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PLASMA BIOCHEMISTRY PROFILES OF JUVENILE GREEN TURTLES (*CHELONIA MYDAS*) FROM THE BAHAMAS WITH A POTENTIAL INFLUENCE OF DIET

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ABSTRACT: Plasma biochemistry profiles aid health assessment of marine turtles, but knowledge of the influence of regional biological factors (e.g., habitat, diet) on marine turtle blood plasma values is limited. To investigate the influence of diet on plasma biochemistry values in juvenile green turtles (*Chelonia mydas*), we used carbon and nitrogen stable isotopes to provide a quantitative estimate of forage items in green turtles feeding at two distinct areas (Bonefish Hole and South Flats) in Bimini, Bahamas. Plasma samples were obtained from 13 turtles in Bonefish Hole (a mangrove tidal estuary) and 15 turtles in South Flats (an open water seagrass bed) in 2018. All turtles appeared outwardly healthy. Sessile filter feeders contributed the largest proportion of diet in Bonefish Hole, and seagrass contributed the highest proportion of diet in South Flats. Turtles at Bonefish Hole presented significantly lower cholesterol, total protein, phosphorus, triglycerides, and aspartate transaminase compared to South Flats. Across all turtles, those feeding primarily on red algae presented the highest uric acid and alkaline phosphatase, and turtles with a seagrass-dominated diet had the highest cholesterol. Understanding dietary influence on plasma biochemistry may help explain variances seen in local health and nutritional evaluations, and the trends reported can aid the interpretation of plasma analyte values in marine turtles.

Key words: Bahamas, *Chelonia mydas*, ecosystem health, foraging ecology, plasma biochemistry, stable isotope.

INTRODUCTION

Plasma biochemistry is an effective analytical tool for monitoring the health and condition of wildlife (Aguirre and Balazs 2000). Studies focusing on biochemistry profiles have identified specific analytes that may influence physiology, metabolic state, and health status in both terrestrial and marine species (Tryland 2006). In marine ecosystems, biochemistry profiles have been used to evaluate the health and nutritional status for aquatic mammals (Schwacke et al. 2009), fish (Harms et al. 2002), and marine turtles (Aguirre et al. 1995). For marine turtles, plasma biochemistry reference intervals have been established for wild populations to serve as a clinical baseline aiding the interpretation of health status. We define healthy as being overtly free from disease and a functional member of the population (Flint et al. 2010).

Plasma analyte values outside reference intervals can indicate chronic abnormalities (Stacy et al. 2018), infectious disease (Aguirre and Balazs 2000), stress (Deem et al. 2009), or other physiological changes (Flint et al. 2010). However, variation in blood plasma analytes can also be found in clinically stable individuals, reflecting differences in ecological and environmental factors that influence individual physiology (Espinoza-Romo et al. 2018). A limited number of studies have explored how different factors such as size (Bolten and Bjorndal 1992), gender (Hamann et al. 2006), habitat, genetics (Herbst and Jackson 2003), migratory status (Stamper et al. 2005), and diet (Whiting et al. 2007) of marine turtle

species can influence blood plasma analytes at an individual level.

Diet of marine turtles can influence plasma analytes due to physical and chemical differences of forage items, including concentrations of metabolites (Hay et al. 1987), elements, and macronutrients (Bjorndal 1980). These differences can affect marine turtle food intake, digestion, and nutrient deficiencies, which are reflected in plasma analyte values (Whiting et al. 2007). Understanding biochemical responses in marine turtles to different diets can assist interpretation of health and nutritional evaluations (Page-Karjian et al. 2015). However, studies directly exploring the influence of diet on plasma biochemistry in marine turtles are mainly limited to captive green turtles (*Chelonia mydas;* Stringer et al. 2010; Bloodgood et al. 2019) and captive Kemp's ridley turtles (*Lepidochelys kempii*; Anderson et al. 2011). Only one study to date has investigated the influence of different diets on plasma biochemistry values between ecologically distinct foraging populations of wild green turtles (Whiting et al. 2007). Gastric lavage was used in the Whiting et al. (2007) study to determine the diet of turtles and compared plasma analyte values between an algal- and a seagrass-dominated diet. However, because wild green turtles have been reported to consume other forage items, including animals (e.g., tunicates, sponges, small crustaceans; Amorocho and Reina 2007), studies exploring how diet influences plasma analytes should consider the full array and proportion of forage items consumed by turtles, not just algae and seagrass, to fully understand how variability in diet may contribute to plasma analyte variability.

We considered the contribution of different forage items to the diet of wild juvenile green turtles at two distinct foraging grounds in Bimini, Bahamas, and their influence on plasma biochemical profiles. Carbon and nitrogen stable isotopic analyses were used to assess individual green turtle diet because it provides a quantitative dietary description as opposed to a qualitative list of diet components in a lavage sample as used in Whiting et al. (2007). Reporting baseline blood plasma profiles for wild marine turtle populations and exploring potential dietary influences can facilitate comparisons among populations and detection of changes in nutritional status, thus optimizing health assessments. These data will enhance our understanding of the implications of future anthropogenic and environmental changes affecting prey composition and habitat quality on marine turtle nutrition and health (Deem et al. 2006).

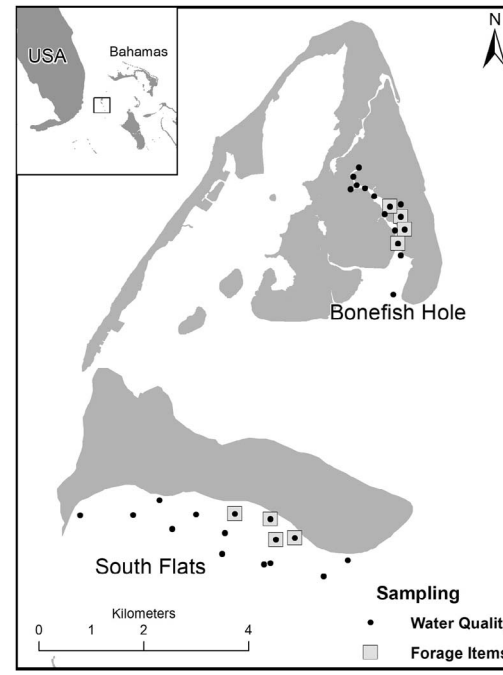

FIGURE 1. Study site (Bimini, Bahamas) showing regions where forage items and water quality samples were collected in July 2018 at Bonefish Hole and South Flats for a study of the influence of diet on plasma biochemistry values of green turtles (*Chelonia mydas*).

MATERIALS AND METHODS

Study site

Bimini, Bahamas (25°44′N, 79°16′W) located on the northwest side of the Great Bahamas Bank, approximately 86 km east of Miami, Florida, US, comprises two small islands (North and South Bimini) separated by a 0.15-km wide channel (Fig. 1). Since 1997, North Bimini has experienced extensive urbanization with development of a vast tourist complex, via site clearing, mangrove

cutting, and channel dredging (Trave and Sheaves 2014). Two distinct green turtle foraging sites exist in Bimini: a mangrove tidal estuary of ca. 0.22 km^2 at the north island, and an open water coastal seagrass bed at the south island (Bonefish Hole [BH] and South Flats [SF], respectively; Fig. 1; Gillis et al. 2018). Green algae and turtle grass (*Thalassia testudinum*) are the most widely distributed green turtle forage groups throughout BH and SF, and a high concentration of red algae is found at BH (Fuentes et al. 2019).

Environmental parameters

Fifteen sites were randomly selected at each of the foraging grounds to assess habitat quality in July 2018. We measured pH and dissolved oxygen (mg/L) at each location using a water quality YSI-sonde (Pro-plus Instrument, Xylem Inc., Rye Brook, New York, USA). Water samples (1 L) were hand-pumped and filtered through 0.7-µm glass fiber filters (Whatman, Maidstone, UK: GF/F grade, precombusted at 450 C) using a precleaned Nalgene filter tower (Nalge Nunc International Corporation, Rochester, New York, USA). Samples were stored in acid-washed polycarbonate bottles (1 L), transported on ice in the dark, and then frozen within 3 h until further analyses. Samples were examined for dissolved inorganic nutrients including nitrate (mg N/L), nitrite (mg N/L), and ammonium (µg N/L) on an Astoria Analyzer (Astoria-Pacific, Inc., Clackamas, Oregon, USA) according to established methods (Mann et al. 2012) at the University of New Hampshire Water Quality Analysis Laboratory (Durham, New Hampshire, USA). Dissolved organic carbon (DOC) was analyzed via high-temperature combustion on a Shimadzu TOC-L CPH (Shimadzu Corporation, Kyoto, Japan; Mann et al. 2012; Johnston et al. 2018). We calculated DOC as the mean of three and five injections using a six-point standard curve using established protocols (Spencer et al. 2014), and the coefficient of variance was always $<2\%$ (Luzius et al. 2018). Fecal coliform counts were determined using a membrane filtration method by Biosan Laboratories (Warren, Michigan, USA). We filtered 100 mL of each sample through a 0.45-µm sterile filter, and the filter was aseptically placed onto an M-Endo plate (Remel™, Lenexa, Kansas, USA) and incubated for 4 (SD=2) h at 33.5 (SD=0.5) C. The number of bacteria colonies on the membrane filter was counted and reported as colony-forming units (cfu)/100 mL. Fecal coliform results presented here are likely an underestimation of in-water values because freezing water samples can decrease coliform counts. Our results still allow for comparison between sites because our methodology was consistent across both areas.

Turtle capture

Green turtles were captured during two field trips in July and August 2018 using the rodeo technique (Limpus and Walter 1980). Upon capture, turtles were brought on the boat for processing. We measured straight and curved carapace lengths (SCL and CCL, respectively) to 0.1 cm from the nuchal notch to the posterior-most edge of the marginal scutes using calipers and a flexible measuring tape, respectively (Bolten 1999). Body weight (±0.1 kg [BW]) was measured using a hanging balance (PHS100, Pesola, Schindellegi, Switzerland). Turtle life stage was determined by CCL, where individuals with CCL <65.0 cm were classified as juveniles (Flint et al. 2010). Each captured turtle was tagged with one uniquely coded Inconel flipper tag on the trailing edge of each front flipper (Style 681, National Band and Tag Company, Newport, Kentucky, USA).

Turtle health assessment

A physical examination was completed on each individual turtle (Flint et al. 2010), where visual body condition estimates and the presence of external parasites, leeches, barnacles, and lesions were recorded. The body condition index (BCI) was calculated using the standard equation $BCI=(BW/SCL^3)\times 10{,}000$ (Bjorndal et al. 2000). Captured turtles were checked for fibropapillomatosis (FP; Work and Balazs 1999) based on the presence of external tumors. Blood samples (5.0–10.0 mL) were collected (Owens and Ruiz 1980). Samples were taken using heparinized evacuated syringes fitted with sterile 22 gauge 40-mm needles, transferred to 10 mL vacutainer tubes, and immediately placed in coolers (4 C). Samples were centrifuged (PowerSpin™ LX Centrifuge, UNICO, Dayton, New Jersey, USA) at $1{,}400 \times G$ for 10 min within 7 h of collection. Plasma was separated and frozen in cryovials (–20 C) for 12 wk until analysis. The chemistry analyses were performed using a Vitros 250 chemistry analyzer (Ortho, Rochester, New York, USA) at the University of Miami Avian and Wildlife Laboratory, Miami, Florida. The following plasma variables were analyzed: alkaline phosphatase (ALP), amylase, aspartate transaminase (AST), creatine kinase (CK), calcium, cholesterol, gamma-glutamyl transferase, potassium, lipase, magnesium, sodium, chloride, carbon dioxide, glucose, urea, anion gap, osmolality, phosphorus, uric acid, total protein, and triglycerides. We report analytes of specific interest based on the potential to differentiate populations (Whiting et al. 2007) or health status (Flint et al. 2010) and to limit parameters that could be influenced by sampling error (Flint et al. 2019).

Turtle foraging strategy

Epidermis samples (i.e., skin tissue) were collected from the dorsal surface of the turtles' necks using a sterile steel razor blade (Gillis et al. 2018). Potential green turtle forage items (Gillis et al. 2018) were collected from four randomly selected locations at each foraging ground with high turtle density (Fuentes et al. 2019; Fig. 1). Nonplant samples were preserved in dry salt, and plant samples were pressed until analysis.

Samples were cleaned of residual dry salt and dried at 70 C. Dried samples were homogenized and measured for analysis (Gillis et al. 2018). Carbon and nitrogen stable isotopic values and elemental concentrations were measured with Continuous Flow Elemental Analyzer Isotope Ratio Mass Spectrometry at the University of South Florida, College of Marine Science, Stable Isotope Biogeochemistry Laboratory (St. Petersburg, Florida, USA) using standard procedures (Werner et al. 1999). Isotope compositions were measured on a ThermoFinnigan DeltaV+ IRMS-FlashIRMS Fast Oven EA-ConFLo IV system (Thermo Fisher Scientific, Waltham, Massachusetts, USA), are reported in per mil (‰) notation, and are scaled to Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric air (^{15}N). Secondary reference materials (US National Institute of Standards and Technology [Gaithersburg, Maryland, USA; NIST] 8574 $\delta^{13}C$=+37.63±0.10‰, $\delta^{15}N$=+47.57±0.22‰, %N=9.52, %C=40.81, C:N [molar]=5.0; NIST 8573 $\delta^{13}C$=–26.39±0.09‰, $\delta^{15}N$=–4.52±0.12‰ %N=9.52, C=%40.81, C:N [molar]=5.0; Werner and Brand 2001; Qi et al. 2003; Coplen et al. 2006) were used to normalize raw measurements to the Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric air ($\delta^{15}N$) scales and to calibrate elemental N, C, and C:N. Measurement uncertainties, expressed as ±1 SD of n=56 measurements of a laboratory reference material (NIST 1577b $\delta^{13}C$=–21.69±0.14‰, $\delta^{15}N$=7.83±0.16‰, N=9.95±0.48, C=48.04±.71%, C:N [molar]=5.63±0.27), were ±0.14‰ for $\delta^{13}C$±0.23‰ for $\delta^{15}N$, ±3.79% RSD for %N, ±4.97%RSD for %C, and ±1.97% RSD for C:N.

Statistical analysis

Environmental parameters, turtle size, BCI, plasma analytes, $\delta^{13}C$, and $\delta^{15}N$ were compared between BH and SF using Welch's t-test due to unequal sample sizes between location. For parameters without a normal distribution, a Mann-Whitney U-test was used. A Bayesian mixing model package for R (MixSIAR; Moore and Semmens 2008; Stock and Semmens 2016) was used to establish the probable contributions of each forage group to the diet of turtles at each foraging site. Prior to analysis with MixSIAR, putative prey items were grouped into categories based on life history similarities (Lemons et al. 2011). Nonplant species (e.g., Demospongiae) were grouped together as sessile filter feeders. The two dominant algal groups (red and green algae) within the forage sites were grouped separately due to differences in photosynthetic pathways that could affect isotopic signatures (Beer and Israel 1986; Cole and Sheath 1990). All species of seagrass were grouped together. Green turtle discrimination factors for skin (0.17±0.03‰ for $\delta^{13}C$ and 2.80±0.11‰ for $\delta^{15}N$; Seminoff et al. 2006) were applied to account for isotopic discrimination during digestion. Correlations between turtle size, plasma analytes, and epidermis isotope values were evaluated using Spearman rank correlations. Significant correlations were identified at $P<0.05$. Individual turtle diet determined via MixSIAR was separated into foraging groups based on which prey items contributed most to individual diet. All values are reported as mean (SD), unless otherwise indicated.

RESULTS

Environmental parameters

Summary information for environmental parameters is presented in Supplementary Materials (Table S1). The values for pH, dissolved oxygen, and ammonium were significantly lower, and DOC concentration was significantly higher in BH compared to SF. A broader range of DOC concentrations were exhibited in BH because samples were taken throughout the mangrove tidal estuary. The lowest DOC concentration (1.67 mg/L) was found at the entrance of BH, with the highest marine influence, and the highest concentration of DOC (4.54 mg/L) was located at the farthest point sampled from the entrance of BH. There were higher concentrations of fecal coliforms in BH (29.52±23.75 cfu/100 mL) than SF (8.50±8.70 cfu/100mL).

Turtle capture

Twenty-eight green turtles were captured during the two sampling trips (BH, n=13; SF, n=15). All turtles were classified as juveniles. The SCL, CCL, and BW of captured turtles indicated that they were significantly different between the two foraging grounds (Table 1).

TABLE 1. Descriptive statistics for size, body condition index, and plasma biochemistry values of juvenile green turtles (*Chelonia mydas*) captured from two different foraging locations in Bimini, Bahamas, in July and August 2018 for a study of the influence of diet on plasma biochemistry values. Bold data indicates significant differences between the two sites. The body condition index was calculated using the equation derived from Bjorndal et al. (2000).

		Bonefish Hole			South Flats			
Parameter[a]	Unit	Mean (SD)	Median	Range	Mean (SD)	Median	Range	*P* value
CCL	**cm**	**40.1 (5.5)**	**41.2**	**25.0–46.8**	**45.4 (5.3)**	**48.0**	**34.2–52.5**	**0.015[b]**
SCL	**cm**	**37.6 (5.4)**	**38.6**	**22.2–44.1**	**43.1 (5.1)**	**44.8**	**32.2–49.8**	**0.011[b]**
BW	**kg**	**6.9 (2.8)**	**7.5**	**1.2–12.1**	**10.6 (4.0)**	**10.2**	**4.5–17.3**	**0.009[b]**
BCI	$kg/cm^3 \times 10{,}000$	1.2 (0.2)	1.19	1.0–1.5	1.3 (0.2)	1.27	1.0–1.5	0.490[b]
ALP	U/L	30 (18)	25	10–80	45 (20)	48	10–84	0.053[b]
Amylase	U/L	333 (117)	319	151–557	324 (129)	295	138–578	0.865[b]
AST	**U/L**	**133 (21)**	**145**	**90–158**	**174 (50)**	**176**	**87–275**	**0.010[b]**
CK	U/L	843 (520)	819	242–1,837	1,944 (2,194)	994	437–7,856	0.140[c]
Cholesterol	**mg/dL**	**113 (46)**	**109**	**45–217**	**181 (57)**	**174**	**84–286**	**0.002[b]**
Glucose	mg/dL	105 (14)	104	85–123	112 (15)	117	79–129	0.214[b]
Urea	mg/dL	9 (6)	9	2–19	5 (3)	5	3–13	0.132[c]
Phosphorus	**mg/dL**	**7 (2)**	**7**	**4–10**	**8 (1)**	**8**	**6–10**	**0.037[b]**
Uric acid	mg/dL	3 (1)	2	1–5	3 (2)	3	1–6	0.394[c]
Total protein	**g/dL**	**3.1 (0.6)**	**3**	**2.1–4.1**	**3.8 (0.5)**	**3.9**	**2.6–4.5**	**0.001[b]**
Triglycerides	**mg/dL**	**132 (56)**	**135**	**72–285**	**225 (106)**	**235**	**61–377**	**0.029[c]**

[a] CCL = curved carapace length; SCL = straight carapace length; BW = body weight; BCI = body condition index; ALP = alkaline phosphatase; AST = aspartate transaminase; CK = creatine kinase.
[b] Welch's *t*-test.
[c] Mann-Whitney *U*-test.

Turtles in SF were slightly larger than those in BH (Fig. 2).

Turtle health assessment

The BCI was not significantly different between sites (Table 1). One of the turtles at BH was reported with FP tumors; however, no biochemical parameters demonstrated an indication of clinical abnormality, so this turtle was reported throughout the paper with the larger sample group of non-FP turtles. None of the turtles at SF exhibited FP. No leeches, barnacles, or other parasites were visually present on any of the individuals captured.

Turtle plasma biochemistry

Summary information for eleven plasma analytes of interest is presented in Table 1, and all 21 measured analytes are reported in Supplementary Materials (Table S2). Significant differences between BH and SF were found for AST, cholesterol, phosphorus, total protein, and triglycerides (Table 1). Individual plasma profiles were compared to published reference ranges of clinically stable green turtles (Flint et al. 2010) and are presented in Supplementary Materials (Table S3). All individuals were clinically stable based on comparison to reference intervals, but 11/28 turtles (2/13 turtles from BH; 9/15 turtles from SF) were under excretory compromise due to elevated levels of ALP, CK, calcium, and uric acid (Table S3).

Correlation between isotopic values and biochemistry parameters

Several plasma analytes correlated with turtle size, other analytes, $\delta^{13}C$, and/or $\delta^{15}N$ (Table 2). Seven correlations were common to both foraging sites: turtle CCL and BW, BW and BCI, CCL and total protein, BW and total protein, phosphorus and glucose, and cholesterol and triglycerides were positively correlated, and $\delta^{13}C$ and $\delta^{15}N$ were negatively

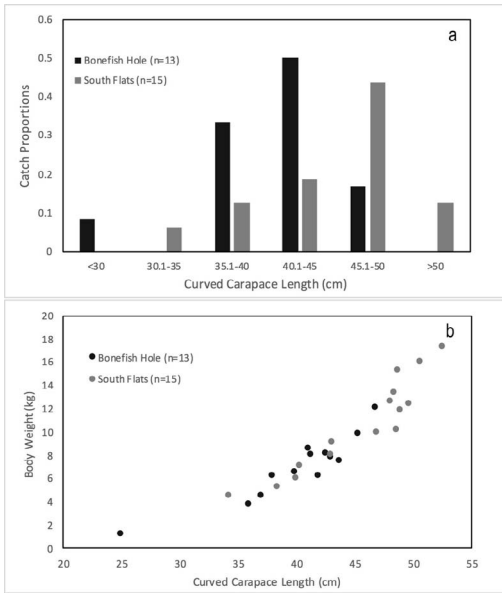

FIGURE 2. (a) Proportion of green turtles (*Chelonia mydas*) in each size class and (b) body weight as a function of carapace length for green turtles captured at each foraging site during two field trips in July and August 2018.

correlated. Other correlations were site specific: 14 for BH and two for SF (Table 2).

Stable isotope values and turtle diet composition

There was a significant difference in epidermis $\delta^{13}C$ (Mann-Whitney *U*-test; $P=0.003$) and $\delta^{15}N$ (Welch's *t*-test; $P=0.009$) between sites with turtles in BH exhibiting depleted $\delta^{13}C$ and enriched $\delta^{15}N$ compared to SF. Carbon and nitrogen isotope values were determined for prey species across two foraging sites (Table 3). Prey items from BH items exhibited more depleted $\delta^{13}C$ and slightly more enriched $\delta^{15}N$ values compared to SF (Table 3).

In BH, sessile filter feeders (28.0±16.7%, 95% credible interval [CI]=1.6–61.9%) contributed the largest proportion to diet, followed by green algae (23.2±17.4%, CI=1.0–65.5%), seagrass (23.2±16.5%, CI=1.2–61.0%), red algae (20.4±14.6%, CI=1.0–54.9%), and mangrove contributing the least to diet (5.1±6.6%, CI=0.1–23.0%; Fig. 3a). In SF, seagrass (33.5±16.3%, CI=4.8–66.3%) contributed the highest proportion to diet, followed by green algae (26.2±17.2%, CI=1.8–65.5%), red algae (20.0±12.2%, CI=1.7–47.5%), and sessile filter feeders (20.4±15.3%, CI=1.0–57.2%; Fig. 3b). Turtles in BH were characterized as having a mixed diet ($n=9$) or feeding primarily on algae and sessile filter feeders ($n=2$), or feeding primarily on seagrass and sessile filter feeders ($n=2$). Turtles in SF were characterized with a mixed diet ($n=7$) or feeding primarily on seagrass ($n=6$) or red algae ($n=2$; Supplementary Material Table S4).

Biochemical analytes were compared between turtles with differing diets in each location (Table 4). A mixed diet was used when multiple prey items contributed between 19–30% of individual diet. When a prey item contributed ≥40% of diet, it was considered to be the main diet choice. Plasma analytes were compared between turtles with different diets. For all turtles sampled across the two foraging sites, individuals feeding on algae and sessile filter feeders had the lowest cholesterol, phosphorus, total protein, and triglyceride levels. Individuals primarily feeding on red algae presented the highest levels of ALP, CK, and uric acid (Table 4).

DISCUSSION

Knowledge of diet in juvenile green turtles foraging in Bimini, Bahamas, aided interpretation of their plasma biochemical profiles and helped explain differences between two distinct foraging grounds. Turtles at BH presented significantly lower phosphorus, AST, cholesterol, total protein, and triglycerides when compared to turtles in SF. This might be driven by the different foraging strategies found between the two locations, with turtles at BH consuming primarily sessile filter feeders, whereas turtles in SF consumed mainly seagrass, consistent with data from previous years (Gillis et al. 2018).

Differences in AST levels are difficult to interpret because plasma AST can be found in many tissues and is not organ specific (Ehsanpour et al. 2015). Increases in AST have been related to muscle damage, capture

TABLE 2. Significant correlations between size measurements, plasma analytes, and epidermis isotope values of green turtles (*Chelonia mydas*) captured from two different foraging locations in Bimini, Bahamas, in July and August 2018 for a study of the influence of diet on plasma biochemistry values. Bold values indicate a positive correlation, and nonbolded values specify a negative correlation.[a]

		Parameter															
Parameter	Units	CCL	BW	BCI	ALP	Amylase	AST	CK	Chol	Gluc	Urea	P	UA	TP	Trig	δN	δC
BW	kg	**1,2**[b]															
BCI	kg/cm^3×10,000		**1,2**[b]														
ALP	U/L																
Amylase	U/L																
AST	U/L					**1**											
CK	U/L																
Chol	mg/dL		**1**					**1**									
Gluc	mg/dL																
Urea	mg/dL																
P	mg/dL								**1**	**1,2**[b]							
UA	mg/dL				**1**					**2**							
TP	g/dL	**1,2**[b]	**1,2**[b]						**1**	**1**							
Trig	mg/dL							**1**	**1,2**[b]		1	**1**		**1**			
δN	‰							**2**					1				
δC	‰							**1**					**1**			1,2[b]	

[a] CCL = curved carapace length; BW = body weight; BCI = body condition index; ALP = alkaline phosphatase; AST = aspartate transaminase; CK = creatine kinase; Chol = cholesterol; Gluc = glucose; P = phosphorus; UA = uric acid; TP = total protein; Trig = triglycerides.

[b] 1 = green turtles at Bonefish Hole, Bimini, Bahamas; 2 = green turtles at South Flats, Bimini, Bahamas.

stress, and hepatocellular damage as a potential response to contaminants in green turtles (Álvarez-Varas et al. 2017). Aspartate transaminase concentrations have also been reported to rise while feeding in foraging areas (Anderson et al. 2011) and in larger turtles due to higher liver enzyme activity in animals with a higher mass (Prieto-Torres et al. 2013). Elevated AST levels, considered with greater cholesterol, protein, and triglycerides values in SF, could be explained by the slightly larger foraging group consuming more in the open

TABLE 3. Stable isotope values for green turtle (*Chelonia mydas*) epidermis and prey groups (BH:43 samples; SF:61 samples) used in mixing model analysis to determine green turtle diet. Samples were collected from two different green turtle foraging locations in Bimini, Bahamas, in July and August 2018 for a study on the influence of diet on plasma biochemistry values.[a]

	δ^{13}C (‰)						δ^{15}N (‰)					
	Bonefish Hole			South Flats			Bonefish Hole			South Flats		
	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max
Turtle epidermis	–12.4 (3.1)	–15.5	–5.7	–8.1 (2.3)	–14.0	–5.8	4.1 (1.4)	1.5	5.9	2.5 (1.6)	–0.4	5.1
Sessile filter feeders	–12.1 (4.8)	–16.8	–3.1	–10.1 (2.6)	–12.0	–5.7	2.0 (4.1)	–3.6	6.0	1.6 (2.5)	–3.4	3.5
Red algae	–17.3 (2.7)	–20.6	–12.2	–13.8 (2.0)	–16.5	–11.1	1.3 (0.2)	0.9	1.5	1.7 (1.0)	0.3	2.6
Green algae	–14.2 (2.5)	–19.1	–10.2	–7.0 (3.1)	–12.8	–1.3	0.5 (1.1)	–2.9	2.0	–0.7 (2.9)	–8.1	2.6
Seagrass	–10.2 (1.6)	–11.2	–8.3	–5.3 (1.5)	–7.3	–2.5	–0.3 (3.2)	–3.9	2.2	–1.7 (3.0)	–7.6	3.9
Mangrove	–25.8 (0.3)	–26.3	–25.6	NA	NA	NA	–1.3 (1.0)	–2.9	–0.01	NA	NA	NA

[a] NA = not applicable.

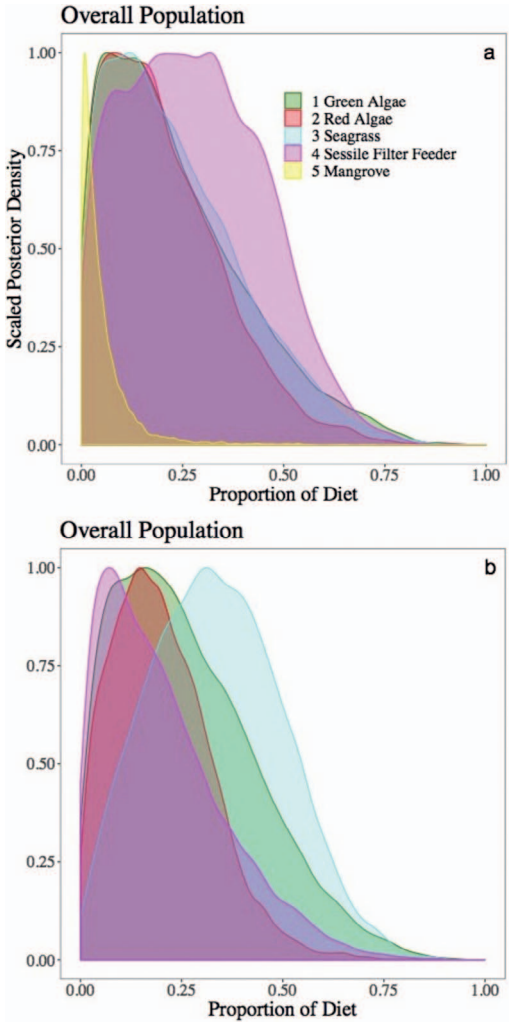

FIGURE 3. Posterior density plots from MixSIAR output indicating the percent contribution of each forage group in the diet of green turtles (*Chelonia mydas*) from (a) Bonefish Hole and (b) South Flats in July and August 2018.

seagrass beds, resulting in higher enzymatic activity. Indeed, higher levels of cholesterol and protein, as found in SF, have been shown to increase following feeding (Arthur et al. 2008), and, in clinically stable turtles with good body condition, increases in protein and triglycerides have been suggested to represent a food-rich environment (Álvarez-Varas et al. 2017).

Despite increased omnivory, BH turtles had significantly lower protein levels than SF turtles. Protein in the blood of chelonians may provide a measurement of nutritional status (Mader 2006; Chaffin et al. 2008). The lowest protein values, along with lowest cholesterol and triglycerides, were found in BH turtles feeding on algae and sessile filter feeders. These turtles were considered in good body condition, so nutrient deficiency was not considered to cause lower protein levels. Size positively correlated with total protein at both sites, consistent with other studies in green (Bolten and Bjorndal 1992; Whiting et al. 2007), loggerhead (*Caretta caretta*; Kelly et al. 2015), hawksbill (*Eretmochelys imbricata*; Whiting et al. 2014), and olive ridley (Espinoza-Romo et al. 2018) turtles. These results support protein in the blood as an indicator of growth functions related to protein assimilation, rather than protein consumption (Osborne et al. 2010). The lower protein values in BH, considered with smaller size of observed individuals and increased protection in a mangrove tidal estuary, could indicate the most recent recruitment of Bimini turtles. Protein levels in Bimini turtles are on the lower end of published ranges for juvenile green turtles in the Bahamas (2.6–6.9 g/dL; Bolten and Bjorndal 1992) and Puerto Rico (3.3–6.6 g/dL; Page-Karjian et al. 2015), with the range of values in BH turtles (2.1–4.1 g/dL) being very similar to that of newly recruited green turtles in Australia (1.8–4.0 g/dL; Whiting et al. 2007).

Biochemistry profiles of green turtles in this study showed no indication of clinical disease, and most blood plasma parameters were within the range of published reference intervals for clinically stable green turtles in Australia (Flint et al. 2010). Few analyte values in our study were outside reference intervals (Table S3). Reference intervals from Flint et al. (2010), although narrower than biochemical ranges for green turtles in other areas (Bolten and Bjorndal 1992; Whiting et al. 2007; Page-Karjian et al. 2015), were used as a comparison because they were established using a large data set (n=211), rigorous evaluation of clinical health, and recommended statistical analysis (Flint et al. 2010; Friedrichs et al. 2012; Flint et al. 2015).

TABLE 4. Plasma biochemistry values for juvenile green turtles (*Chelonia mydas*) captured at two foraging grounds in Bimini Bahamas, Bonefish Hole and South Flats, in July and August 2018. Plasma biochemistry values are separated by individual diet. Individual green turtle diet was determined via stable isotope model summary statistics and separated into foraging groups based on which prey items contributed the most to individual diet. Bold data indicates significant differences between the two sites.

		Bonefish Hole, mean (SD)			South Flats, mean (SD)		
Parameter[a]	Unit	Mixed (n=9)	Algae and SFF[b] (n=2)	Seagrass and SFF[b] (n=2)	Seagrass (n=6)	Mixed (n=7)	Red algae (n=2)
CCL	**cm**	**39.4 (6.2)**	**39.9 (2.8)**	**43.4 (4.9)**	**44.2 (6.4)**	**45.4 (4.9)**	**49.3 (0.5)**
BW	**kg**	**6.6 (2.8)**	**6.2 (0.0)**	**9.3 (4.0)**	**10.1 (4.5)**	**10.6 (4.4)**	**12.2 (0.4)**
ALP	U/L	31 (22)	30 (9)	30 (7)	51 (15)	34 (18)	68 (23)
Amylase	U/L	307 (97)	394 (89)	382 (248)	293 (115)	371 (148)	251 (42)
AST	**U/L**	**132 (24)**	**132 (13)**	**139 (27)**	**183 (68)**	**171 (39)**	**156 (46)**
CK	U/L	854 (522)	386 (135)	1,248 (543)	732 (229)	2,258 (1,913)	4,476 (4,781)
Cholesterol	**mg/dL**	**118 (48)**	**97 (17)**	**101 (79)**	**199 (77)**	**167 (34)**	**176 (81)**
Glucose	mg/dL	6 (0.7)	5 (0.7)	5 (1)	7 (0.7)	6 (0.9)	7 (0.9)
Urea	mg/dL	3 (2)	5 (0.8)	5 (2)	2 (0.5)	2 (2)	2 (0)
Phosphorus	**mg/dL**	**2 (0.4)**	**2 (0.2)**	**3 (1)**	**3 (0.4)**	**3 (0.3)**	**3 (0.6)**
Uric acid	mg/dL	161 (73)	89 (17)	223 (80)	194 (55)	164 (114)	297 (8)
Total protein	**g/dL**	**3.1 (0.5)**	**2.8 (0.3)**	**3.3 (1.2)**	**3.8 (0.3)**	**3.8 (0.7)**	**3.9 (0)**
Triglycerides	**mg/dL**	**147 (59)**	**88 (15)**	**111 (55)**	**224 (103)**	**213 (112)**	**271 (150)**

[a] CCL = curved carapace length; BW = body weight; ALP = alkaline phosphatase; AST = aspartate transaminase; CK = creatine kinase.
[b] SFF = sessile filter feeders.

Levels of some analytes, particularly AST and CK, can be influenced by sampling and capture stress (Whiting et al. 2007; Bloodgood et al. 2019); however, all turtles in our study were handled using the same methodology to minimize biochemical differences due to sampling technique (Arthur et al. 2008). Individual turtle profiles in our study revealed elevated levels of plasma ALP, CK, calcium, and uric acid compared to reference intervals, indicating an excretory compromise in these individuals. Of the turtles that we sampled, 11/28 (9/15 from SF and 2/13 from BF) had elevated levels of these analytes (Table S3). The elevated analytes, in addition to significantly higher AST levels, and indication of excretory stress suggested a potential renal or liver compromise in SF turtles (March et al. 2018). Turtles in SF with a diet primarily of red algae had the highest levels of ALP, CK, and uric acid. The primary species of red algae distributed throughout Bimini, *Laurencia intricata*, produces diverse halogenated secondary metabolites (Suzuki et al. 2002). Halogenated C-15 enynes have been documented in samples of *L. intricata* from Bermuda and have been suggested to act as predator deterrents for herbivorous mollusks that sequester these compounds (Cardellina et al. 1982). There are potentially secondary metabolites in the algae consumed by green turtles in Bimini that inhibit nutrient digestion and assimilation, resulting in higher levels of organ enzymatic activity in these individuals.

Despite some turtles presenting blood plasma parameters outside published reference intervals, all but one turtle were documented as clinically stable based on physical examination, so illness and injury were not considered when interpreting biochemical values. Previous data in Bimini revealed a high percentage of turtles in BH presenting FP (Gillis et al. 2018). It is possible that turtles in this study had internal fibropapillomas, early onset indicators of FP, or other subclinical disease that we were not able to detect in external examination or

biochemical profiles. Further methods to quantify pathological status in Bimini turtles are necessary to understand disease prevalence at these foraging grounds because these data reveal disparity in FP presence between this study and data from previous years (Gillis et al. 2018). It is possible that the shallow coastal waters of Bimini, and other areas of the Bahamas, present an area of early FP manifestation. It has been suggested that environmental stressors, such as chemical contamination, play a role in the development of FP (Jones et al. 2016). Events associated with increased tourism and development, such as channel dredging in North Bimini, can be related to increased bioavailability of metals and contaminants released from sediment (Flint et al. 2015). A link has been demonstrated between marine turtle biochemistry and heavy metals (Álvarez-Varas et al. 2017). Future research should determine environmental stressors in this area associated with increased anthropogenic activity, such as heavy metal concentration in sediment, water column, and marine turtle prey (Villa et al. 2019).

Green turtle prey represent a route for dietary exposure of contaminants. Seagrasses, a very important dietary component for green turtles, are proficient accumulators of metals, sequestering them from both the water and sediment environment in which they grow (Flint et al. 2015). Similarly, filter feeders, which were reported to be an important food item for turtles in this study and in Pacific Mexico (Seminoff et al. 2002), Hawaii (Russell et al. 2011), and the Caribbean (Stringell et al. 2016), have the potential to act as biomonitors for heavy metals or other environmental disturbances (de Mestre et al. 2012). Our environmental data revealed increased values of nitrate, nitrite, and fecal coliform in BH, which could reflect increased anthropogenic activity on the North Island. However, a wider range of ammonium values was found in SF, and thus long-term data from a larger spatial extent are needed to draw conclusions related to water and ecosystem quality. To obtain further insights into how individual turtle health is influenced by anthropogenic stressors a suite of health parameters is needed including hematology, toxicology, and molecular testing for chelonid fibropapilloma-associated herpesvirus (Page-Karjian et al. 2015).

Further advances to this and similar studies could use blood tissue instead of epidermis for carbon and nitrogen stable isotopic analyses, because blood tissue reflects recently consumed exogenous nutrients at time of blood sampling (Haywood et al. 2019). Epidermis tissue was used in our study because it provides more long-term information on nutrients assimilated from an individual's diet due to a relatively slow (3–5 wk) rate of isotope replenishment in this tissue type for marine turtles (Reich et al. 2008). Although isotope analysis was the preferred method in our study to quantify individual forage proportions, stomach content analysis could also have been used to confirm forage items and add a priori knowledge into the stable isotope mixing model to provide a more robust comparison of individual diet and overcome the biases of both methodologies (Layman et al. 2012). Our study would also have benefited from larger sample sizes to calculate biochemical reference intervals for this location (Geffré et al. 2009) and elucidate potential confounding factors of diet, size, and foraging location to determine whether our results are random or if the trends presented are a reflection of the influence of local factors on biochemical profiles in Bimini turtles.

Regardless of the limitations of our study, our results provide a baseline into natural variations in green turtle plasma analyte values due to geographic location and diet. Importantly, our results indicated that, even if foraging at disparate but adjacent sites, green turtles can have different diets and different biochemical profiles, and thus when interpreting plasma analyte values individual diet must be considered. Given knowledge of foraging strategy, future assessments can utilize the blood data provided to aid in the interpretation of plasma analyte values in health and nutritional evaluations in marine turtle populations.

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SUPPLEMENTARY MATERIAL

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