intake (Mader 2006), or use of Na heparin during the sample acquisition process. As venipuncture methods for both the UNSUPPL and the SUPPL groups were standardized, it is highly unlikely that the increased levels seen in the SUPPL compared to the UNSUPPL populations were related to sample handling. As these animals showed no clinical signs of dehydration, the most likely cause of the difference in Na levels seen was the increased dietary intake by the SUPPL populations, who, in many cases, were fed processed foods.

The BUN values for both the UNSUPPL and SUPPL groups fell outside previously reported reference ranges for green sea turtles (Bolten and Bjorndal 1992; Jacobson et al. 2008). However, the SUPPL population had a median that extended 10 times beyond the median demonstrated for the UNSUPPL population, and the values for BUN in the SUPPL population were at the high end of established reference ranges. BUN reflects catabolism and is typically high in carnivorous turtles and low in herbivores like green sea turtles. The elevated BUN levels found in the SUPPL group are most likely the result of a provisioned diet high in protein.

Cholesterol and triglyceride levels for both the UNSUPPL and SUPPL populations fell within reference ranges established in other foraging green sea turtle populations (Bolten and Bjorndal 1992; Jacobson et al. 2008; Labrada-Martagón et al. 2011). Recent studies have demonstrated that elevated serum triglycerides are a reliable indicator of recent feeding in green sea turtles (Price et al. 2013). The increased levels in the SUPPL population may be because these animals were more likely to have recently consumed a high-calorie meal prior to capture and venipuncture. Triglyceride levels were found to be positively correlated with body mass in red-sided garter snakes (*Thamnophis sirtalis parietalis*; Whittier and Mason 1996), and our SUPPL populations were significantly larger than our UNSUPPL populations. Cholesterol is monitored in humans as an indicator of heart disease. Little information is available regarding heart disease in reptiles, and in a study examining arterial lesions in 200 captive reptiles that succumbed in captivity, intimal lesions containing lipids were uncommon (Finlayson and Woods 1977). Three animals in the SUPPL group had an increased lipemic index. This is most likely the result of the recent ingestion of a meal. Triglyceride and cholesterol levels as they relate to

disease states in reptiles warrant further examination as limited information is available on how or if these analytes relate to disease processes.

Plasma protein electrophoresis

Seven animals tested were found to have a prealbumin fraction with values ranging from 0.01 to 0.35. In previous studies, prealbumin fractions have been found in green, leatherback, and loggerhead sea turtles, but they were not common or found in high concentrations (Gicking et al. 2004; Deem et al. 2006, 2009; Jacobson et al. 2008; Perrault et al. 2014). The values for our study population were higher than values previously reported for greens in Florida. There was no significant difference between the UNSUPPL and SUPPL groups. The prealbumin band has been studied extensively in other species (Chang et al. 1999; Harr 2002) but was not a focus of our study. Our sample population fell within previously established reference ranges for green sea turtles in the southern Bahamas for albumin, with mean values for the SUPPL group falling within range for greens in Florida but values for the UNSUPPL lower than ranges reported in the same Florida population (Jacobson et al. 2008). The albumin:globulin ratio for our sampled populations was lower than values reported for green sea turtles in Florida (Jacobson et al. 2008). The SUPPL group had significantly higher values than the UNSUPPL group. The mean beta globulin levels in both our SUPPL and UNSUPPL populations were higher than previously reported values in green sea turtles sampled in Florida (Jacobson et al. 2008). The SUPPL group had a higher beta globulin level. The mean gamma globulin levels in both our UNSUPPL and SUPPL groups were lower than previously reported for greens in Florida (Jacobson et al. 2008). The UNSUPPL group had a higher level of gamma globulins. Three classes of immunoglobulins have been identified in the green sea turtle (Benedict and Pollard 1972). As immunoglobulins are the primary component of gamma globulins, an increase in gamma globulin levels would be expected with antibody production (Graczyk et al. 1995) in foraging sea turtles. The low levels of gamma globulins found in our study populations indicate that our study animals most likely had limited exposure to antigens, with the SUPPL group having even less exposure than the UNSUPPL, perhaps as a result of their increased foraging site fidelity related to the supplementation activities. Although no systematic studies on site fidelity have been undertaken, tagged turtles are repeatedly sighted in the same supplemented areas, and an injured animal removed on two occasions from a west coast supplemented site to two different east coast locations returned to the same west coast site within 2 days of release (J. Horrocks, pers. comm).

Vitamin panels

Vitamin A plays a role in many critical physiological processes, such as appropriate skin, mucous membrane, vision, immune system, and reproductive system function (Hand et al. 2000). Vitamin A is found in herbivorous diets typically in the form of beta-carotene, which is then converted to retinol by the liver in species that have the ability to do so. Carnivorous and omnivorous animals consuming whole-prey diets are consuming preformed vitamin A. The SUPPL turtles demonstrated significantly higher levels of vitamin A, measured as all-*trans* retinol, perhaps because they are being provisioned in many cases with whole fresh fish and thus consuming a high level of preformed vitamin A. To confirm this, future studies should be performed to investigate levels of circulating esters in these study populations.

Vitamin E functions within the body as an antioxidant, primarily involved in the protection of cell membranes from metabolism and lipid oxidation byproducts (Dierenfield 1989). The SUPPL population in our study had significantly higher levels of Vitamin E ($P<0.001$) than the UNSUPPL population,

most likely because of the higher fat intake and thus uptake of fat-soluble nutrients that may otherwise not be available.

Lutein and zeaxanthin are carotenoids found in green leafy plants that serve antioxidant properties primarily in the eye. The UNSUPPL group in this study had significantly higher levels of these carotenoids, likely because they are feeding on a natural, primarily herbivorous diet that would include substantial quantities of *T. testudinum*, whereas the SUPPL populations are consuming food items high in animal protein and carbohydrates.

Mineral panels

Magnesium plays a key role in a number of physiological processes, including cell membrane function and bone formation (Thrall 2006). It is also thought to potentially function in parathyroid hormone regulation, and in a recent study examining the diagnostic value of certain analytes in cases of renal insufficiency, including Mg, elevated Mg levels were found to be a useful diagnostic indicator (Thrall 2006; Jones personal communication). The UNSUPPL population in this study had significantly higher Mg levels than the SUPPL population. Further investigation and monitoring would be needed to determine if these UNSUPPL animals suffer from renal insufficiency or if their diet is simply higher in Mg.

Increased Cu levels can be the result of increased mobilization of stored Cu or the result of short-term exposure through ingestion (Caurant et al. 1999; Anan et al. 2001; Stockham and Scott 2008; Rios-mena-Rodriguez 2011). Herbivorous green sea turtles tend to have lower metal levels when compared to carnivorous or omnivorous species, as trace elements typically bioaccumulate (Storelli et al. 1998; Godley et al. 1999; Ikonomopoulou et al. 2011). As the UNSUPPL population of greens in this study is feeding on a more natural diet primarily composed of sea grasses and algae, they would be expected to have lower Cu values than the SUPPL greens in this study that are consuming large portions of fish offal combined with other animal proteins.

Phosphorus levels for the UNSUPPL and SUPPL populations did not fall outside of previously established reference ranges for foraging green sea turtles (Bolten and Bjorndal 1992), but the SUPPL population had a significantly higher level of P ($P=0.006$) than the UNSUPPL population. Elevations in P levels in reptiles are affected by nutrition and husbandry and are typically higher in animals with renal insufficiency. In our experience based on clinical rehabilitation cases, the difference in plasma concentrations seen here are most likely due to diet, as, in captivity, higher P levels have been documented in turtles on a seafood diet versus a vegetable diet. Repeated measures and renal biopsies would need to be undertaken and compared to determine if renal insufficiency was developing in this SUPPL group.

The SUPPL group had higher levels of Se than the INSUPPL group. As Vitamin E and Se have a synergistic relationship, the higher levels observed in the SUPPL group could be a result of the high levels of vitamin E demonstrated in this group (Watts 1994). Higher levels of Se in the SUPPL animals could also be the result of a diet that is higher in Se. Limited information is available on Se ranges in green sea turtles and their prey items and this warrants further investigation.

CONCLUSIONS

The SUPPL turtles have been habituated to view humans as a food source. This exposes animals to increased risk of injuries such as boat strikes in the short term, and in the longer term to capture as they migrate as subadults and adults through regional waters where open harvests for sea turtles still exist (Horrocks et al. 2007). For these reasons alone, supplementation may have a negative impact on endangered green turtles around Barbados. However, as this industry has become extremely popular and is a significant source of income to

participating tour operators, elimination of this activity is unlikely. Alternatives and recommendations have been provided by Horrocks et al. (2007), including the restriction of numbers of sites and of boats in the areas, the establishment of a code of conduct for both tour participants and operators, and the offering of alternative sources of food that are comparable to the natural diet of greens in the wild. The authors support these recommendations. An additional recommendation to decrease negative behaviors as a result of provisioning would be to set up potential feeding stations where more natural food items are provided that are anchored and set up independent of people and boats, that are stocked once per day, and for which set times are provided during which boats can pass to view turtles from the vessel. However, these feeding sites would have to be carefully regulated, as even a well-balanced diet fed in excess could result in obesity and health problems for these animals.

In addition to the behavioral impacts, our study has shown that provisioning is altering important biochemical parameters in the SUPPL group that have the potential to affect several disease processes, including liver disease, gout, and potential cardiovascular issues, if not ceased or altered to provide a nutritionally appropriate diet for green sea turtles. Additionally, a health monitoring program that includes annual health assessments should be established in this SUPPL population to determine the long-term health effects and if these altered parameters are truly detrimental and will lead to disease manifestation.

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