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Trophic Ecology of Green Turtles (Chelonia mydas) From Dry Tortugas National Park, Florida

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TROPHIC ECOLOGY OF GREEN TURTLES (*CHELONIA MYDAS*) FROM DRY TORTUGAS NATIONAL PARK, FLORIDA

By

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ABSTRACT

Located 100 km west of Key West, Florida, Dry Tortugas National Park (DRTO) is a largely untouched subtropical marine ecosystem that serves as an important developmental habitat, nesting ground, and foraging area for several species of sea turtles, including green turtles. The Park supports a recovering population of green turtles comprised of resident juveniles, subadults, and adults of both sexes; nesting females include residents and migrating females that only return to nest. Stable isotope analysis has been applied widely to describe the trophic ecology of green turtles, from urbanized bays with significant anthropogenic input, to relatively pristine ecosystems with healthy populations at carrying capacity. However, there is a paucity of published literature about the trophic ecology of green turtles in DRTO. This study describes the trophic ecology occupied by two distinct size groups (61 green turtles < 60 cm (SCL) and 98 green turtles > 60 cm (SCL)). Flipper tissue and plasma were analyzed for stable isotopic composition of C and N. Flipper tissue values for $\delta^{15}N$ (3.41‰ to 9.69‰) and $\delta^{13}C$ (-22.43‰ to -5.38‰) fall within literature values for green turtles, and the wide range of values indicated they could potentially feed at multiple trophic levels. Understanding the trophic ecology of this population of green sea turtles is instrumental to effective management and habitat preservation strategies in DRTO.

Keywords: Stable isotopes, Herbivory, Seagrass ecosystem, $\delta^{13}C$, $\delta^{15}N$, Florida Keys National Marine Sanctuary

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1. INTRODUCTION

Sea turtle populations are a shadow of their former levels, as commercial exploitation in the late 1800's brought populations down to 10% of former stocks. Green sea turtles were originally thought to be primarily seagrass consumers; however, recent studies indicate that diet varies depending on geographic location and resource availability. Green sea turtles in the Caribbean still hold true to the original paradigm of seagrass-dependence. Prior studies in the Caribbean indicate that green turtles are primarily herbivores (Bjorndal, 1980, Bjorndal, Lutz, & Limpus, 1997, Vander Zanden et al. 2013) and green turtles in the Bahamas crop patches of seagrass and consume the young cropped blades (Bjorndal, 1980, Fourqurean et al. 2010). Green turtle populations in the Mediterranean also begin grazing on seagrasses after recruitment (Cardona et al. 2010). Green turtles in Moreton Bay, Australia primarily feed on algae (Brand-Gardner, Limpus, & Lanyon, 1999). A study by Burkholder, Heithaus, and Fourqurean (2011) in Australia shows individual diet variation between individuals suggesting that nutrient content of green turtle prey items (seagrass, algae, and gelatinous macroplankton) may influence foraging behavior. Currently, all species of sea turtles are under pressure from land development, habitat degradation, light pollution, pollution, potential bycatch in fisheries, direct harvest, and a changing seagrass landscape in the Caribbean due to the introduction of non-native species (Bräutigam & Eckert 2006, Willette et al., 2014).

Conservation of green turtle habitat is instrumental in protecting the species, which has been historically classified as "endangered" on the *International Union for Conservation of Nature* red list (assessed in 2004) since 1986. Effective May 2016 the National Marine Fisheries Service, the National Oceanic and Atmospheric Administration, and the United States Fish and Wildlife Service revised the "endangered" listing of the green turtle and instead list 11 distinct population segments (DPSs), with eight threatened DPSs and three endangered DPSs. The Florida and Caribbean population fall in the North Atlantic DPS which is classified as "Threatened". The green turtles in Dry Tortugas National Park (DRTO) are a recovering population that has resident juveniles, subadults, and adults of both sexes. Nesting females include residents that never leave and migratory females that only return to nest (Hart et al., 2013). Historically, when green turtle numbers were higher, the seagrass beds at the Dry Tortugas were

grazed down to the blade-sheath junction (Thayer et al. 1982; McClenachan et al., 2006; Van Houtan & Pimm, 2007). Presently, grazed and maintained patches of *Thalassia testudinum* are found at the Dry Tortugas (K. Hart pers. comm), but anecdotally the areas of these grazed patches are much less that historically observed.

1.1. Dry Tortugas National Park

Dry Tortugas National Park encompasses 100 mi^2 within the Florida Keys National Marine Sanctuary (FKNMS). The park is composed of several areas (Fig. 1B), the NCZ that encompasses 50% of the park, the Historic Adaptive Use Zone (HAU) which is 3% of the park, and the RNA. The NPS and the Florida Fish and Wildlife Conservation Commission (FWC) created the 46 mi^2 RNA in 2007, this accounts for 46% of the park. The RNA is a 46 mi^2 marine reserve in which fishing and other detrimental activities to the ecosystem are prohibited in order to preserve and promote marine biodiversity. The Dry Tortugas are an important developmental habitat and foraging ground for several species of sea turtles and a key nesting ground for green turtles *Chelonia mydas* (Linnaeus, 1758) and loggerhead sea turtles *Caretta caretta* (Linnaeus, 1758) (Hart et al., 2010; 2013). In 2010 over 50% (186 of 369) of the sea turtle nests that were monitored in DRTO belonged to green turtles. The third largest nesting season was 10 years prior and hosted 181 nests (NPS, 2010). In 2016 there were 397 nests. East Key hosted approximately 44% of the nests. Throughout DRTO, 19% of nests belonged to green turtles (77 nests) and 81% belonged to loggerhead turtles (320 nests). This is the lowest number of green turtle nests documented since 2004, though it was expected to be a low year due to the cyclical nature in sea turtle nesting behavior.

Male turtles are often underrepresented in the literature due to their pelagic nature and capture difficulty (Godley et al., 2008). Through acoustic tagging and satellite tracking of green sea turtles at DRTO in previous years, we now know where the majority of these tagged turtles are spending their time. Tagged green turtles are detected in all areas of the park; with nesting green turtles having a distinct core use area in the Research Natural Area (RNA). The Natural Cultural Zone (NCZ) (Fig. 1B), outside of the RNA is a heavily utilized core use area for green turtle males and sub-adults (Hart et al., 2012). In 2010 and 2011, Hart el al. (2013) satellite tagged 11 nesting green turtles. 9

out of the 11 turtles utilized other areas within the FKNMS, the Marquesas, the southern end Biscayne National Park, and Everglades National Park. The remaining 3 stayed within park boundaries. The study also examined the benthic habitat at Pulaski Shoal and Northkey Harbor at DRTO using Along-Track Reef-Imaging System (ATRIS) and found seagrass habitat with greater than 75% coverage at 21.9% of the overall study site; presence of seagrass increased to 42% in the 'hotspot' where multiple turtle activity centers overlapped.

1.2. Green turtle biology

The green turtle, belonging to the family Cheloniidae, is one of seven extant species of sea turtles of what once was a varied group of cryptodiran turtles. Cryptodiran turtles are identified by the mechanism that allows them to close their jaw and the method in which they retract their head (Gaffney 1975; Gaffney & Meylan, 1988). Green turtles have an oval shaped shell and a single claw on each flipper (Eckert et al., 1999). Adult green turtles can weigh up to 230 kg and can have a straight carapace length (SCL) exceeding 1 m (Eckert et al., 1999).

Proper nutrition among green turtles is necessary for growth and fecundity (Hadjichristophorou & Grove, 1983). Females nest every one to three years, returning to their natal beaches to lay their eggs. During a nesting season, female green sea turtles may return to the nesting beach up to seven times and lay clutches containing an average of 100-130 eggs each; at DRTO, Hart et al. 2013 documented up to 6 clutches per female. Green turtle nesting season in Florida can begin in April, but primarily starts in May and continues through October (Meylan et al., 1995). Upon hatching, the turtles will begin the pelagic phase of their life cycle, which lasts for several years. During this pelagic phase juveniles are omnivorous, floating around Sargassum mats, feeding on a variety of items including cnidarians, molluscs, and crustaceans (Bjorndal, 1985 & 1997). As juveniles, green turtles recruit to neritic areas abundant in seagrass or marine algae after their pelagic phase and are thought at this time to make the switch to a primarily vegetative diet (Bjorndal, 1980; Musick & Limpus, 1997). Green sea turtles in the North Atlantic reach sexual maturity at approximately 44 years (Goshe et al., 2010). Mating events are

thought to occur near nesting beaches. At internesting habitats, turtles congregate from wide-ranging foraging areas (Hamann et al., 2010).

1.3. Conservation efforts

Green turtles are distributed globally through tropical and temperate latitudes (30° N to 30° S) (Godley et al., 2001). Several international treaties and agreements have been initiated that work towards protecting sea turtles. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an agreement signed by 180 participating nations to protect native animals and plants in the wild by monitoring and prohibiting trade of protected organisms, green turtles are listed in Appendix I, which provides the highest level of protection. The Convention on the Conservation of Migratory Species of Wild Animals (CMS) is a treaty formed under the United Nations Environmental Program, green turtles are listed in Appendices I and II, which pertain to endangered migratory species and migratory species conserved through agreements, respectively. The Inter-American Convention (IAC) is an intergovernmental treaty that serves to take actions benefitting sea turtles. It is the only international treaty designed solely for marine turtles.

On the national level, the National Marine Fisheries Service (NMFS) puts measures into effect to reduce sea turtle interactions in fisheries through the Endangered Species Act and the Magnuson-Stevens Fishery Conservation and Management Act. Regulations to protect sea turtles include Sea Turtle Observer requirements aboard vessels in select fisheries, turtle excluder devices (TED), fishing gear reviews and continuing to review sea turtle interactions in fisheries to prepare environmental impact statements and recovery plans.

Aside from legislation and treaties, local sea turtle conservation includes monitoring of nesting beaches, nest relocation, and "head starting" turtles. A study by Godfrey (1995) showed relocating eggs reduced hatchling success. While not ideal, nest relocation can be useful under the right circumstances (i.e., accepting partial loss in place of the potential for total loss). "Head started" turtles are hatched in captivity and released under optimal conditions (Garcí, 2003). Conservation efforts need to focus on adults as

well. Studies by Lazar et al. (2004) and Tomas et al. (2002) indicated that adult turtles have a larger effect on the population than hatchling mortalities.

Conservation success largely depends on the ability to enforce and maintain regulations. A study conducted by Humber, Godley, and Broderick (2014) concluded that the legal take of sea turtles, not accounting for poaching and bycatch, in the 42 countries that still permit a marine turtle fishery was greater than 42,000 turtles annually. In areas where efforts are successful, we now face the realization of growing herbivore populations, reduction of apex predators, and anthropogenic influences on already impacted seagrass ecosystems (Jackson et al., 2001; Fourqurean et al., 2010, Heithaus et al., 2014). Grazing areas with rebounding populations are found to have lower diversity and biomass, and potentially not sustain rising sea turtle numbers ultimately causing ecosystem collapse. (Heithaus et al., 2007; Fourqurean et al., 2010; Lal et al., 2010; Christianen et al., 2012). The global reduction in shark populations (Ferretti et al., 2010) has affected seagrass ecosystems similar to the extirpation of the grey wolf (*Canis lupus*) from Yellowstone National Park (Ripple & Beschta 2012); without the presence of an apex predator, primary producers were grazed by herbivores to the point of inducing trophic downgrading (Estes et al., 2011).

1.4. Stable Isotope Analysis

Stable isotopes are forms of elements that have a different number of neutrons in the nucleus, are not hazardous, and remain stable for long periods of time (Fry, 2006). “Heavy” stable isotopes have an extra neutron in the nucleus. Heavier stable isotopes react more slowly because of the extra neutron(s) which form bonds that are harder to break, while lighter isotopes form bonds that are more easily broken apart. The apportioning of isotopes between products is due to the different isotopic masses and a chemical difference is known as fractionation (Hoefs 2009). The processes driving fractionation are isotope exchange reactions and kinetic processes, such as evaporation, dissociation reactions, biologically mediated reactions, and diffusion (Hoefs 2009). Using calculated stable isotope values scientists infer an animal’s dietary life history including foraging and resource use.

The principle elements that are analyzed from tissue samples for dietary studies are carbon and nitrogen (Post 2002, Fry, 2006). The isotopes of these elements that are useful in SIA are ^{13}C and ^{15}N. For analysis, samples are combusted by mass spectrometer to release gasses containing carbon and nitrogen. Magnets inside the spectrometer pull the heavier and lighter isotopes in different paths, with the lighter isotopes "falling" out first. The sum of the values of the heavy and light isotopes allows the calculation of the ratio in the sample.

^{13}C values are useful because they can help determine the basal resource food web an organism is feeding in (marine vs terrestrial, planktonic, detrital, etc.) (DeNiro & Epstein, 1978; Post 2002), since different primary producers have very different fractionation compared to their CO2 source, leading to distinct stable isotope compositions of organic compounds synthesized by different plant groups, and very little fractionation of those plant compounds when they are consumed by heterotrophs. Studies have shown that marine and terrestrial ^{13}C values do not overlap (DeNiro & Epstein, 1978). The ratio of ^{13}C to ^{12}C is represented with $\delta^{13}C$ (Fry 2006).

Nitrogen isotopes exhibit a trophic enrichment with each step in the food chain as the lighter isotope ^{14}N is preferentially excreted at each trophic level resulting in a more enriched ^{15}N in the bodies of the heterotrophs compared to their food (DeNiro & Epstein, 1981), this can provide a relative trophic position at which the organism is feeding. Enrichment of $\delta^{15}N$ in marine organisms can be linked to terrestrial inputs, even though there are biological processes that enrich $\delta^{15}N$ (Anderson et al., 2011). The ratio of ^{15}N to ^{14}N is represented with $\delta^{15}N$.

Different tissue types represent different time scales of isotope assimilation because of tissue specific turnover rates (Fry, 2006). Isotope incorporation rates were experimentally determined to be approximately 2-3 months for green turtles, significantly longer in adults (~300 days) and discrimination factors were also determined experimentally (Seminoff et al., 2006 & Vander Zanden et al., 2014). The incorporation rate for epidermis in another rapidly growing ectotherm, juvenile loggerheads, is approximately 4 mo, faster than the incorporation rate of adults, which have slower growth rates (Reich et al., 2008). Plasma has a very fast turnover rate. Flipper tissue, analogous to epidermis, is used in the majority of the studies. Another sample type,

carapace scute punches are unique because it captures dietary information in the layers it is composed of (Reich et al. 2007). Layers of scute approximately 50 µm thick are thought to represent the isotopic signal of several months (Reich et al. 2008 & Vander Zanden et al. 2010). These layers can show a time series or the accumulated average of the green sea turtles dietary information. Analysis of several tissue types allows us to piece together a mosaic of snapshots and can help us identify variations in their diet over both short and long time frames, potentially revealing differences in diet from their foraging habitats, prior to nesting beach arrival, to their diet during the inter-nesting period.

1.5. Effects of lipids in Stable Isotope Analysis

The lipid concentration in a sample can significantly affect $\delta^{13}C$ values. High lipid concentrations can induce a 3 to 4 ‰ more negative $\delta^{13}C$ value in a sample. Lipid extraction techniques chemically removes the lipids from samples using a solvent, creating a sample set that has evenly low lipid levels. Lipid extraction should be used when lipid content is variable among the consumers or consumers and prey, and when there is a difference of <10-12 ‰ in the $\delta^{13}C$ signature between consumers and prey (Post et al., 2007). A concern when using lipid extraction on tissue samples is that it may cause fractionation to occur in $\delta^{15}N$ (Pinnegar & Polunin 1999, Sotiropoulos et al., 2004).

A review of recent publications shows that lipid extraction is widely used when analyzing tissue from sea turtles (Table 3). Another alternative to the time-consuming lipid extraction is a *post hoc* mathematical normalization technique initially investigated by McConnaughey and McRoy (1979), but further refined by Post et al. in 2007. This *post hoc* method exploits the relationship between C:N, % lipid, and $\delta^{13}C$. While the equation may not be suitable for a variety of species, it is reliable when using it on values derived from marine organisms, such as our study species the green turtle.

1.6. Previous stable isotope studies conducted on sea turtles

One of the earliest studies to use stable isotope analysis on sea turtles to determine trophic relationships was conducted by Godley et al. (1998). This study compared stable

isotope values across several species of sea turtles, including green turtles, hawksbill turtle (*Eretmochelys imbricata*), loggerheads, and leatherbacks (*Dermochelys coriacea*). Bone, carapace, and nest contents were collected, but not for each species. The green turtle tissue samples showed an enrichment of δ15N that indicated a diet that was not completely herbivorous, which we know today to be true for green turtles outside of the Caribbean (Burkholder et al., 2011; Carman et al., 2012; Hatase et al., 2006).

Seminoff et al. (2006) performed a diet switching experiment in which eight juvenile green turtles were fed a specific diet for over 600 days to normalize their carbon and nitrogen isotopic signature to better understand the discrimination factors of these two elements across multiple tissue types. The study provided stable isotope ratios of green turtles in a controlled setting. Whole blood (WB), red blood cells (RBC), blood plasma (BP), and epidermis (EPI) were analyzed for δ13C and δ15N. Samples were taken at Day 371 and at Day 619. The change (Δdt) in the δ13C and δ15N isotopic signature from day 371 and day 619 was measurable (see Table 1). The results from this experiment gave tissue specific discrimination factors for green sea turtles, instead of the generic 0 to 1‰ for $\delta^{13}C$ and 4 to 5‰ for $\delta^{15}N$. The tissue specific discrimination factors give us better resolution when calculating mixing models of prey contribution to a green turtle's diet.

Vander Zanden et al. (2012) expanded on Seminoff's work by examining the variation in carbon and nitrogen stable isotope values of captive green turtles against variation of isotope values found in a wild population of Caribbean green turtles. The study examined discrimination factors of four tissue types (epidermis, dermis, serum, and red blood cells) in captive juvenile and adult green turtles fed an isotopically consistent diet (Table 1). Results indicated that variation is dependent on life stage and tissue composition. Discrimination factors for both juveniles and adults were different than the ones derived from Seminoff's study, and were found to vary based on tissue type, diet, species, and growth rate. Applying the discrimination factors calculated from the Caribbean green turtles in Vander Zanden's study to our data will ensure the most likely differences in diets are represented as accurately as possible with the data provided.

Mixing models have been used extensively with stable isotope datasets to make predictions on possible resource use by consumers within an ecosystem. At the infancy of

diet reconstruction with stable isotopes, simple linear models were used and were unable to account for more than one isotope. Currently, Bayesian models are often used.

1.7. Research questions

The focus of this project was to analyze the isotopic signatures from several tissue types collected since 2008 from both male and female green turtles sampled at the Dry Tortugas to describe the trophic ecology of this recovering sea turtle population. I proposed several questions for this study:

1. Is there a difference between the stable isotope signatures of blood, flipper, and homogenized scute?
2. Do stable isotope values differ in green turtles of different size classes?
3. Do stable isotope values of adult green turtles differ with gender?
4. Is there a difference in stable isotope values from satellite tagged "Resident" and "Non-resident" turtles?
5. What is the potential contribution of prey items to DRTO green turtle diets?

2. MATERIALS AND METHODS

2.1. Study site

We conducted this study in Dry Tortugas National Park, Florida, which is located approximately 100 km west of Key West, Florida (Figure 1). The park was initially established in 1935 as "Fort Jefferson National Monument" it was later reestablished as "Dry Tortugas National Park". Geologically, the Dry Tortugas are a cluster of carbonate banks and sand shoals resembling an atoll (Mallinson et al., 2003). Seven islands make up the Dry Tortugas, the largest island being Loggerhead Key measuring ~1.5 km long x ~250 m wide and is home to the Dry Tortugas Lighthouse. Fort Jefferson is located (24°37'41.34"N, 82°52'19.02"W) adjacent to Loggerhead Key on Garden Key. Bush Key and Long Key lie to the east of Garden Key. Continuing to the northeast are Hospital, Middle and East Key, which is the smallest, measuring ~400 m long x ~100 m wide.

I sampled green turtles from within the boundaries of the Tortugas Ecological Reserve as well as the Research Natural Area (RNA). The most abundant seagrass found

around the Dry Tortugas is *Thalassia testudinum*. We captured juvenile turtles in the shallows adjacent to Garden Key and Bush Key and adults by Pulaski Shoals, located 10 km northeast of Garden Key (Fig. 1B).

2.2. Field methods

From 2008-2015 we collected samples from green turtles. We sampled from nesting females and free swimming/foraging individuals. Sampling took place annually both before and throughout the nesting season. Turtle capture and work-up of nesting females followed methods employed by Hart et al. (2013). Established protocols for taking biological samples and marking each animal were followed (NMFS-SEFSC, 2008). We caught juvenile green turtles using a dip net while standing at the bow of our research vessel. We caught adult green turtles using the 'rodeo' technique (Ehrhart & Ogren, 1999). The 'rodeo' technique relies on spotting a turtle from an underway vessel and having two individuals equipped with mask, fins, and snorkel diving on top of the turtle during the turtle's surface interval, effectively restraining it, and bringing it aboard the vessel to work-up. This provides a minimally invasive, fast processing alternative to using nets or other capture techniques. We restrained female turtles on the nesting beach in a four section corral.

2.2.1. Sample collection

We measured curved and straight carapace length (CCL, SCL) and curved and straight carapace width (CCW, SCW). Measurements were taken from the midpoint of the precentral scute to the posterior tip of the postcentral scute. Immediately after taking measurements we applied Inconel flipper tags and Passive Integrated Transponder (PIT) tags. Soft tissue was collected from the right rear flipper using a sterile 6mm Sklar biopsy punch. Approximately 2ml of whole blood was taken from the dorsal cervical sinus with a needle and 2 ml syringe (Owens & Ruiz 1980). The whole blood was then spun down into the pellet and supernatant, red blood cells and plasma, respectively

2.2.2. Gastric lavage

We collected diet samples from individuals via gastric lavage following the methods of Forbes and Limpus (1993). We carefully placed each captured turtle in an upside-down position on a researcher's lap, with its head lower than its body. We carefully opened the mouth, inserted flexible rubber tubing, pumped seawater into the stomach, and flushed out and collected recently-consumed food items. We filtered each sample, placed it into a 500 ml tube, and stored it frozen until later identification in the laboratory. After thawing each frozen sample, we separated diet items into the following categories: grass, algae, detritus, crustaceans, mollusks, other invertebrates, sand, coral, unknown solids, and unknown gelatinous material. Using a dissecting scope, we identified each item to the lowest taxonomic level possible. We measured dry weight of each food category. Lavage samples were placed in a tare inside the drying oven at 70°F for 3 to 7 days, depending on the size of the sample and then weighed.

2.3. Sample Preparation and Isotope Analysis

We thawed tissue samples, rinsed them with distilled water, dried them at approximately 60°C for up to 48hrs, and then pulverized the samples using a mortar and pestle to a fine powder. Carapace samples were rinsed with distilled water, dried at <60°C for up to 48hrs, cut into smaller pieces with scissors, and then ground to a fine powder. Plasma samples were thawed, poured out over glassware, and dried at < 60°C for at least 24 h, scraped off the glassware, and then pulverized with a mortar and pestle to a fine powder. Proper lab protocols were taken to avoid contamination.

We tared 3.3 x 5mm tin capsules and measure out 0.60-0.70mg of sample into them. All encapsulated samples were analyzed for stable carbon and stable nitrogen isotopes at the Florida International University Department of Biological Sciences Stable Isotope Lab. The Stable Isotope Lab at Florida International University uses a continuous flow IRMS machine coupled to elemental analyzers, specifically, a Finnigan *Delta* C EA-IRMS. Standardized notation of $\delta\,^{13}C$ and $\delta\,^{15}N$ was determined by DeNiro and Epstein (1981, 1978). Best practices for terminology were reported by Bond and Hobson (2012) and is represented as follows:

$$\delta^{\text{heavy/light}}X = \frac{(^{\text{heavy}}X/^{\text{light}}X)_{\text{sample}}}{(^{\text{heavy}}X/^{\text{light}}X)_{\text{standard}}} - 1$$

$^{\text{heavy}}X/^{\text{light}}X$ are the ratios of heavy to light isotopes (^{13}C:^{12}C, ^{15}N:^{14}N) in the sample and standard, respectively. Carbon stable isotope ratios are reported relative to the international standards of Pee Dee Belemnite (PDB) or the equivalent Vienna PDB (VPDB) standard. Nitrogen stable isotope ratios are reported relative to the standards of atmospheric nitrogen (AIR). Standard error for this study based on internal glycine standards is ± 0.18 ‰ for $\delta^{15}N$ and ± 0.10 ‰ for $\delta^{13}C$. Internal standards were run every 6 to 8 experimental samples to ensure proper system calibration.

2.3.1. Lipid extraction

The equation ($\delta^{13}C_{\text{normalized}} = \delta^{13}C_{\text{untreated}} - 3.32 + 0.99 \times C{:}N$) developed by Post et al. (2007) was used for samples > 3.5 Carbon to Nitrogen ratio, as I did not extract lipids.

2.3.2. Literature stable isotope values and discrimination factors

Stable isotope values from the literature were used as resources for the mixing model SIAR in R. Tropical marine seagrasses including: *Thalassia.testudinum, Halodule wrightii*, and *Syringodium filiforme*, were grouped into a "Seagrass" functional unit. Macroalgae including the Rhodophyta *Laurencia spp*., the Phaeophyta *Dictyota spp*., and the Chlorophyta *Halimeda spp*., were also grouped into a functional unit designated "Macroalgae". The Schyphozoan *Aurelia aurita* was included as potential prey as well. See Table 3. I used discrimination factors published in Vander Zanden et al's., (2014) study on inherent variation and discrimination factors of green turtles (Table 4).

2.4. Statistical analysis

All statistical analyses were carried out with the program R (R Development Core Team 2011). For certain analyses green turtles were binned to specific length groups: < 60 cm SCL and > 60 cm SCL, or gender. Turtles were binned into these groups as past

studies have seen diet shifts around 60 cm SCL (Arthur et al., 2008, Cardona et al., 2010. The relationship of length (SCL) and stable isotope values were examined with linear models. Gender differences between males and females based on flipper stable isotope values were tested for homogeneity of variances using a robust Forsyth Levene-Brown test followed by an Analysis of Covariance. Isotopic differences from flipper tissue between turtles that were classified as "resident" or "non-resident" were tested using a Mann-Whitney-Wilcoxon test. Differences between stable isotope values of flipper tissue and plasma based on size bins of green turtles < 60 cm and turtles > 60 cm were tested using repeated measures ANOVA, accounting for individuals. Basic descriptive statistics were used on the gastric lavage data.

To better understand the potential dietary contribution of resources to sea turtle diet, SIAR (Stable Isotope Analysis in R- Jackson et al. 2011) a Bayesian-mixing model package in R was used. SIAR solves for the most probable dietary proportions based on the food sources and consumers and allows for uncertainties such as discrimination factors. Bayesian models assume that the data is fixed and that parameters are probabilistic, contrary to earlier frequentist mixing models which have the parameters as being fixed. The Bayesian method realistically reflects the potential plasticity of resource use by an organism through calculating probabilities.

3. RESULTS

Flipper Tissue

I analyzed 159 flipper samples (Table 5). The δ^{13}C values of green turtles < 60 cm SCL (n = 61) ranged from –16.57 to –7.05 ‰ ($\bar{x}$ = –11.30 ± 2.58 ‰) and δ^{15}N values ranged from +6.14 to +10.61 ‰ ($\bar{x}$ = +8.22 ± 1.07 ‰). Straight carapace length of turtles < 60 cm ranged from 22.3 to 51.5 cm ($\bar{x}$ = 35.7 ± 7.1). The δ^{13}C values of turtles > 60 cm (n = 98) ranged from –13.09 to –5.38 ‰ ($\bar{x}$ = –7.90 ± 1.18 ‰) and δ^{15}N values ranged from +3.70 to +9.53 ‰ ($\bar{x}$ = +7.04 ± 1.04 ‰). Straight carapace length of turtles > 60 cm ranged from 65.3 to 111.7 cm ($\bar{x}$ = 93.66 ± 9.62).

3.1. Correlation of length to stable isotope values

I examined the relationship of SCL on $\delta^{13}C$ and on $\delta^{15}N$ values of all individuals with linear models. The relationship between $\delta^{13}C$ and SCL was fit with a log model. The relationship between $\delta^{15}N$ and SCL used a standard linear model. There was a significant effect on $\delta^{13}C$ and $\delta^{15}N$ by SCL ($\delta^{13}C$ $F_{1, 157} = 211.1$, $p < .05$; $\delta^{15}N$: $F_{1, 157} = 44.58$, $p < .05$). The log model of SCL and $\delta^{13}C$ explained ~ 61% of the variation in the data ($r^2 = 0.605$)(Fig. 3). The variation between $\delta^{15}N$ and SCL had a weaker correlation, ~21% of the variation is explained by the model ($r^2 = 0.213$, Fig. 4).

3.2. Comparison between genders

I used the robust Brown Forsyth Levene-type test and found no significant difference between the variance of $\delta^{13}C$ or $\delta^{15}N$ values of males and females ($\delta^{13}C$: $F = 2.1$, $p = 0.15$; $\delta^{15}N$: $F = 0.37$, $p = 0.55$). Testing the relationship of the stable isotope values and gender, I found that there was no significant effect on $\delta^{13}C$ based on gender when controlling for length ($p > .05$). Additionally, there was no significant effect on $\delta^{15}N$ based on gender when controlling for length ($p > .05$).

3.3. Resident v. non-resident comparison

I used a Mann-Whitney-Wilcoxon test to see if there were differences in the stable isotope signatures between satellite tagged turtles that were considered “Resident” v. turtles that were considered “Non-resident”. No significant difference was found between the two groups ($\delta^{13}C$: W = 95.5, p = 0.23; $\delta^{15}N$: W = 117.5, p = 0.71).

3.4.Comparison of different tissue types within and across length bins

I investigated if there is a difference between the stable isotope values of flipper and plasma samples based on turtles binned into two groups. The binned groups were turtles < 60 cm SCL and turtles > 60 cm SCL. I used a repeated measures ANOVA accounting for individual differences with a dataset that included 25 turtles that had both tissue types; 9 turtles were < 60 cm SCL and 16 turtles were > 60 cm SCL. There was no significant difference in the $\delta^{13}C$ values of flipper tissue and plasma within the < 60 cm group or the > 60 cm group (Figure 5). Examining the $\delta^{15}N$ values of flipper and plasma

within the < 60 cm group and the > 60 cm group showed significant differences between the tissue types in both size groups (ANOVA, δ^{15}N < 60 cm $p < 0.01$, δ^{15}N > 60 cm $p < 0.001$) (Table 7)(Figure 6).

There were significant differences in the δ^{13}C values of flipper tissue between the < 60 cm group and the > 60 cm group (ANOVA, δ^{13}C, flipper tissue $p < 0.001$). There were also significant differences in the δ^{13}C values of plasma between the < 60 cm group and the > 60 cm group (ANOVA, δ^{13}C , plasma $p < 0.001$) (Table 7)(Figure 5). The same trends of significance were found in the δ^{15}N values of flipper tissue and plasma between the < 60 cm group and the > 60 cm group (ANOVA, δ^{15}N, flipper tissue $p < 0.001$; δ^{15}N, plasma $p < 0.01$)(Table 7)(Figure 6) .

3.5. Prey contribution to DRTO green turtle diet

3.5.1. Gastric lavage

Lavage results for juvenile turtles in 2008 (n = 10) showed that all turtles had recently consumed seagrass. *Thalassia testudinum* was found in the majority of turtles (n=7, 70%), followed by *Halodule wrightii*, and lastly *Syringodium filiforme*. One turtle recently consumed both *Thalassia testudinum* and *Syringodium filiforme* (n = 1, 10%), and two recently consumed only *Halodule wrightii* (n = 2, 20%). A turtle that only had *Halodule wrightii* in their crop also ingested tiny jellyfish (< 1 cm), most likely *Cassiopea* sp. *Thalassia testudinum* dry weight ranged from 0.015 to 0.05 g ($\bar{x} = 0.026 \pm 0.013$). *Halodule wrightii* dry weight ranged from 0.01 to 1.125 g ($\bar{x} = 0.377 \pm 0.324$). *Syringodium filiforme* dry weight was 0.004 g.

3.5.2. Mixing models

The model run with flipper samples indicated that multiple resources contribute to green turtle diet in the Tortugas in both length bins. Green turtle < 60 cm (SCL) are reported to have assimilated most of their energy from the seagrasses, up to 55%, with less contribution from the macroalgae, and moon jellyfish. Resource use in this group appears to be more generalist in nature (Figure 10). Green turtles > 60 cm (SCL) have a much different resource use than turtles < 60 cm (SCL) (Figure 11). The turtles in this group almost exclusively consumed seagrass. Proportion of seagrasses to the diets of

green turtles > 60 cm (SCL) was up to 88%. Macroalgae consumption was severely depressed in relation to turtles < 60 cm, .03 % v. ~43 % .

I also investigated the outliers of the length bins based on points selected from outside of the 95% confidence interval calculated from a linear model with $\delta^{13}C$ and $\delta^{15}N$ as my axes. Again, I initially visualized the data on a biplot, but only using the outlying points. The boxplots of the dietary contributions to the outlying individuals of the length bins (< 60 cm (SCL) and > 60 cm (SCL) show an increase in the proportion of seagrass in the diet of green turtles < 60 cm, as well as a slight increase of the moon jellyfish (~2 %) to their diet and less contribution from the macroalgae (38 %) (Figure 12). Outlying green turtles > 60 cm (SCL) still have seagrass contribution as a main proponent, a negligible amount macroalgae (~ .04 %) and similar contribution from the moon jellyfish as compared to the entire population (Figure 13) (Table.

4. DISCUSSION

4.1. Effect of length on stable isotopes values

The relationship between length and stable isotope values presents us with the classical example of green sea turtles as the mature, starting to feed lower trophically, i.e., on seagrasses. We can see this in the isotope plots, $\delta^{15}N$ values lowering as size increases and $\delta^{13}C$ values increasing, moving away from macroalgae signatures and towards seagrass isotope signature range. This pattern is consistent with past studies that captured a variety of green turtle size classes and were able to show the classic shift to herbivory as turtles matured (Arthur et al., 2008, Cardona et al., 2010).

4.2. Gender comparison

Male and female green turtles were not significantly different from each other isotopically in this study, indicating similar dietary preferences, even when accounting for length. Considering the females migrate to and from different foraging areas there was potential to see a difference between sexes, indicating resource, but no differences were found. Vander Zanden et al. (2013) found no difference between male and female green turtles from Nicaraguan foraging sites.

4.3. Resident v. non-resident comparison

Comparing the satellite tagged groups of "Resident" and "Non-resident", turtles whose satellite tracks stayed within DRTO v. turtles that left to other areas, showed no significant differences between the groups. Taking into account the turnover rate of flipper tissue for this analysis we can infer that the groups were not at the nesting site and still near their foraging areas. This supports the result that the resident green turtles at the Dry Tortugas fed on an isotopically equivalent diet as green turtles that migrated. The "Non-resident" group was tracked to areas near the Marquesas, which is 75km away from DRTO and closer to Key West, FL. Other foraging grounds that were visited include seagrass habitat adjacent to Everglades National Park, Key Largo, Mexico, near the Yucatan, and one tracked into the Gulf of Mexico (Hart et al., 2013, K.M. Hart unpubl. data, seaturtle.org).

4.4.Comparison of tissue types and length bins

Carbon stable isotope data for flipper and plasma from green turtles < 60 cm (SCL) or > 60 cm (SCL) from the Dry Tortugas were not significantly different from each other. This indicates that between the time periods these tissues represent, green turtles were feeding on the same resources. The smaller group was feeding on a diet more depleted in its Carbon signature and is validated by the values from both tissues. There was a significant difference in the comparison of $\delta^{13}C$ across size groups. $\delta^{13}C$ values increased from the smaller size group to the larger, supporting the diet shift from a more depleted diet, to a more enriched herbivorous diet. In the analysis of $\delta^{15}N$ there were significant differences between the sample types within and across size groups. The pattern in boxplots of Figure 5 could be describing the shift from their initial carnivorous feeding strategy prior to recruiting to neritic foraging areas and agrees with stable isotope values for several early life stages (New recruit) as outlined by Arthur et al. (2008).

Dry Tortugas green turtle flipper tissue $\delta^{13}C$ values, -16.57 ‰ to -5.38 ‰, are similar and within the ranges of other Caribbean green turtle populations. Vander Zanden et al. (2013) found skin $\delta^{13}C$ ranging of -12.2 ‰ to -4.5 ‰ from two sites in the Bahamas, $\delta^{13}C$ values ranging from -14.7 ‰ to -7.3 ‰ from 2 sites in Nicaragua, $\delta^{13}C$ values ranging from -15.7 ‰ to -9.0 ‰ from St. Joe Bay, FL, and $\delta^{13}C$ values ranging

from -17.0 ‰ to -5.3 ‰ at Tortuguero Beach, Costa Rica. The flipper tissue $\delta^{13}C$ values are also within range, but slightly more enriched than known omnivorous populations found in Australia (-22.4 ‰ to -9.8 ‰, Burkholder et al., 2011) and the eastern Pacific (-8.9 ‰ to -13.7 ‰, Lemons et al., 2011). The range of $\delta^{15}N$ values, 3.70 ‰ to 10.61 ‰, indicates that the population occupies more than one trophic level, but is doing so through disparate groups. The juvenile turtles had the most enriched $\delta^{15}N$ values ($\overline{x}$ = +8.22 ± 1.07 ‰) which was expected. This could be due to the potential omnivory in their diet. Turtles that were sampled showed no signs of poor nutrition. Overall, mean body condition index scores for green turtles in DRTO during the months of May through August were over 1.2, which represents a score of "Very Good" (Reintsma 2015, FAU).

4.5. Prey contribution to DRTO green turtle diet

Lavage results support that juvenile green turtles in the Dry Tortugas are omnivores. We found ingestion of small jellyfish, *Cassiopea* sp, the "upside-down" jellyfish which lives on the seafloor, but only in a single turtle that had recently consumed *Halodule sp.* This occurrence brings up the question if the juveniles could be purposefully selecting patches where jellyfish are present. Aside from the single turtle that had consumed the jellyfish all the others were found to only have consumed seagrass recently.

I visualized the data in SIAR, and generated a biplot with stable isotope values of individual turtles with potential prey resources (Fig. 8). Proportions from the stable isotope mixing models indicated an omnivorous feeding regime for both all turtles and outliers in the < 60 cm (SCL) size group. The > 60 cm (SCL) group heavily depended on seagrass as its primary food source. I added the moon jellyfish, *Aurelia aurita*, as green turtles are known predators. The model is lacking *Cassiopea sp.* as none were collected for stable isotope analysis in this study. *Cassiopea* are physiologically different than Aurelia. Aurelia consumes zooplankton through the use of its tentacles, *Cassiopea* have symbiotic zooxanthellae that photosynthesize and contribute to its host's nutrient budgets. A similar jellyfish in the same order as *Cassiopea*, *Mastigas sp.*, was shown to be able to be almost wholly supplied with its daily carbon demand from its host zooxanthellae with the remainder coming from predatory feeding on zooplankton

(McCloskey, Muscatine, & Wilkerson, 1994). Additionally, $\delta^{13}C$ values were similar between the zooxanthellae and the mesogleal tissue of *Mastigas sp.* You could potentially, see a similarity between the $\delta^{15}N$ values of zooplankton and *Cassiopea*. If juvenile green turtles in this study were consuming *Cassiopea*, the $\delta^{15}N$ values of zooplankton in the literature, even with theoretical enrichment to represent it, does not explain the enriched $\delta^{15}N$ values. The model is also lacking proper geometry in its resource pool. An additional resource which is depleted in nitrogen and enriched in carbon would allow better resolution of diet in this green turtle population.

The upper value of $\delta^{15}N$ for seagrasses from the FKNMS is 5.4 for *T. testudinum* (Campbell & Fourqurean, 2009) even trophic enrichment does not encompass the bulk $\delta^{15}N$ values of green turtles in the Dry Tortugas. Employing Compound Specific Stable Isotope Analysis of Amino Acids could help resolve the foraging ecology of this population, as it helps illuminate the differences in baseline $\delta^{15}N$ and differences in trophic position.

5. CONCLUSIONS AND FUTURE RESEARCH

Green turtles in the Dry Tortugas follow the traditional model of Caribbean green turtles. Stable isotope analysis captures the shift from omnivory to herbivory as the DRTO population of green turtle grows in size. The location of the Dry Tortugas in the Gulf of Mexico grants the migratory green turtle access to a different suite of places sets it apart from other Caribbean green turtle populations. Even with the varied locations visited by this population stable isotope analysis still supports that the adults are primarily herbivores, seagrass and macroalgae were consistently utilized as a resource in their diet. The contribution of seagrass as a major resource to the DRTO green turtles as demonstrated by lavage and SIA supports and strengthens the protection of this population thriving in the waters designated as a National Park. This study improves our understanding of this species at an important foraging and nesting ground. Future research includes the layering of scute samples to determine individual foraging patterns, and collecting additional samples to firmly answer the unexplained $\delta^{15}N$ values, and to contribute to region wide isoscapes.

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Table 1: Mean and variance of $\delta^{13}C$ and $\delta^{15}N$ values from tissue samples at isotopic equilibrium from green turtles from previous studies

	***C. mydas*(adult n=30, juveniles n=40)[a]**		***C. mydas*(juveniles n=8)[b]**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Diet	-23.05 (.29)	2.49 (.05)	-19.03 (.97)	6.24 (.24)
Diet (lipid extracted)			-18.64 (.20)	6.21 (.34)
Adults:				
Epidermis	-21.44 (.08)	6.57 (.14)		
Dermis	-20.47 (1.14)	7.47 (.29)		
Serum/Plasma	-22.80 (.08)	6.70 (.12)		
Red blood cells	-22.75 (.04)	5.01 (.07)		
Whole blood				
Juveniles:				
Epidermis	-21.18 (.03)	6.31 (.11)	-18.54 (.04)	9.00 (.32)
Dermis	-20.88 (.05)	6.69 (.16)		
Serum/Plasma	-21.89 (.02)	6.59 (.08)	-19.18 (.05)	9.14 (.03)
Red blood cells	-22.54 (.03)	4.89 (.09)	-20.15 (.03)	6.52 (.04)
Whole blood			-19.94	6.99

[a] Vander Zanden et al. 2012

[b] Seminoff et al. 2006

Table 2: Number of flipper tissue samples in specific length bins

Length bin	Flipper tissue
< 60 cm	61
≥ 60 cm	98
Total:	159

Table 3: Studies that used lipid extraction during stable isotope analysis on different species and tissues of sea turtles

Publication	Study species	Tissue used	Used lipid extraction	Significant difference between extracted and non-extracted tissues?
Hatase et al. (2002)	Loggerhead turtle	Egg yolk	Yes	Not reported
Caut et al. (2008)	Leatherback turtle	Egg yolk and blood (plasma and red blood cells)	Yes. On egg yolk.	Not reported
Burkholder et al (2011)	Green turtle	Flipper tissue	Yes	No significant difference
Lemons at al. (2011)	Green turtle	Epidermis and prey species	Yes	Not reported
Seminoff et al. (2012)	Leatherback turtle	Epidermis	Yes	Not reported
Vander Zanden et al. (2013)	Green turtle	Epidermis	Yes	Not reported
Vander Zanden et al. (2014)	Green turtle	Dermis	Yes	No significant difference
Hall et al. (2015)	Loggerhead turtle	Plasma	No. Used correction method from Post et al. 2007.	N/A

Table 4: Stable isotope values of potential resources found at the Dry Tortugas and the surrounding areas used in this study

Reference	Location		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
			Range	Mean	Range	Mean
Campbell and Fourqurean (2009)	Florida Keys National Marine Sanctuary	*Thalassia testudinum*	-13.0 to -5.3	-8.6	-2.2 to 5.4	2.0
		Halodule wrightii	-13.2 to -7.8	-10.6	-3.5 to 4.0	1.0
		Syringodium filiforme	-8.4 to -3.5	-6.2	-1.6 to 4.7	1.6
Behringer and Butler (2006)	Ocean-side, Florida key's reef tract	*Laurencia spp*		-12.7		2.6
		Ircinia strobilina		-10.8		1.7
Lamb et al. (2012)	Florida key's reef tract	*Dictyota spp*		-15.0		2.4
		Halimeda spp		-17.0		1.6
Rooker et al. (2006)	Gulf of Mexico	*Sargassum spp*		-16.5		2.6
D'Ambra et al. (2014)	Gulf of Mexico	*Aurelia sp*		-18.7		11.8
Mompéan et al. (2013)	Subtropical North Atlantic	Zooplankton		-9.64		2.32

Table 5: Discrimination factors for green turtles from the literature

	Chelonia mydas adults (n = 30)[a]	*C. mydas* juveniles (n = 40)[a]	*C. mydas* juveniles (n = 8)[b]
$\delta^{13}C$			
Epidermis	1.62 ± 0.61	1.84 ± 0.56	0.17 ± 0.08
Dermis	2.58 ± 1.19	2.18 ± 0.59	N/A
Whole Blood	N/A	N/A	-0.92 ± 0.06
Serum/plasma	0.24 ± 0.61	1.16 ± 0.56	-0.12 ± 0.03
Red blood Cell	0.30 ± 0.58	0.51 ± 0.56	-1.11 ± 0.05
$\delta^{15}N$			
Epidermis	4.04 ± 0.44	3.77 ± 0.40	2.80 ± 0.11
Dermis	4.93 ± 0.59	4.15 ± 0.47	N/A
Whole Blood	N/A	N/A	0.57 ± 0.09
Serum/plasma	4.17 ± 0.41	2.92 ± 0.08	2.92 ± 0.03
Red blood Cell	2.48 ± 0.35	0.22 ± 0.08	0.22 ± 0.03

[a] Vander Zanden et al. 2012
[b] Seminoff et al. 2006

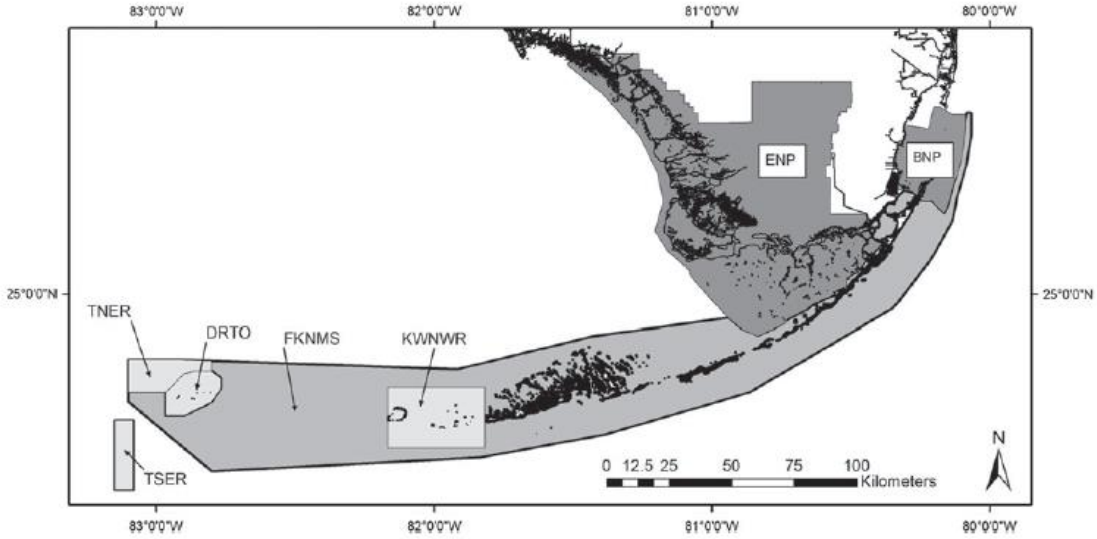
YEAR	SIZE CLASS	N	FLIPPER STABLE ISOTOPE VALUES			
			$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
			Range	Mean ± SD	Range	Mean ± SD (‰)
2008	Juvenile	16	–14.57 to –7.05	–9.97 ± 2.02	+6.14 to +9.84	+7.87 ± 0.88
	Sub-adult	N/A	N/A	N/A	N/A	N/A
	Adult	N/A	N/A	N/A	N/A	N/A
2009	Juvenile	4	–12.90 to –8.79	–10.89 ± 1.73	+6.98 to 10.61	+8.71 ± 1.53
	Sub-adult	5	–9.03 to –6.74	–7.74 ± 1.01	+5.29 to +8.04	+6.58 ± 1.01
	Adult	6	–9.04 to –6.20	–7.29 ± 1.04	+6.80 to +8.02	+7.62 ± 0.46
2010	Juvenile	8	–10.76 to –7.42	–8.93 ± 1.20	+6.49 to +9.63	+7.68 ± 1.17
	Sub-adult	2	–9.03 to –6.74	–7.74 ± 1.01	+5.29 to +8.04	+6.58 ± 1.01
	Adult	5	–8.09 to –7.41	–7.72 ± 0.25	+6.66 to +9.30	+7.95 ± 0.99
2011	Juvenile	9	–14.55 to –7.83	–11.77 ± 2.29	+7.27 to 10.53	+8.63 ± 1.00
	Sub-adult	6	–10.58 to –7.36	–8.61 ± 1.10	+5.51 to +8.18	+7.20 ± 0.93
	Adult	16	–10.25 to –6.37	–8.38 ± 1.08	+5.82 to +9.36	+7.37 ± 0.99
2012	Juvenile	3	–16.57 to –9.52	–13.09 ± 3.53	+8.42 to 10.32	+9.35 ± 0.95
	Sub-adult	1	–7.53	N/A	+7.39	N/A
	Adult	7	–13.08 to –7.26	–8.66 ± 1.98	+5.62 to +7.85	+6.80 ± 0.75
2013	Juvenile	3	–15.27 to –8.28	–13.09 ± 3.53	+6.88 to +9.81	+7.92 ± 1.64
	Sub-adult	N/A	N/A	N/A	N/A	N/A
	Adult	11	–9.39 to –6.68	–8.27 ± 0.90	+3.70 to +9.53	+7.03 ± 1.93
2014	Juvenile	3	–15.20 to –9.17	13.13 ± 3.43	+7.53 to +9.15	+8.35 ± 0.81
	Sub-adult	1	-8.46	N/A	+7.24	N/A
	Adult	5	–8.99 to –5.38	–7.71 ± 1.48	+4.88 to +8.28	+6.79 ± 1.36
2015	Juvenile	15	–15.59 to –8.65	–13.00 ± 2.17	+6.48 to 10.18	+8.33 ± 0.98
	Sub-adult	1	–7.58	N/A	+5.68	N/A
	Adult	30	–10.53 to –6.16	–7.57 ± 1.10	+5.41 to +8.22	+6.85 ± 0.66

Table 6: Mean stable isotope values for green turtle flipper tissue samples collected from 2008-2015 in Dry Tortugas National Park

Table 7: Stable isotope mixing model (SIAR) results with potential prey contribution to the diets of green turtles at DRTO. Data was arranged by length groups of < 60 cm (SCL) and > 60 cm (SCL) for all the turtles and turtles considered outliers as described in the Methods. Values are the 5th and 95th percentile with the mean values in parentheses.

Group	N	Seagrasses	Macroalgae	Aurelia sp. (Moon jellyfish
All samples				
< 60 cm (SCL)	61	0.34−0.55 (0.45)	0.19−0.42 (0.31)	0.21−0.27 (0.24)
> 60 cm (SCL)	98	0.84−0.88 (0.86)	0.0−0.03 (0.13)	0.11−0.15 (0.13)
Outliers				
< 60 cm (SCL)	39	0.40−0.65 (0.52)	0.09−0.38 (0.23)	0.19−0.29 (0.24)
> 60 cm (SCL)	77	0.83−0.88 (0.85)	0.0−0.05 (0.02)	0.11−0.15 (0.13)

(A)



(B)

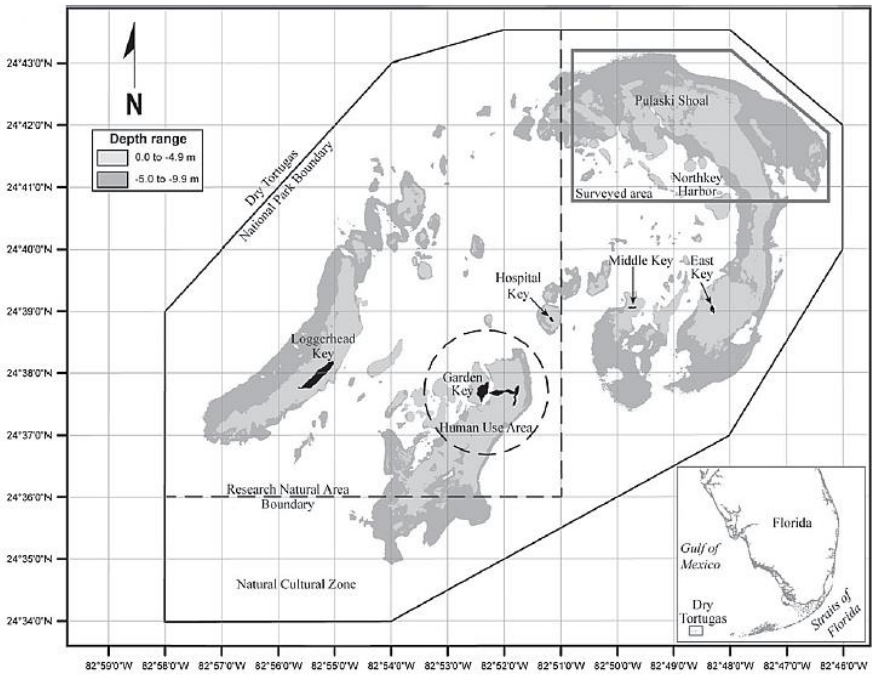


Figure 1: Location of Dry Tortugas National Park where green turtles were targeted. Reprinted from "Habitat use of breeding green turtles Chelonia mydas tagged in Dry Tortugas National Park: making use of local and regional MPAs", Hart et al., 2013, *Biological Conservation*, *161*, 144.

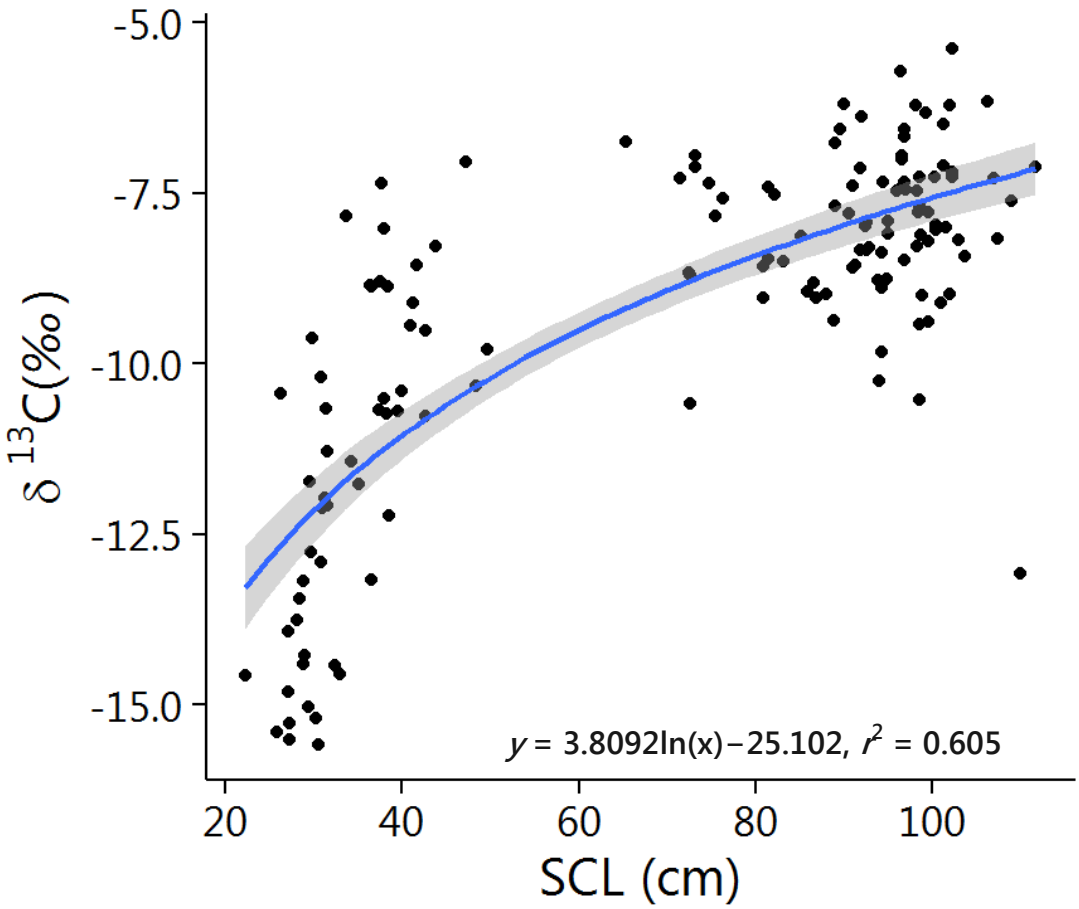


Figure 2: Correlation of straight carapace length (SCL, cm) on $\delta^{13}C$

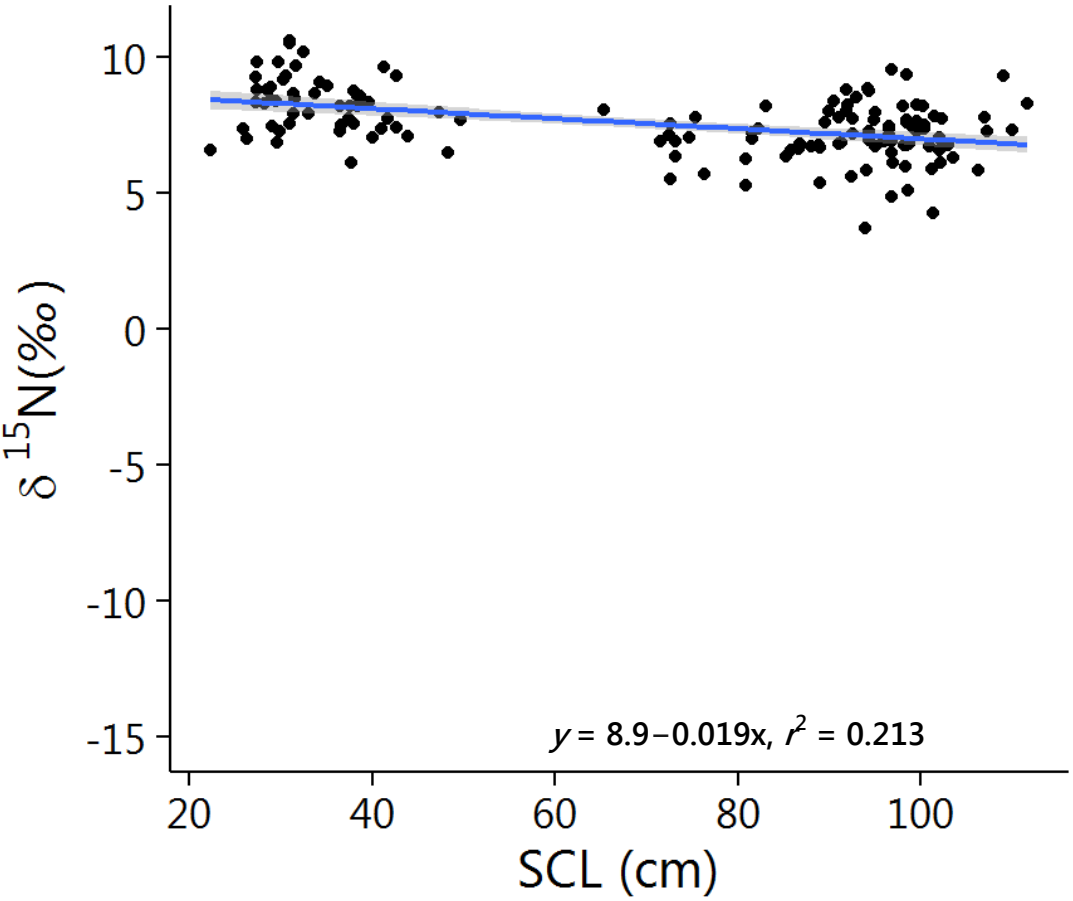


Figure 3: Correlation of straight carapace length (SCL, cm) on $\delta^{15}N$

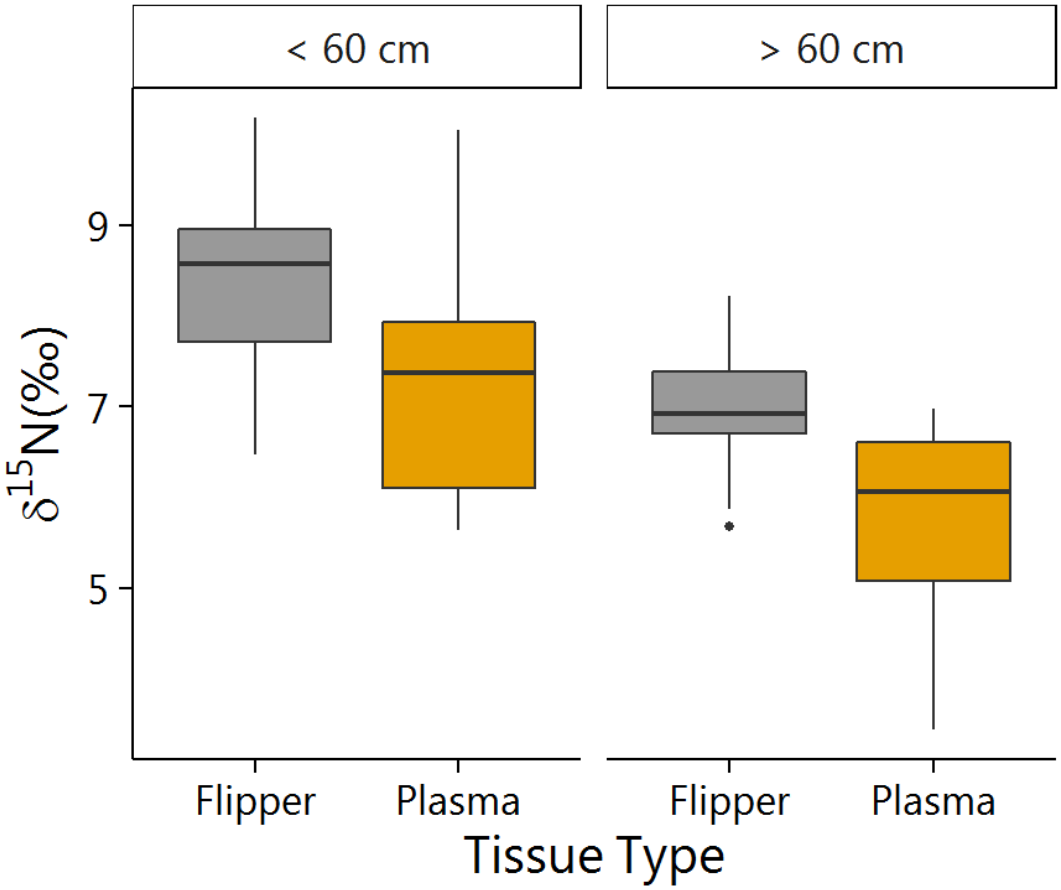


Figure 4: Boxplot displaying significant differences between flipper and plasma $\delta^{15}N$ values **within** the < 60 cm group and **within** > 60 cm group.

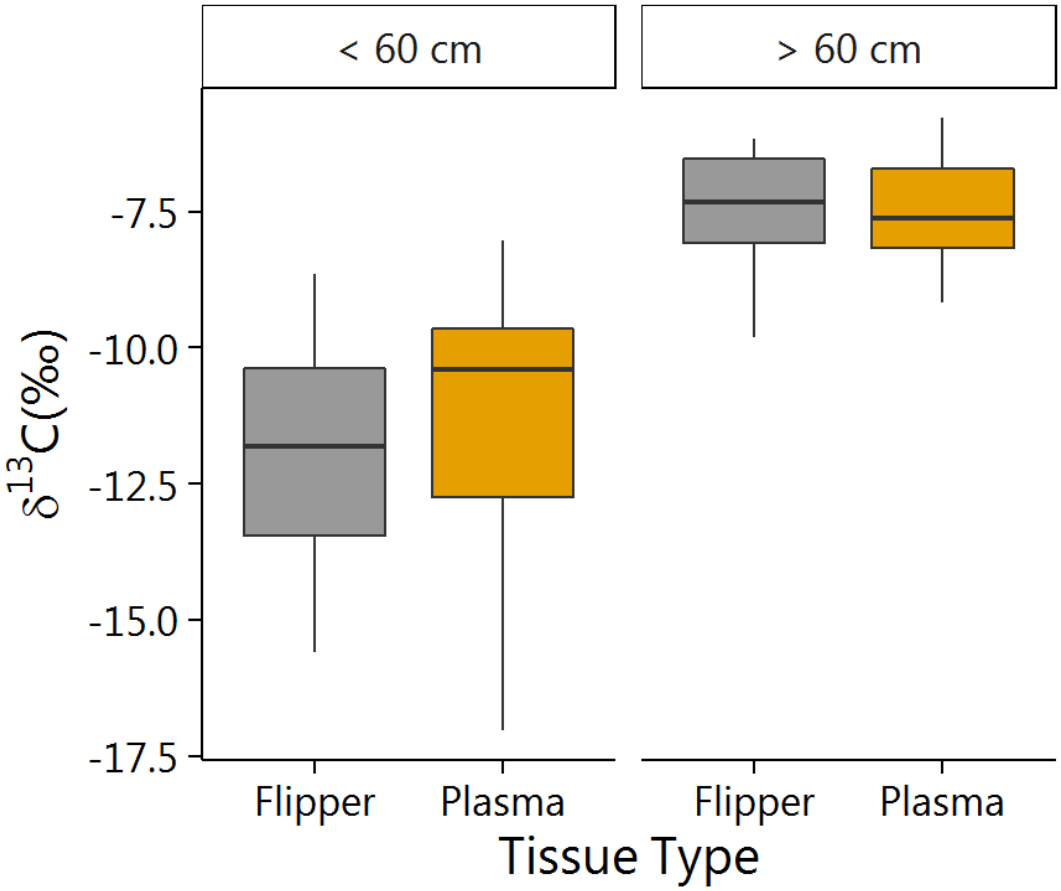


Figure 5: Boxplot displaying significant differences in flipper and plasma $\delta^{13}C$ **across** length groups.

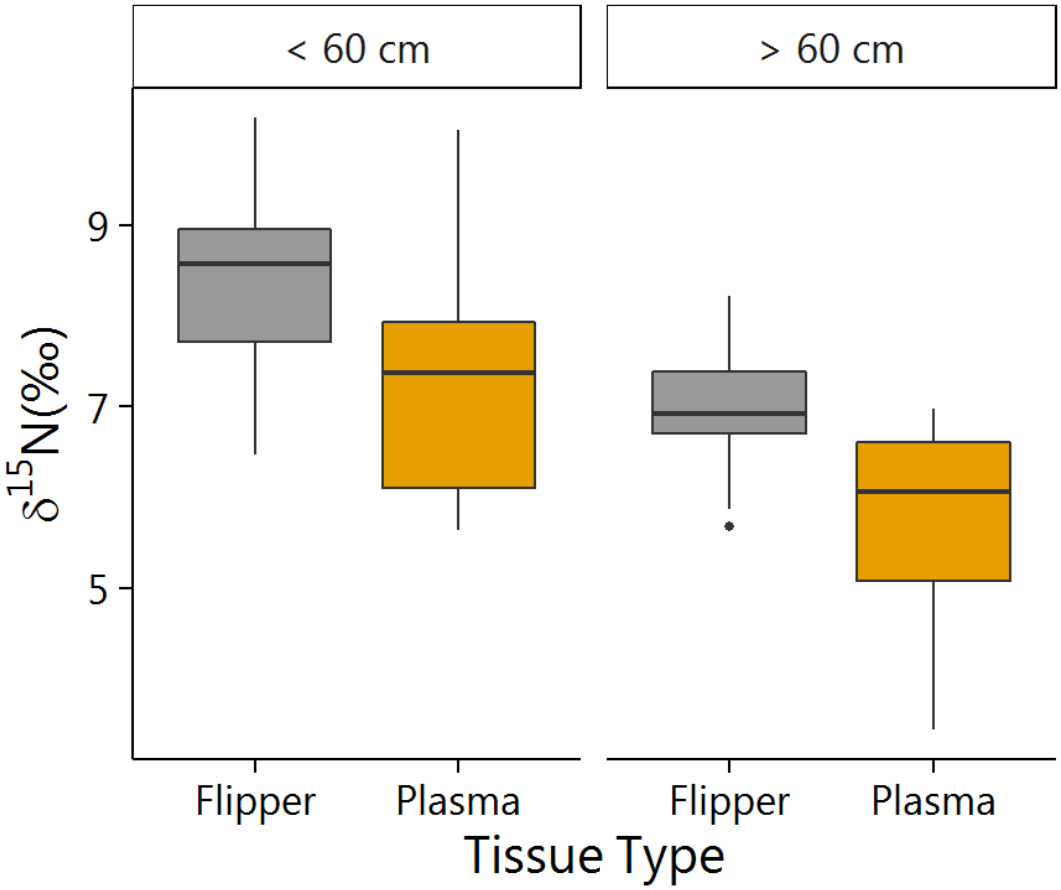


Figure 6: Boxplot displaying significant differences in flipper and plasma δ^{15}N **across** length groups.

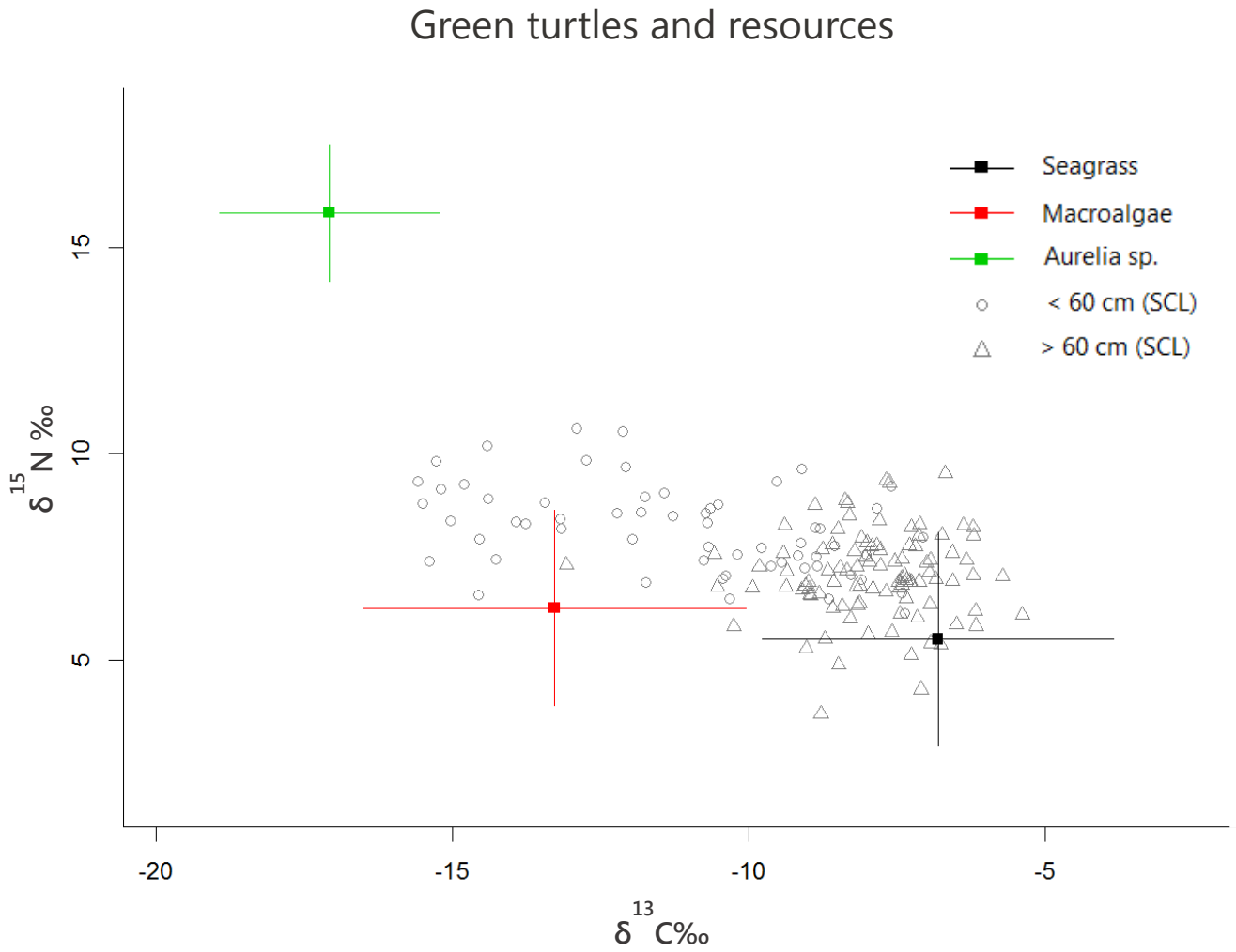


Figure 7: Isotopic biplot of individual green turtles and potential resources calculated with discrimination factors.

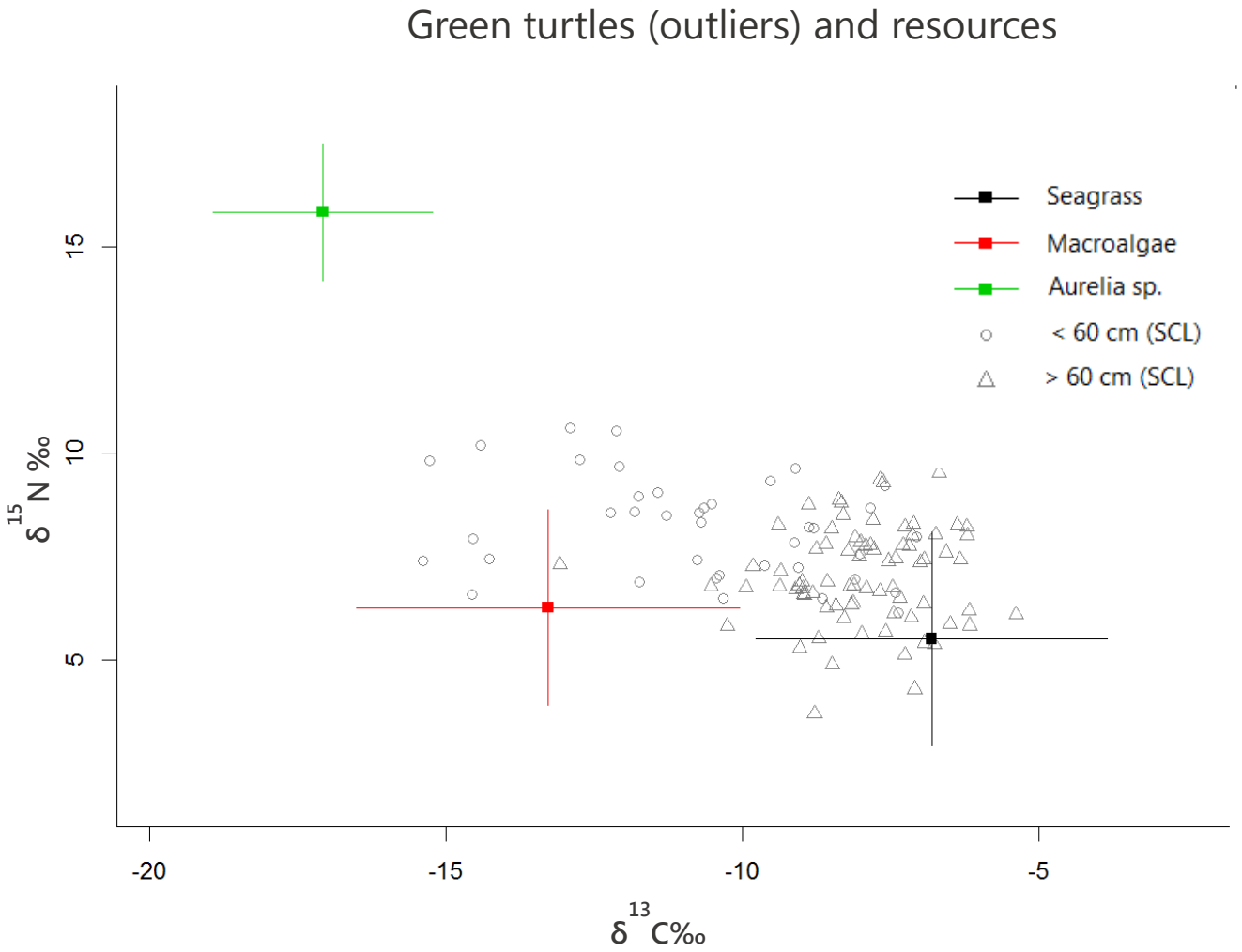


Figure 8: Isotopic biplot of the outliers (individuals removed from inside of the 95% confidence interval calculated with a linear regression) potential resources calculated with discrimination factors.

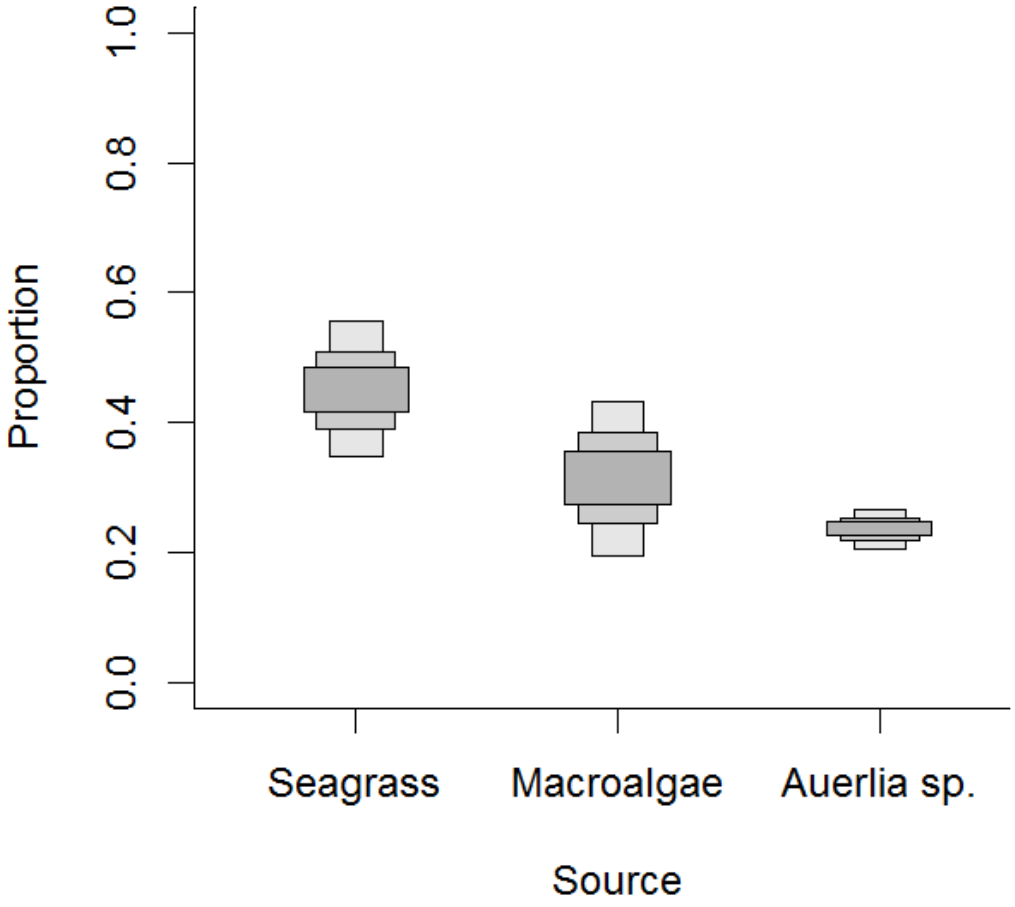


Figure 9: Potential contribution of resources from DRTO and the Caribbean green turtles< 60 cm (SCL) according to SIAR (95, 75, and 50% confidence intervals).

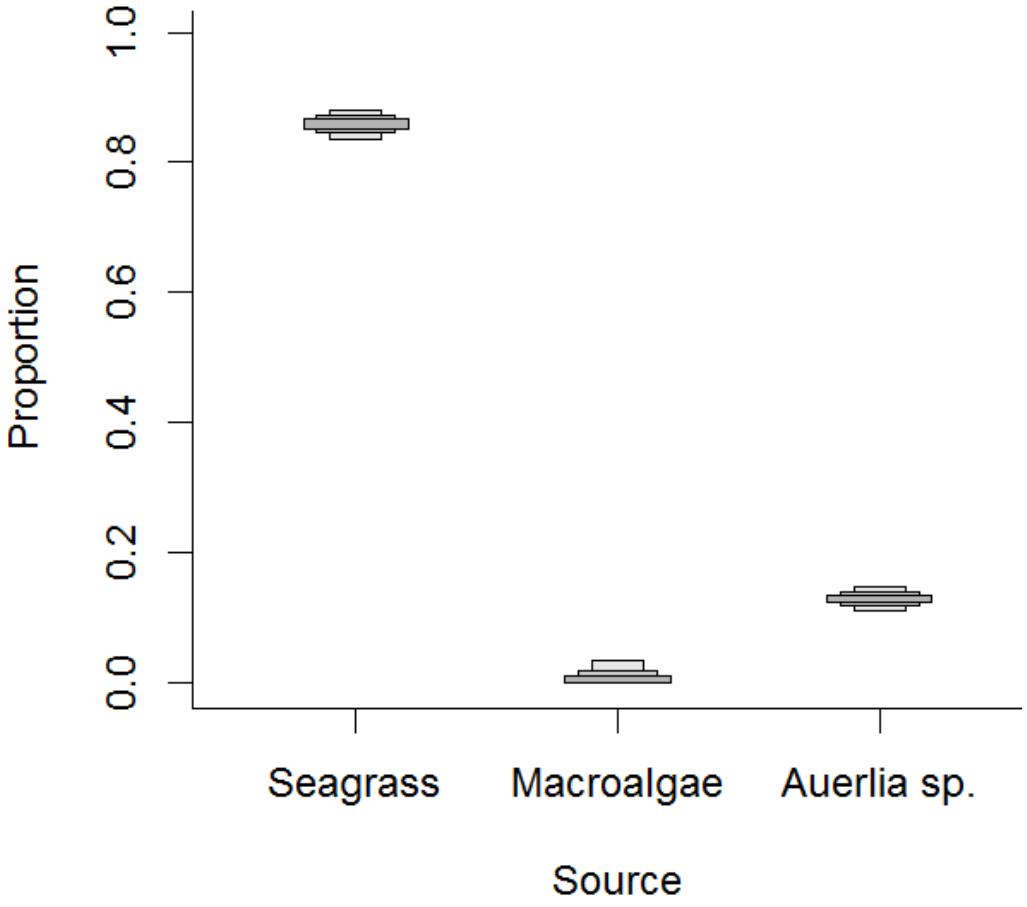


Figure 10: Potential contribution of resources from DRTO and the Caribbean to green turtles > 60 cm (SCL) according to SIAR (95, 75, and 50% confidence intervals).

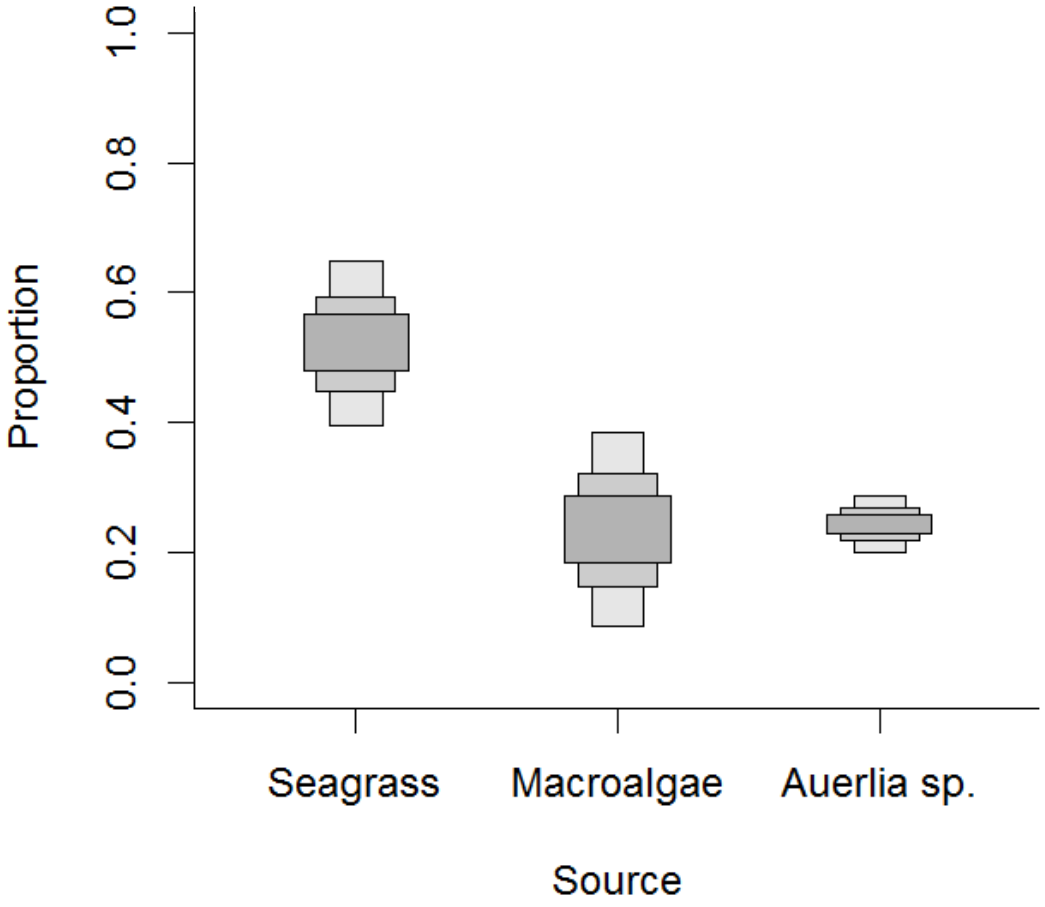


Figure 11: Potential contribution of resources from DRTO and the Caribbean to green turtle < 60 cm (SCL) considered outliers (individuals removed from inside of the 95% confidence interval calculated with a linear regression) according to SIAR (95, 75, and 50% confidence intervals).

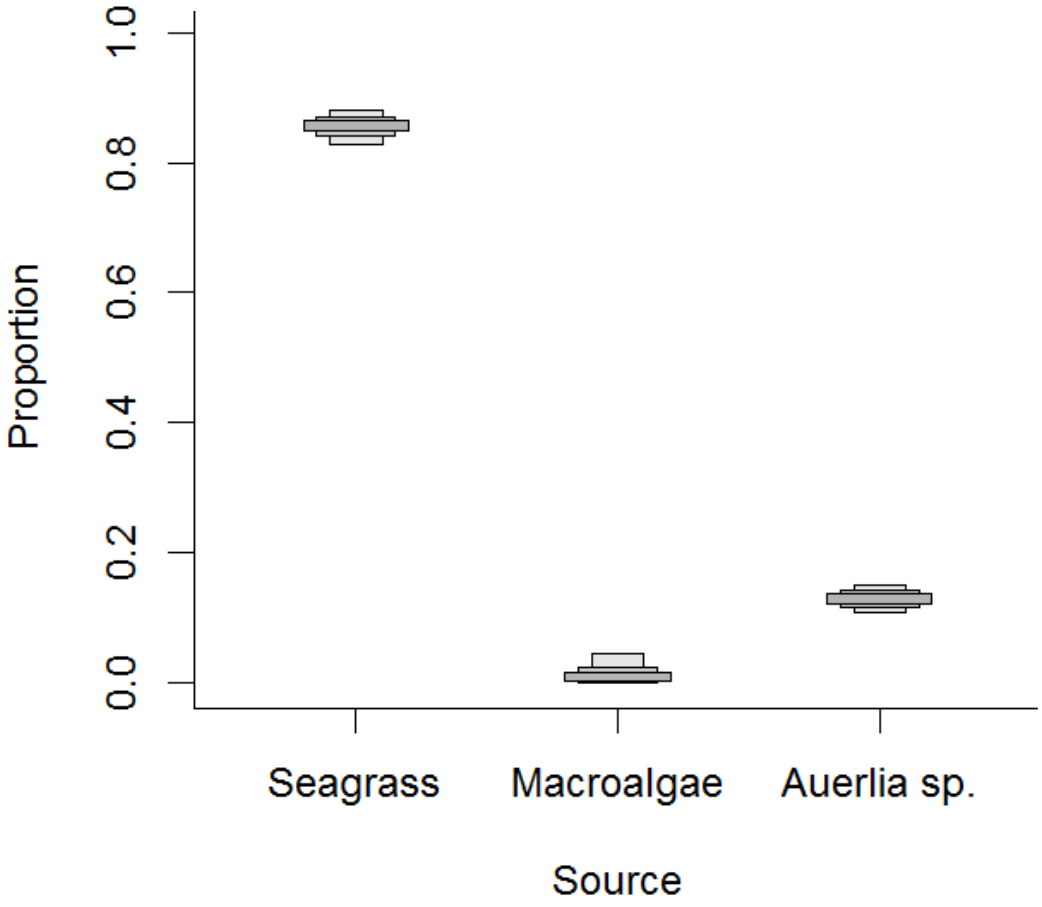


Figure 12: Potential contribution of resources from DRTO and the Caribbean to green turtle > 60 cm (SCL) considered outliers (individuals removed from inside of the 95% confidence interval calculated with a linear regression) according to SIAR (95, 75, and 50% confidence intervals).