

# Trophic ecology of a green turtle breeding population

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**ABSTRACT:** While many migratory marine organisms converge at breeding areas, identifying foraging strategies away from these reproductive sites can be challenging. Adult female green turtles *Chelonia mydas* regularly migrate thousands of kilometers between nesting and foraging areas, making it difficult to identify foraging habitats that support nesting populations and to understand their feeding strategies. In this study, we use stable isotope analysis to investigate the trophic ecology and spatial distribution of foraging green turtles in the Greater Caribbean. Further, we explore the possibility that adult green turtles, originally considered to be herbivores, may, like their counterparts in the Pacific Ocean, display carnivorous feeding strategies. The wide range of carbon and nitrogen isotope values in bulk epidermis observed in the nesting population at Tortuguero, Costa Rica, could indicate that these turtles feed over several trophic levels. Isotopic niches—or the range of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, which can be used as a proxy for ecological niche—varied among the 5 green turtle foraging aggregations sampled. Similarly, the isotopic composition of the primary producer *Thalassia testudinum* also varied substantially with geographic location. However, compound-specific stable isotope analysis of amino acids (AA-CSIA) indicated that individuals in the nesting population with different bulk  $\delta^{15}\text{N}$  values feed at the same trophic position. The combined results suggest that spatial differences in the isotopic composition of seagrass at the base of the food web, rather than differences in turtle foraging strategy, contribute to the isotopic variation in the nesting population. This study improves understanding of the foraging ecology of a highly dispersed and migratory species.

**KEY WORDS:** *Chelonia mydas* · *Thalassia testudinum* · Compound-specific stable isotope analyses · Amino acids · Carbon · Nitrogen · Herbivory · Caribbean

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## INTRODUCTION

Many marine organisms migrate far from their reproductive grounds during the nonbreeding season. It remains a challenge to identify foraging strategies away from breeding sites, as diving organisms may have cryptic foraging habits and may spread over highly dispersed areas. Stable isotope analysis has become an increasingly useful tool to examine resource

use patterns of migratory marine vertebrates, as satellite tracking costs can be prohibitive, and stomach content analysis can provide only a brief history of diet items that may be biased toward more detectable prey species. Sampling individuals congregated at breeding grounds for stable isotope analysis can provide the opportunity to assess resource use in foraging areas prior to the breeding period (Cherel et al. 2006, 2009, Phillips et al. 2009, Witteveen et al. 2009).

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Green turtles *Chelonia mydas* are an example of a highly migratory species, with nesting populations composed of individuals from multiple foraging grounds, often separated by hundreds or thousands of kilometers (Harrison & Bjorndal 2006). Sea turtles make regular migrations between foraging grounds and breeding areas and—based on satellite tracking and recapture of tagged individuals—often show fidelity to both areas (Limpus et al. 1992, Broderick et al. 2007). Thus, sampling a single breeding population permits the study of foraging ecology of females that originate from widely dispersed foraging aggregations. For most marine turtle nesting populations, the distribution of foraging grounds, the proportion of nesters from each foraging ground, and the variation in diets among and between foraging grounds are poorly understood but are important for the conservation of the breeding stock.

Green turtles are the only herbivorous sea turtle species, though omnivory and carnivory are common among young juveniles using oceanic habitats (Bjorndal 1997). In the Greater Caribbean, green turtles typically recruit to neritic, or coastal, habitats by 6 yr of age, where they switch to a herbivorous diet that continues into adulthood (Bjorndal 1997, Zug & Glor 1998, Reich et al. 2007). However, adult green turtles consuming primarily animal matter have been observed in other regions, mainly in the Pacific (Hatase et al. 2006, Amorocho & Reina 2007, Arthur et al. 2007, Burkholder et al. 2011, Lemons et al. 2011, Rodriguez-Baron et al. 2011), and some adult green turtles continue to maintain an oceanic, carnivorous foraging strategy as adults (Hatase et al. 2006), suggesting considerable flexibility in the diet of this species.

Previous analyses of stomach and feces content indicate that Greater Caribbean green turtles are herbivorous, with a diet composed predominately of seagrasses and/or algae (reviewed by Bjorndal 1997). Small amounts of animal matter, primarily sponges, are consumed by Caribbean green turtles (Mortimer 1981, Bjorndal 1990), which could potentially contribute disproportionately to their nutrition, given the accessibility of nitrogen in sponges compared with that in seagrass (Bjorndal 1985). Green turtles in the Caribbean have not been observed to maintain an oceanic or carnivorous diet after the oceanic stage, yet evidence of this foraging strategy would suggest a new ecological role for the population. Turtles using alternative foraging strategies might have been missed as a result of the methods used to evaluate their foraging ecology. Previous diet studies have occurred in known foraging habitats in nearshore en-

vironments and would fail to identify turtles feeding offshore. Additionally, fishery-dependent tag return data in the Caribbean originate primarily from coastal waters, therefore biasing recapture information to turtles that use neritic habitats (Troëng et al. 2005).

Stable isotope analysis has become increasingly advantageous for revealing resource use patterns in highly migratory marine vertebrates (Rubenstein & Hobson 2004). More specifically, the isotopic niche provides a metric with which to compare assimilated diet and habitat differences among and/or within populations (Layman et al. 2007a, Martínez del Rio et al. 2009, Navarro et al. 2011). An isotopic niche is a proxy for an ecological niche and is influenced by what individuals consume (bionomic factors), as well as where they live (scenopoetic factors), and is typically represented by 2 or more stable isotope measurements of tissues from of a consumer (Newsome et al. 2007). Carbon isotope values ( $\delta^{13}\text{C}$ ) have been used as habitat indicators because they reflect those of the primary producers in a given environment, while nitrogen isotope values ( $\delta^{15}\text{N}$ ) have been used to reflect an organism's trophic position (DeNiro & Epstein 1978, 1981, Post 2002). However, these distinctions are not always clear, as many other factors can influence  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values at the base of the food web, particularly in marine environments (e.g. Hannides et al. 2009, Graham et al. 2010, Dale et al. 2011, McMahon et al. 2011, O'Malley et al. 2012, Pajuelo et al. 2012a, Seminoff et al. 2012).

Values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  vary naturally with location as a result of biogeochemical processes that affect nutrient isotopic compositions and can create gradients such as those related to latitude or proximity to shore (Rubenstein & Hobson 2004, Somes et al. 2010). Because the tissues of organisms reflect the isotope compositions of carbon and nitrogen in their habitats, stable isotope analysis can be used to infer geographic origins and differentiate among populations (Rubenstein & Hobson 2004, Ramos et al. 2009). Therefore, individuals sampled at breeding grounds can provide the opportunity to identify distinct isotopic features of foraging areas and patterns of migratory connectivity (Cherel et al. 2006, 2007).

Compound-specific stable nitrogen isotope analysis of amino acids (AA-CSIA) can determine whether variations in bulk (total tissue)  $\delta^{15}\text{N}$  values are due to differences in baseline  $\delta^{15}\text{N}$  values or differences in trophic position (e.g. herbivory vs. carnivory). Previous studies using AA-CSIA have quantified trophic levels of marine organisms without having to characterize baseline  $\delta^{15}\text{N}$  values (Popp et al. 2007, Han-

nides et al. 2009, Dale et al. 2011, Seminoff et al. 2012), which is particularly useful for systems in which  $\delta^{15}\text{N}$  values of primary producers vary spatially or temporally.

We assess the trophic ecology and foraging ground distribution of green turtles nesting at Tortuguero in NE Costa Rica using bulk and compound-specific isotopic analyses. We also investigate the potential for carnivory, and the ecological niche occupied by this Caribbean green turtle population through multiple approaches. First, we evaluate both trophic and habitat contributions to carbon and nitrogen isotope compositions of the nesting population by comparing the isotopic niche of the nesting population and those of multiple foraging aggregations. Next, we explore the extent to which the biogeochemistry of a particular foraging ground contributes to green turtle stable isotope values using isotopic variability in the primary producer, *Thalassia testudinum*, which has been identified as the main component of Caribbean green turtle diets (Bjorndal 1997). Finally, we further refine baseline and trophic contributions to bulk epidermis nitrogen isotopic composition of the nesting population using AA-CSIA.

## MATERIALS AND METHODS

### Sample collection and preparation

Epidermis samples were collected from 376 green turtles *Chelonia mydas* from 1 nesting beach (Tortuguero, Costa Rica) and 5 known green turtle foraging grounds that range across the Greater Caribbean: Union Creek, Great Inagua, Bahamas; Clarence Town Harbour, Long Island, Bahamas; Puerto Cabezas, North Atlantic Autonomous Region (RAAN), Nicaragua; Pearl Cays and Man O' War/Tyra Cays area, South Atlantic Autonomous Region (RAAS) Nicaragua; and St. Joseph Bay, Florida, USA (Fig. 1). Tortuguero hosts the largest rookery of green turtles in the Atlantic by an order of magnitude (Chaloupka et al. 2008). Recoveries of >4600 flipper tags applied to individuals nesting at Tortuguero indicate that these turtles travel throughout the Greater Caribbean from the Florida Keys to northern Brazil (Troëng et al. 2005). The large majority of flipper tag

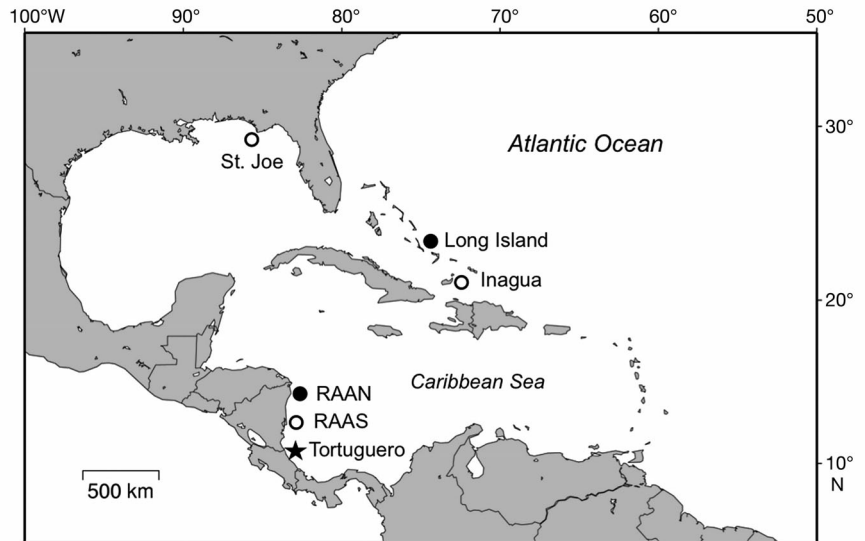


Fig. 1. Map of 5 foraging grounds (circles) and 1 nesting beach (star) where green turtles *Chelonia mydas* were sampled. *Thalassia testudinum* samples were collected at the 3 foraging grounds with open circles. RAAN and RAAS: North and South Atlantic Autonomous Region, respectively

returns come from the seagrass beds of Nicaragua (Troëng et al. 2005), but this observation is likely biased by the take of turtles in this area, as there is an extensive turtle fishery in the region. Only adult females were sampled at the nesting beach. Adults and juveniles of both sexes were sampled from the 2 Nicaraguan sites; only juveniles were sampled at the other foraging grounds, although adults are known to forage in those areas (Table 1). Sex was only determined in adult green turtles by observing tail length. Size is reported as curved carapace length (CCL) (Bolten 1999); methods of measurement are provided in the supplement at [www.int-res.com/articles/suppl/m476p237.pdf](http://www.int-res.com/articles/suppl/m476p237.pdf).

Epidermis samples from turtles nesting at Tortuguero were collected from May to July (approximately the first third of the nesting season) in 2007 and 2009. Rates of isotopic incorporation have not been reported for green turtles. However, the isotopic turnover time of epidermis in juvenile loggerhead sea turtles *Caretta caretta* is approximately 4 mo (Reich et al. 2008), and the time period represented in adults is likely much longer, as the rates of isotopic incorporation slow with reduced growth rates (Reich et al. 2008) and increasing body mass (Bauchinger & McWilliams 2009). Therefore, we assumed the isotopic composition of these samples reflects the diet in the foraging habitat during the months preceding migration to the nesting beach. Two females (Aurora and Chica) were also fitted with satellite transmitters; the routes are available online (Sea Turtle Conservancy 2012). Sam-

Table 1. *Chelonia mydas*. Number of green turtles, size range, and year sampled at each of the 5 foraging grounds and the nesting beach location (Tortuguero). Two estimates of isotopic niche area (convex hull area and Bayesian ellipse area) were calculated for each site, as well as isotopic means  $\pm$  SE and minimum/maximum values. CCL: curved carapace length. RAAN and RAAS: North and South Atlantic Autonomous Region, respectively

Site name	Country	Number of individuals sampled	Size range CCL (cm)	Year sampled	Convex hull area	Bayesian ellipse area	Mean $\delta^{13}\text{C}$ (‰) (min., max.)	Mean $\delta^{15}\text{N}$ (‰) (min., max.)
Inagua	Bahamas	62	38.9–65.5	2008, 2009	18.5	4.0	$-6.4 \pm 0.1$ (-8.8, -4.5)	$1.7 \pm 0.4$ (-1.9, 5.2)
Long Island	Bahamas	9	30.8–44.8	2010	7.5	6.1	$-9.4 \pm 0.7$ (-12.2, -6.4)	$5.2 \pm 0.4$ (3.5, 7.1)
RAAN	Nicaragua	110	85.0–106.6	2010	20.3	2.7	$-9.0 \pm 0.1$ (-14.7, -7.3)	$5.6 \pm 0.1$ (3.1, 7.9)
RAAS	Nicaragua	73	69.5–106.0	2009–2011	10.7	1.8	$-10.0 \pm 0.1$ (-13.0, -8.2)	$6.6 \pm 0.1$ (4.2, 7.9)
St. Joe Bay, Florida	USA	20	31.7–60.5	2010	13.7	5.3	$-12.3 \pm 0.5$ (-15.7, -9.0)	$8.1 \pm 0.4$ (4.9, 11.1)
Tortuguero Beach	Costa Rica	102	93.7–122.1	2007, 2009	44.9	8.4	$-9.3 \pm 0.2$ (-17.0, -5.3)	$6.6 \pm 0.1$ (3.0, 9.4)

ples at foraging grounds were obtained from green turtles caught via net, hand capture, en route to slaughter, or from stranded cold-stunned animals. Turtles were determined to be residents at the foraging area in which they were captured based on site-specific criteria. All turtles at the 2 Bahamian sites had previously been captured in the same location a year or more prior to the sampling date and were identified by flipper tags. At the 2 Nicaraguan foraging grounds, turtles were not sampled during the months in which migration to or from the nesting grounds occurs (i.e. sampling occurred from January to May). Outside of the migration period, green turtles found in these regions are likely residents (Campbell 2003). Samples from turtles in Florida were collected during a cold stunning event in January of 2010, and, at that site, we excluded juvenile turtles that could have recently recruited from oceanic foraging grounds by selecting individuals that were  $>31$  cm CCL (the minimum size of recaptured turtles at the sites in the Bahamas; Table 1).

Skin samples were collected from the neck region between the front flipper and head, just below the carapace, using a sterile 6 mm Miltex biopsy punch and were preserved in 70% ethanol until processing. Stable isotope values of green turtle skin are not significantly affected by preservation in 70% ethanol (Barrow et al. 2008). All

skin samples were rinsed in deionized water and cleaned with an isopropyl alcohol swab prior to preparation. Epidermis was separated from the dermis using a scalpel blade, and the epidermis was diced and dried at 60°C for 24 h. Lipids were removed from epidermis using an ASE300 accelerated solvent extractor (Dionex) and petroleum ether solvent for 3 consecutive cycles consisting of 5 min of heating to 100°C and pressurization to 1500 PSI (103 bar), 5 min static, purging, and then flushing with additional solvent.

Healthy leaf blades of the seagrass *Thalassia testudinum* were collected from 3 of the 5 foraging locations (Fig. 1, Table 2). Epiphytes were removed from blades with gloved fingers, and seagrass samples were dried at the field site or frozen for transport back to the laboratory. All seagrass blades were dried in the laboratory at 60°C for 24 h and ground to  $<1$  mm in a Wiley Mill.

Table 2. *Thalassia testudinum*. Seagrass carbon and nitrogen isotope compositions provided as mean  $\pm$  SE and minimum/maximum values. One sample was collected at each site

Location	Country	Number of sites	Year sampled	Mean $\delta^{13}\text{C}$ (‰) (min., max.)	Mean $\delta^{15}\text{N}$ (‰) (min., max.)
Union Creek, Great Inagua	Bahamas	3	2002	$-6.6 \pm 0.1$ (-7.1, -6.4)	$1.2 \pm 0.2$ (0.4, 1.8)
Pearl Cays, RAAS	Nicaragua	4	2010	$-9.0 \pm 0.7$ (-10.4, -7.8)	$3.2 \pm 0.5$ (2.6, 4.3)
St. Joe Bay, Florida	USA	1	2011	-7.7	5.6

### Sample analyses

Isotopic compositions of bulk epidermis (0.5 to 0.6 mg) and seagrass (0.5 to 4 mg) samples were determined at the Department of Geological Sciences, University of Florida, Gainesville, Florida, using a ECS 4010 elemental analyzer (Costech) interfaced via a ConFlo III to a DeltaPlus XL isotope ratio mass spectrometer (ThermoFisher Scientific). Delta notation was used to express stable isotope abundances, defined as parts per thousand (‰) relative to the standard:

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the corresponding ratios of rare to common isotopes ( $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ) in the sample and international standard, respectively. Vienna Pee Dee Belemnite was used as the standard for  $^{13}\text{C}$ , and atmospheric  $\text{N}_2$ , for  $^{15}\text{N}$ . The reference material USGS40 (L-glutamic acid) was used to normalize all results. The standard deviation of the reference material was 0.13‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $n = 58$ ). Repeated measurements of a laboratory reference material, loggerhead scute, were used to examine consistency in a homogeneous sample with similar isotopic composition to the epidermis samples. The standard deviation of this laboratory reference material was 0.12‰ for  $\delta^{13}\text{C}$  values and 0.18‰ for  $\delta^{15}\text{N}$  values ( $n = 21$ ).

Nitrogen isotopic composition of amino acids was analyzed for 4 *Thalassia testudinum* samples collected in southern Nicaragua and 1 *T. testudinum* sample collected in Inagua, Bahamas, and for 6 green turtles nesting at Tortuguero, using a subsample of the epidermis sample used for bulk tissue analysis. Green turtle samples selected for AA-CSIA represent the range of bulk epidermis  $\delta^{15}\text{N}$  values observed in the nesting population (solid triangles in Fig. 2a). Approximately 5 mg (5.0 to 5.4 mg) of homogenized turtle tissue and 30 mg (30.2 to 33.8 mg) of seagrass were hydrolyzed using 6 N hydrochloric acid and then derivatized to produce trifluoroacetic amino acid esters using methods previously described (Macko et al. 1997, Popp et al. 2007). Nitrogen isotopic compositions of individual amino acids were determined using a Delta V Plus mass spectrometer (ThermoFisher Scientific) interfaced with a Trace GC gas chromatograph (ThermoFisher Scientific) through a GC-C III combustion furnace (980°C), reduction furnace (650°C), and liquid N cold trap as described by Dale et al. (2011) and Hannides et al. (2009). Internal reference

materials, norleucine and amino adipic acid, were used to normalize measured  $\delta^{15}\text{N}$  values. Each sample was analyzed in triplicate, and data are presented as the means of 3 analyses. Standard deviations for all amino acids averaged 0.6‰ (range: 0.2 to 1.1‰).

### Turtle trophic position

Fractional trophic position estimates based on AA-CSIA rely on the  $\delta^{15}\text{N}$  values of 2 types of amino acids. 'Trophic' amino acids (TrAA: e.g. alanine, glutamic acid, and leucine; sensu Popp et al. 2007) are enriched in  $^{15}\text{N}$  relative to prey presumably due to transamination and deamination reactions that cleave the carbon-nitrogen bond (Chikaraishi et al. 2007). 'Source' amino acids (SrcAA: e.g. glycine and phenylalanine; sensu Popp et al. 2007) remain relatively unchanged in their nitrogen isotope composition due to an absence of or reduction in metabolic processes that break C-N bonds (Chikaraishi et al. 2007). The fractional trophic positions (TP) of green turtles were calculated using 2 variations of the equation proposed by Chikaraishi et al. (2009). First, turtle TP was calculated using nitrogen isotopic compositions of glutamic acid (Glu) and phenylalanine (Phe) representing 'trophic' and 'source' amino acids, respectively:

$$\text{TP}_{\text{Glu/Phe}} = \left( \frac{(\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}}) + \beta_{\text{Glu-Phe}}}{\text{TEF}_{\text{Glu-Phe}}} \right) + 1 \quad (2)$$

where  $\beta_{\text{Glu-Phe}}$  is the difference between Glu and Phe in the primary producer. In aquatic primary producers, a general pattern has been observed across >25 photoautotrophs with  $\beta_{\text{Glu-Phe}} = -3.4\%$  (Chikaraishi et al. 2009, 2010). However, here we demonstrate that seagrass amino acid biosynthesis is more similar to that of terrestrial  $\text{C}_3$  plants rather than macroalgae and phytoplankton (see 'Results'), and, hence, for the purposes of this study, we use a  $\beta_{\text{Glu-Phe}}$  value of 8.4‰ ( $\text{C}_3$  plants; Chikaraishi et al. 2010) to calculate turtle  $\text{TP}_{\text{Glu/Phe}}$ . The trophic enrichment factor (TEF) is the expected enrichment in  $^{15}\text{N}$  with each trophic step for TrAAs and SrcAAs (Chikaraishi et al. 2010) as calculated by:

$$\text{TEF}_{\text{Glu-Phe}} = \Delta\delta^{15}\text{N}_{\text{Glu}(\text{consumer-diet})} - \Delta\delta^{15}\text{N}_{\text{Phe}(\text{consumer-diet})} \quad (3)$$

Controlled feeding studies using herbivorous zooplankton and young carnivorous fish have yielded  $\text{TEF}_{\text{Glu-Phe}} = 7.6\%$  (Chikaraishi et al. 2009), and here we adopt this value to calculate turtle  $\text{TP}_{\text{Glu/Phe}}$ .

Second, turtle TP was calculated using a combination of all available TrAAs and SrcAAs such that:

$$TP_{Tr/Src} = \left( \frac{(\delta^{15}N_{Tr} - \delta^{15}N_{Src}) + \beta_{Tr-Src}}{TEF_{Tr-Src}} \right) + 1 \quad (4)$$

where  $\delta^{15}N_{Tr}$  is the weighted mean  $\delta^{15}N$  value of turtle TrAAs alanine (Ala), leucine (Leu), aspartic acid (Asp), and glutamic acid (Glu), and  $\delta^{15}N_{Src}$  is the weighted mean of SrcAAs phenylalanine (Phe), serine (Ser), glycine (Gly), tyrosine (Tyr), and lysine (Lys). These amino acid classifications are based on McClelland & Montoya (2002), Popp et al. (2007), and Sherwood et al. (2011). Valine (Val), isoleucine (Ile), and threonine (Thr) were omitted from this analysis because they were not measured in all turtle samples, and proline (Pro) was not reported because it co-eluted with an unidentified compound in the green turtle samples. Arginine (Arg) was measured in both green turtles and seagrass, but there are no published data on which to base arginine  $\beta$  or TEF values, so it was not included in  $TP_{Tr/Src}$  calculations. For the purposes of Eq. (4),  $\beta_{Tr-Src}$  was calculated as the difference between weighted means for  $\delta^{15}N_{Tr}$  and  $\delta^{15}N_{Src}$  in seagrass (present study) and terrestrial  $C_3$  plants (Chikaraishi et al. 2010) combined to yield  $\beta_{Tr-Src} = -1.4 \pm 1.8$ . Asp, Lys, and Tyr data were only available for seagrass, but given the consistent relationship observed in seagrass and  $C_3$  plant amino acid nitrogen isotopic composition (Fig. 3), these amino acids were included in the calculation of the  $\beta_{Tr-Src}$  value. The TEF was determined using a weighted mean of TrAA and SrcAA data from the literature. TEF values for Ala, Leu, Glu, Gly, Ser, and Phe were mean values derived from multiple studies (Chikaraishi et al. 2010), and TEF values for Asp, Lys, and Tyr were derived from feeding study results (McClelland & Montoya 2002) to yield  $TEF_{Tr-Src} =$

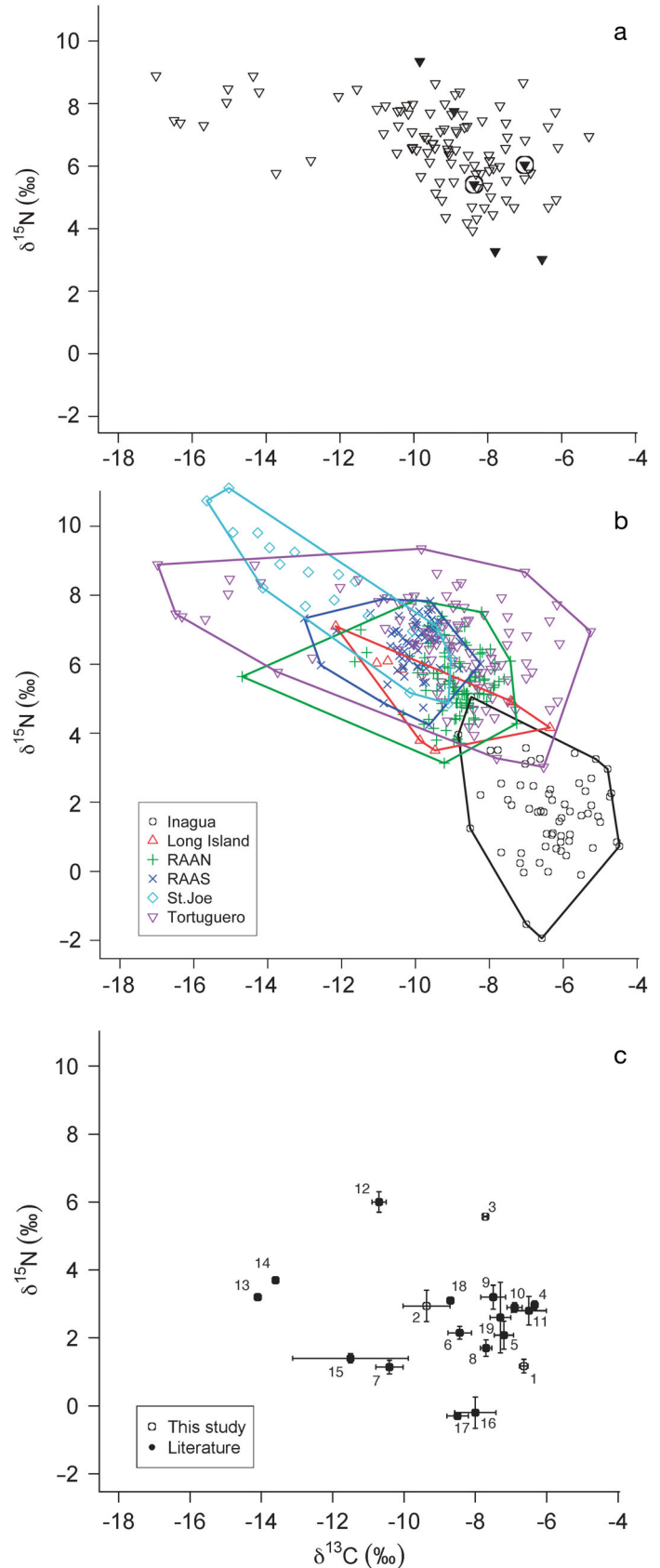


Fig. 2. *Chelonia mydas*, *Thalassia testudinum*. Bulk tissue  $\delta^{13}C$  and  $\delta^{15}N$  values of: (a) green turtle epidermis from the nesting population at Tortuguero, Costa Rica (solid symbols represent the 6 epidermis samples that were used for compound-specific stable isotope analysis of amino acids, and circles around 2 of the solid symbols identify the 2 individuals that were satellite tracked); (b) green turtle epidermis at 5 foraging sites and 1 nesting beach (Tortuguero) (convex hulls represent the isotopic niche for each population); and (c) seagrass samples from 3 green turtle foraging sites (1: Inagua, Bahamas; 2: RAAS, Nicaragua; 3: Florida, USA) in this study, as well as at 15 other sites around the Greater Caribbean. Points are means  $\pm$  SE except for 13 and 14, for which SEs were not available. See Table S1 in the supplement at [www.int-res.com/articles/suppl/m476p237\\_supp.pdf](http://www.int-res.com/articles/suppl/m476p237_supp.pdf) for complete list of sites and sources

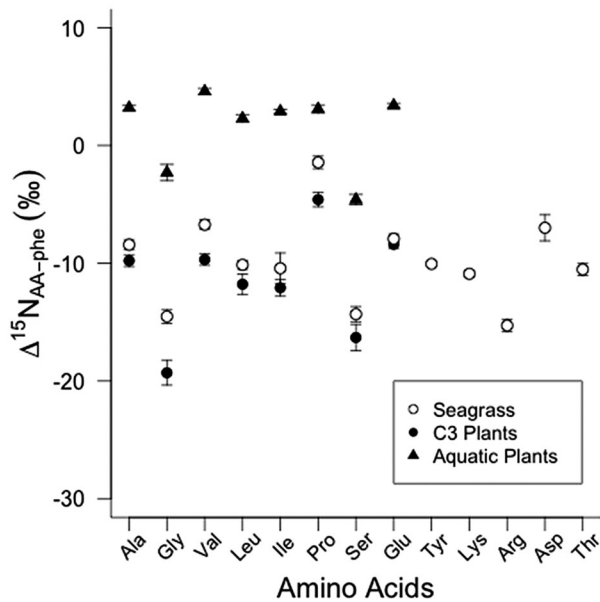


Fig. 3. Difference in  $\delta^{15}\text{N}$  values between each amino acid and phenylalanine ( $\Delta\delta^{15}\text{N}_{\text{AA-Phe}}$ ) for *Thalassia testudinum* seagrass (present study), terrestrial  $\text{C}_3$  plants (Chikaraishi et al. 2010), and 25 aquatic primary producers (Chikaraishi et al. 2009). *T. testudinum* amino acid profile is more similar to that of terrestrial  $\text{C}_3$  plants than to that of other aquatic primary producers. The relationship between seagrass and terrestrial  $\text{C}_3$  plant  $\Delta\delta^{15}\text{N}_{\text{AA-Phe}}$  values is significant for the 8 amino acids for which data are available in both groups ( $p < 0.001$ ;  $\Delta\delta^{15}\text{N}_{\text{AA-Phe}}(\text{seagrass}) = \Delta\delta^{15}\text{N}_{\text{AA-Phe}}(\text{C}_3) \times 0.90(\pm 0.11) + 1.3(\pm 1.4)$ ;  $r^2 = 0.91$ ). Error bars represent  $\pm\text{SE}$ . For amino acid abbreviations see Table 3

$4.2 \pm 0.2$ . The weighted mean incorporates the variance for values that may derive from different probability distributions using the following formula:

$$\bar{x} = \frac{\sum(x_i/\sigma_i^2)}{\sum(1/\sigma_i^2)} \quad (5)$$

where  $\sigma_i^2$  is the analytical uncertainty derived from triplicate analysis of the compound  $\delta^{15}\text{N}$  value. In both forms of the TP calculation, the analytical error associated with multiple measurements of the  $\delta^{15}\text{N}$  values of amino acids and of published uncertainty in TEF and  $\beta$ -values was propagated to determine TP error (e.g. see Dale et al. 2011).

### Data analysis

All statistics were performed using R (R Development Core Team 2011). Comparisons of variance were conducted with the robust Brown-Forsyth version of the Levene test. Isotope niche metrics (convex hull area and Bayesian ellipse area) were calculated

using SIAR (Jackson et al. 2011). Convex hull area is the total area encompassed by all points on a  $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$  bi-plot (Layman et al. 2007a), but this method is particularly sensitive to sample sizes  $< 50$  (Jackson et al. 2011). Because the convex hull area is based on the outer-most points to construct the polygon, extreme values or outliers can heavily influence the resulting area. The ellipse area was proposed as a metric that is unbiased with respect to sample size, and, particularly for the Bayesian method incorporates greater uncertainty with smaller sample sizes, resulting in larger ellipse areas (Jackson et al. 2011). Bayesian ellipses provide information about the core aspects of a population's niche, whereas the convex hull approach includes information about every part of the isotopic niche space occupied (Layman et al. 2012). We provide area estimates using both metrics but plot only convex hulls because we are more interested in the potential range at each foraging ground, rather than the mean of the population.

## RESULTS

The nesting population at Tortuguero exhibited a wide range in bulk epidermis  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig. 2a). Estimates of Bayesian ellipse area for each foraging site resulted in slightly different size rankings than the convex hull area method, but both measurements of the isotopic niche (convex hull area and Bayesian ellipse area) generated smaller niche areas for each foraging aggregation than for the nesting population (Table 1, Fig. 2b).

Males and females were compared in the 2 Nicaragua foraging grounds (RAAN and RAAS) to determine if variance in stable isotope values differed with sex. The variance in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of RAAN males and females did not differ significantly ( $\delta^{13}\text{C}$ :  $t = 0.13$ ,  $p = 0.71$ ;  $\delta^{15}\text{N}$ :  $t = 0.98$ ,  $p = 0.33$ ). The variance in  $\delta^{13}\text{C}$  values of RAAS turtles did not differ significantly between the sexes ( $t = 0.14$ ,  $p = 0.71$ ), whereas females had significantly greater variance in  $\delta^{15}\text{N}$  values than males ( $t = 6.50$ ,  $p = 0.01$ ). Because females exhibited increased variance (and thus a larger range), including adult males in the sample did not alter the convex hull interpretations of the isotopic niche.

Seagrass samples obtained from 3 green turtle *Chelonia mydas* foraging sites had a wider range of mean bulk  $\delta^{15}\text{N}$  values (4.4‰) than  $\delta^{13}\text{C}$  values (2.3‰; Table 2, Fig. 2c), though the variance was not significantly different ( $t = 2.34$ ,  $p = 0.29$ ). Mean nitrogen isotope values in seagrass of Florida  $>$  Nicara-

gua > The Bahamas, and a similar trend was evident in the respective green turtle foraging aggregation means (Tables 1 & 2). Even wider ranges in isotope values of *Thalassia testudinum* were observed when additional sites in the Caribbean were included from the literature ( $\delta^{13}\text{C}$  range = 7.8‰;  $\delta^{15}\text{N}$  range = 6.3‰) (Fig. 2c) (for a complete list of sites and sources see Table S1 in the supplement at [www.int-res.com/articles/suppl/m476p237\\_supp.pdf](http://www.int-res.com/articles/suppl/m476p237_supp.pdf)). These ranges in the primary producer across the Caribbean were nearly as large as those of the Tortuguero nesting population.

Nitrogen isotope fractionation patterns in amino acids of seagrass were found to be more similar to those of terrestrial  $\text{C}_3$  plants than to those of other aquatic primary producers such as macroalgae, phytoplankton, and cyanobacteria (Fig. 3). The mean  $\text{TP}_{\text{Glu/Phe}}$  (calculated using only Glu and Phe) was not significantly different from 2.0 and ranged from  $1.7 \pm 0.3$  to  $2.1 \pm 0.3$  ( $\pm\text{SD}$ ) when the  $\beta$ -value for terrestrial  $\text{C}_3$  plants was used (Table 3, Fig. 4a).  $\text{TP}_{\text{Tr}/\text{Src}}$  (calculated using weighted means of all available TrAAs and SrcAAs) yielded greater variability in the estimate of trophic position, ranging from  $1.0 \pm 0.4$  to  $2.6 \pm 0.4$  (Table 3, Fig. 4a). With most of the  $\text{TP}_{\text{Tr}/\text{Src}}$

values falling below 2.0 (the trophic position for a primary consumer), the results suggest that the assumptions underlying the use of all available TrAAs and SrcAAs require further refinement. The  $\delta^{15}\text{N}$  values of bulk epidermis and Phe, a SrcAA, showed a significant positive relationship (Fig. 4b).

Two of the green turtles that were analyzed for AA-CSIA were also satellite tracked. These turtles (Aurora and Chica) migrated to northern Nicaragua (RAAN) to areas of seagrass habitat before their transmissions ceased (Sea Turtle Conservancy 2012). Their bulk epidermis  $\delta^{15}\text{N}$  values fell inside the RAAN isotopic niche, and their AA-CSIA data indicated herbivorous feeding. Therefore, interpretations made from isotopic data for these individuals were consistent with satellite tracking information.

## DISCUSSION

Multiple lines of evidence indicate that the Tortuguero green turtle *Chelonia mydas* population is composed of herbivores that feed over a wide geographic range of neritic habitats with differences in

Table 3. *Chelonia mydas*, *Thalassia testudinum*. Bulk tissue and amino acid  $\delta^{15}\text{N}$  values (‰) of Tortuguero green turtle epidermis and seagrass. Green turtles were identified by their flipper tag numbers. Seagrass sampling sites include 4 sites within the Pearl Cays, Nicaragua (WCC: Wild Cane Cay; SC: Savanna Cay; MC: Maroon Cay; LR: Long Reef), and 1 in the Bahamas (UC: Union Creek). Trophic amino acids—Glu: glutamic acid; Ala: alanine; Asp: aspartic acid; Leu: leucine; Ile: isoleucine; Val: valine. Source amino acids—Phe: phenylalanine; Gly: glycine; Lys: lysine; Ser: serine; Tyr: tyrosine; Arg: arginine; Thr: threonine. Trophic position (TP) was calculated in 2 ways (see 'Materials and methods'): using only Glu and Phe ( $\text{TP}_{\text{Glu/Phe}}$ ) or a combination of several 'trophic' and 'source' amino acids ( $\text{TP}_{\text{Tr}/\text{Src}}$ )

	Green turtles						Seagrass				
	86070	99251	104857	113826	114103 <sup>a</sup>	114128 <sup>a</sup>	WCC	SC	MC	LR	UC
Bulk	3.3	3.0	9.4	7.8	5.4	6.0	2.6	2.7	4.3	2.2	0.4
<b>Trophic</b>											
Glu <sup>b</sup>	6.7	5.5	13.3	11.5	7.9	9.0	4.7	4.7	5.5	4.0	2.6
Ala <sup>b</sup>	8.4	9.4	14.3	10.6	11.2	11.0	3.8	5.7	–	4.3	–
Asp <sup>b</sup>	6.3	4.4	11.7	9.1	8.2	10.2	4.7	6.5	6.2	4.9	3.9
Leu <sup>b</sup>	6.6	5.1	13.4	9.1	8.8	8.7	1.5	3.2	3.3	2.5	–0.1
Ile	8.5	4.2	12.9	9.7	–	–	0.3	5.2	–	2.3	–
Val	3.6	3.6	12.2	8.6	–	–	6.4	7.3	–	5.2	–
<b>Source</b>											
Phe <sup>b</sup>	9.7	7.3	13.1	12.0	10.0	9.5	13.0	13.4	12.9	12.7	9.2
Gly <sup>b</sup>	6.9	6.9	15.1	10.5	10.0	10.0	–2.9	–1.5	–	–0.3	–5.1
Lys <sup>b</sup>	–0.4	–1.4	4.2	1.4	–0.3	1.1	–	3.0	–	3.0	–0.9
Ser <sup>b</sup>	3.3	5.0	12.0	10.3	6.6	8.6	–3.6	0.3	–1.2	–2.1	–3.8
Tyr <sup>b</sup>	–1.8	0.1	4.4	0.3	1.0	0.4	–	3.0	–	3.0	–0.9
Arg	–1.1	–11.8	0.7	1.5	2.4	1.1	–3.4	–2.4	–0.5	–2.4	–6.6
Thr	–3.7	–3.2	1.7	0.1	–	–0.1	–0.8	4.6	3.5	2.6	–
$\text{TP}_{\text{Glu/Phe}}$	1.7	1.9	2.1	2.0	1.8	2.0	–	–	–	–	–
$\text{TP}_{\text{Tr}/\text{Src}}$	1.4	1.6	1.0	2.6	1.4	1.9	–	–	–	–	–

<sup>a</sup>Turtles were satellite tracked

<sup>b</sup>Amino acids used in trophic position calculations



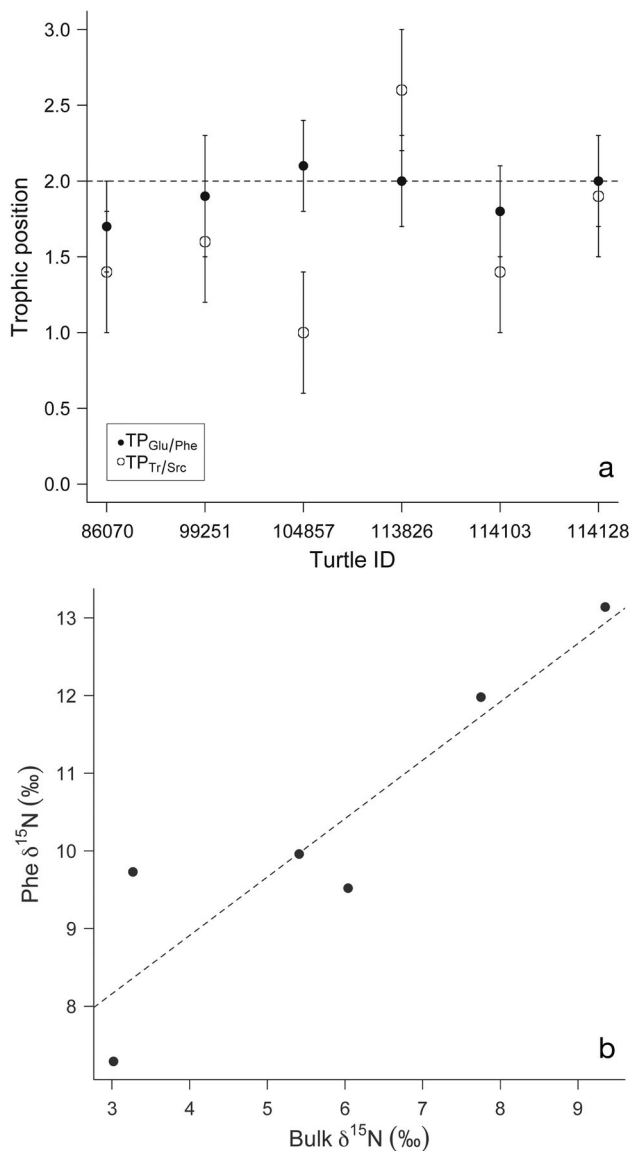


Fig. 4. *Chelonia mydas*. (a) Trophic position was calculated for each green turtle using the nitrogen isotope composition of amino acids through 2 approaches: with glutamic acid (Glu) and phenylalanine (Phe) (TP<sub>Glu/Phe</sub>) or with a combination of 'trophic' and 'source' amino acids (TP<sub>Tr/Src</sub>) (see 'Materials and methods'). The dashed horizontal line indicates the expected trophic position of a herbivore. Bars indicate  $\pm 1$  SD based on the propagated error (see 'Materials and methods'). (b) The relationship between the bulk epidermis  $\delta^{15}\text{N}$  and the 'source' amino acid Phe  $\delta^{15}\text{N}$  values in green turtle epidermis is significant ( $n = 6$ ,  $F = 19.3$ ,  $r^2 = 0.78$ ,  $p = 0.012$ ). Dashed line indicates linear regression (Phe  $\delta^{15}\text{N} = 0.75 \times \text{Bulk } \delta^{15}\text{N} + 5.9$ )

stable isotope compositions of the primary producers. The wide range in bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the nesting population can be explained by distinct isotopic niches at foraging grounds, thus supporting geographic differences as a primary cause for iso-

topic variation among nesting turtles. This is consistent with the prediction of Bearhop et al. (2004) that populations foraging across a range of geographical areas are likely to show more variation in stable isotope values than those from sedentary populations. The nesting population represents a mixture of individuals from a wide range of foraging sites, while each foraging aggregation is comprised of individuals that are relatively site-fixed (Campbell 2003, Bjorndal et al. 2005, Meylan et al. 2011).

The large variation in bulk stable isotope values of *Thalassia testudinum* across the Greater Caribbean and the results of AA-CSIA are fully consistent with baseline differences primarily contributing to variation in the bulk epidermis  $\delta^{15}\text{N}$  values of turtles from the nesting population. Despite a wide range of  $\delta^{15}\text{N}$  values, the close relationship between turtle bulk epidermis  $\delta^{15}\text{N}$  and Phe  $\delta^{15}\text{N}$  values and similar TP<sub>Glu/Phe</sub> estimates indicate that turtles are feeding at a similar trophic position across their geographic range.

Combining all available SrcAA and TrAA data to calculate TP<sub>Tr/Src</sub> resulted in more variable estimates, which may reflect variability among the many amino acids in TEF- or  $\beta$ -values or both. With values that tended to be below the trophic position of 2, this approach yields ecologically implausible results, as primary consumers should have a minimum trophic position of 2. It is possible that some assumptions we make about isotope fractionation of individual amino acids are erroneous, thus underscoring the need for additional studies to quantify TEF- and  $\beta$ -values for the wider range of TrAAs and SrcAAs. In this case, we conclude TP is most robustly estimated by comparing nitrogen isotopic compositions of Glu and Phe. This observation concurs with results of Chikaraishi et al. (2009), which indicate these amino acids have smaller estimation error and greater precision, though this may not be the case for all species or food webs.

We found that the  $\beta$ -value appropriate for seagrass is consistent with that of  $\text{C}_3$  terrestrial plants and is very different from other aquatic primary producers. This is not surprising, given seagrasses are descendant from terrestrial angiosperms and use a  $\text{C}_3$  pathway of photosynthesis (Hemminga & Mateo 1996, Waycott et al. 2006). Trophic position estimates were erroneous when using the aquatic algal producer  $\beta$ -value measured for other aquatic food webs (Chikaraishi et al. 2009), which underscores the need to understand amino acid metabolism in the primary producer at the base of the food web when calculating TP based on amino acid nitrogen isotopic composition.

The sample size for AA-CSIA was limited to 6 individuals due to the cost of this analysis. The trade-off

for this powerful methodology is that sample sizes are often small and encompass a subset of a larger sample base (Dale et al. 2011, Seminoff et al. 2012). Bulk epidermis  $\delta^{15}\text{N}$  values were used to select samples that would be most likely to demonstrate differences in trophic position if they were present.

It is also necessary to understand habitat-derived differences in stable isotope patterns when translating the isotopic niche to the ecological niche (Flaherty & Ben-David 2010). The observed range in  $\delta^{15}\text{N}$  values of 6.3‰ in the nesting population could represent 2 or more trophic levels, if calculated using available bulk nitrogen isotope discrimination factors for epidermis in green turtles (2.8 to 4.0‰; Seminoff et al. 2006, Vander Zanden et al. 2012). In addition, lower  $\delta^{13}\text{C}$  values are found in oceanic/pelagic habitats compared to coastal/benthic habitats (Rubenstein & Hobson 2004). A subset of the nesting population exhibits the combination of low  $\delta^{13}\text{C}$  values and high  $\delta^{15}\text{N}$  values that could be indicative of an oceanic, carnivorous feeding strategy as displayed by juvenile green turtles and loggerheads (Reich et al. 2007, Pajuelo et al. 2010). A carnivorous portion of the Tortuguero nesting population would align with the carnivorous foraging patterns of some Pacific populations determined through stable isotope analysis, stomach contents, satellite tracking, and video analysis (Bjorndal 1997, Hatase et al. 2006, Amorcho & Reina 2007, Arthur et al. 2007, Lemons et al. 2011). Yet neither the AA-CSIA based trophic position estimates nor additional investigation into the nesting population isotopic niche (through comparison with the foraging niches and the seagrass niche) provided evidence of carnivory, thus underscoring the ecological role of the Atlantic green turtle as a primary consumer (reviewed in Bjorndal 1997). These turtles do not exhibit evidence of alternative foraging strategies, possibly due to the extensive seagrass pastures found throughout the Caribbean and a population size that is far from carrying capacity (Bjorndal & Jackson 2003).

Assessing the foraging ecology of the nesting population based on bulk isotopic values and without the additional information used in this study might have led to incorrect interpretations. Therefore, we emphasize that caution must be used when interpreting the isotopic niche of wide-ranging consumers to avoid erroneous conclusions when geographic differences cause isotopic variation across the foraging range. However, stable isotope analysis—often in conjunction with other methods—has successfully been applied to determine the habitat used in the nonbreeding season for many other migratory mar-

ine species, such as Atlantic salmon *Salmo salar* (MacKenzie et al. 2011), leatherback sea turtles *Dermochelys coriacea* (Seminoff et al. 2012), loggerheads *Caretta caretta* (Zbinden et al. 2011, Pajuelo et al. 2012b), wandering albatrosses *Diomedea exulans* (Jaeger et al. 2010), and humpback whales *Megaptera novaeangliae* (Witteveen et al. 2009).

Particularly for sea turtles, assessing geographic origin is critical for understanding population interconnectivity between nesting and foraging areas and is important for population modeling and management. For example, seagrass beds in the coastal waters of Nicaragua are known to host foraging individuals that nest in Tortuguero (Troëng et al. 2005), but estimates of the proportion of this nesting population that come from foraging grounds in Nicaragua vary from 65 to 86% using other methods such as flipper tags, satellite tracking, and genetics (Carr et al. 1978, Troëng et al. 2005, Bolker et al. 2007, Sea Turtle Conservancy 2012). The isotopic niche may provide a complementary method to assess the contribution of particular foraging grounds to the nesting population, but doing so accurately requires isotopic variation among foraging grounds with an understanding of the source of isotopic variability (i.e. diet vs. habitat). While we now have more information about the causes of isotopic variability in this region, we do not yet have enough resolution to adequately determine geographic origin using the isotopic niche. However, additional information on bulk epidermis and compound-specific amino acid isotope values of green turtles at other foraging grounds in conjunction with other methods would likely improve our ability to assess population connectivity in the Greater Caribbean.

## CONCLUSIONS

The Tortuguero nesting population of *Chelonia mydas* appears to be strictly herbivorous and feeds over a wide geographic range, as indicated by results of seagrass stable isotope composition and AA-CSIA of amino acids. We demonstrate that the range in stable isotope values of the Tortuguero nesting population is not related to differences in trophic position but rather is primarily determined by differences in biogeochemistry of a foraging area, which controls the  $\delta^{15}\text{N}$  values of primary producers. While  $\delta^{15}\text{N}$  values are typically used as an indicator of trophic level, we found that baseline differences due to spatial variation in the primary producer greatly influence green turtle  $\delta^{15}\text{N}$  values. Therefore, we caution that

bulk tissue stable isotope values of a highly dispersed or wide-ranging species may be difficult to interpret in the absence of baseline values or without the use of AA-CSIA to understand causes of isotopic variability (see also Seminoff et al. 2012). Information on where and what green turtles eat is critical to protecting the areas in which these turtles spend most of their lives and for assessing the risk of encountering anthropogenic threats such as oil spills or incidental capture in fisheries.

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## **Trophic ecology of a green turtle breeding -population**

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### **Supplement 1. Additional data**

#### **MATERIALS AND METHODS**

To report size as curved carapace length (CCL), conversions were made from alternate length measurements for green turtles *Chelonia mydas* sampled in Nicaragua and Inagua, Bahamas. Conversions to CCL were made using linear regression equations from other turtles measured at those sites. For the Nicaragua sites, direct CCL measurements were available for 32 of the individuals sampled. For the remaining 151 turtles, CCL values were derived from curved plastron length (CPL) measurements based on a regression of 814 adult turtles encompassing the size range of the sample population ( $CCL = 1.089 \times CPL + 11.008$ ,  $r^2 = 0.84$ ) (Lagueux & Campbell unpubl. data). For Inagua, direct CCL measurements were available for 42 of the individuals sampled. For the remaining 20 turtles, CCL values were derived from straight carapace length (SCL) measurements based on a regression of 1421 juvenile green turtles encompassing the size range of the sample population ( $CCL = 1.043 \times SCL - 0.345$ ,  $r^2 = 0.99$ ) (Bjorndal & Bolten unpubl. data).

Table S1. Mean and SE of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *Thalassia testudium* analyzed in this study and collected from the literature for sites in the Greater Caribbean. The standard error was not reported in the original study, it was calculated using the reported standard deviation and sample size. Site ID refers to the identification numbers in Fig. 2c of the main text ('Results')

Site ID	$\delta^{13}\text{C}$ (‰)	SE $\delta^{13}\text{C}$	$\delta^{15}\text{N}$ (‰)	SE $\delta^{15}\text{N}$	n	Location	Source
1	-6.6	0.1	1.2	0.2	9	Union Creek, Inagua, Bahamas	Present study
2	-9.4	0.7	2.9	0.5	4	RAAS, Nicaragua	Present study
3	-7.7	0.1	5.6	0.01	2	St. Joe Bay, Florida, USA	Present study
4	-6.3	0.1	3.0	0.1	2	Tobacco Reef, Belize Barrier Reef, Belize	Abed-Navandi & Dworschak (2005)
5	-7.2	0.3	2.1	0.4	12	Florida Keys Nat'l Marine Sanctuary, USA	Anderson & Fourqurean (2003)
6	-8.4	0.3	2.2	0.2	12	Florida Keys Nat'l Marine Sanctuary, USA	Anderson & Fourqurean (2003)
7	-10.4	0.4	1.1	0.2	12	Florida Keys Nat'l Marine Sanctuary, USA	Anderson & Fourqurean (2003)
8	-7.7	0.2	1.7	0.2	12	Florida Keys Nat'l Marine Sanctuary, USA	Anderson & Fourqurean (2003)
9	-7.5	0.4	3.2	0.4	2	Florida Keys, ocean side, USA	Behringer & Butler (2006)
10	-6.9	0.2	2.9	0.1	2	Florida Keys, impacted bay side, USA	Behringer & Butler (2006)
11	-6.5	0.5	2.8	0.4	2	Florida Keys, non-impacted bay side, USA	Behringer & Butler (2006)
12	-10.7	0.2	6	0.3	10	Florida Bay, USA	Fourqurean & Schrlau (2003)
13	-14.1	-	3.2	-	-	Laguna Joyuda, Puerto Rico	France (1998)
14	-13.6	-	3.7	-	1	Schooner Bank, Florida Bay, USA	Harrigan et al. (1989)
15	-11.5	1.6	1.4	0.1	3	Biscayne Bay, Florida, USA	Kieckbusch et al. (2004)
16	-8	0.6	-0.2	0.5	3	Andros & Grand Bahamas Island, Bahamas	Kieckbusch et al. (2004)
17	-8.5	0.3	-0.3	0	4	Jaragua, Dominican Republic	Tewfik et al. (2005)
18	-8.7	0.1	3.1	0.1	4	Barahona, Dominican Republic	Tewfik et al. (2005)
19	-7.3	0.3	2.6	1.0	3	Twin Cays, Belize	Wooller et al. (2003)

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