Using Stable Isotope Analysis to Assess the Foraging Habits of Palmyra Atoll Green Turtles (*Chelonia mydas*)

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APPROVAL SHEET

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DEDICATION

I dedicate my thesis to my future husband, Justin, and our perfect little boy, Caleb.

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LIST OF ABBREVIATIONS

ISTS	International Sea Turtle Symposium
PANWR	Palmyra Atoll National Wildlife Refuge
$\delta^{13}C$	Stable Carbon Isotope
$\Delta^{13}C$	Discrimination Factor (Difference between Consumer and Prey Carbon Signatures)
$\delta^{15}N$	Stable Nitrogen Isotope
$\Delta^{15}N$	Discrimination Factor (Difference between Consumer and Prey Nitrogen Signatures)
AMNH	American Museum of Natural History
BCI	Body Condition Index
BUSIL	Boston University Stable Isotope Laboratory
CCL	Curved Carapace Length
CCMA	Center for Coastal Monitoring and Assessment
HCL	Hydrochloric Acid
IACUC	Institutional Animal Care and Use Committee
LMM	Linear Mixed Model
NOAA	National Oceanic and Atmospheric Administration
SCL	Straight Carapace Length
SD	Standard Deviation
TL	Tail Length
USFWS	United States Fish and Wildlife Service

ABSTRACT

An Assessment of the Foraging Habits of Palmyra Atoll Green Turtles (*Chelonia mydas*) using Stable Isotope Analysis

Elizabeth Brooke Kelly

Juvenile green turtles (*Chelonia mydas*) undergo an ontogenetic habitat shift to neritic developmental grounds after an initial period of development in the pelagic environment. Post-pelagic juveniles are believed to undergo a switch from omnivory to herbivory upon recruitment to foraging grounds. However, recent evidence suggests that some populations of green turtles may not undergo direct ontogenetic habitat and dietary shifts and may continue to supplement their herbivorous diet with animal products into adulthood. The individual and population level variability of this shift has not been extensively studied and further support is needed to substantiate if these are widespread phenomena. The Palmyra Atoll National Wildlife Refuge (PANWR), a remote uninhabited atoll in the central Pacific, currently serves as a mixed-stage foraging ground for juvenile, subadult and adult green turtles, yet little is known about the ecology of this population. The presence and timing of ontogenetic shifts, stage-specific foraging habits, and spatial variation in foraging habits, are all unresolved questions regarding of green turtle foraging ecology in the Palmyra Atoll population.

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provide information on diet and trophic position allowing for a more thorough understanding of the ontogeny of Palmyra Atoll green turtle foraging behavior. The carapace, or dorsal section of a sea turtle shell, is composed of keratin covered bony plates called scutes. Scute tissue is often used for isotopic studies because keratin is metabolically inactive thus retaining the isotopic signature of the assimilated elements of the diet from the time of tissue synthesis. Isotopic signatures of successive scute layers may therefore be analyzed to identify when dietary shifts occur during recruitment to foraging grounds. In addition, comparison of isotopic signatures in the most recently deposited tissue layer allows for examination of spatial variation and stage-specific foraging habits in the Palmyra Atoll population.

Two scute biopsy samples, posterior (oldest tissue) and anterior (younger tissue), were collected from live-captured green turtle from 2008 to 2010. Size classes were determined using curved carapace length (CCL) as a proxy for age resulting in juvenile (30.0 – 64.9 cm CCL), sub-adult (65 – 84.9 CCL) and adult (85 - 120 CCL) size classes. A total of 28 juveniles, 26 sub-adults and 21 adults were sampled. Prey items, such as epiphytic turf algae, macroalgae and sponges, were also collected for stable isotope analyses.

Analysis of the $\delta^{13}C$ and $\delta^{15}N$ values of successive 50 μm layers from juvenile posterior biopsy samples (n = 17) allowed for examination of resource use over time, providing insight on the timing of ontogenetic shifts. Analysis of layer isotopic signatures using a linear mixed model provided insight on the timing of ontogenetic shifts. In addition, the $\delta^{13}C$ and $\delta^{15}N$ values of the most recently deposited tissue layer (i.e. inner anterior layer, n = 75) were used to examine stage-specific habits and describe spatial variation in foraging habits.

Palmyra Atoll juvenile green turtles do not exhibit $\delta^{15}N$ values that could be attributed to a drop in trophic level or a switch from omnivory to herbivory. It is possible that the enriched nitrogen found in algae is helping to maintain the enriched nitrogen levels seen in recent juvenile

green turtle recruits. High levels of individual variation in layer $\delta^{13}C$ and $\delta^{15}N$ suggest that this may be a generalist population. In addition, differing $\delta^{15}N$ signatures among size classes (Kruskal-Wallis, $p = 0.001$) suggests that individuals may forage at different trophic levels. Enriched $\delta^{15}N$ signatures may signify ingestion of animal products. These stage-specific habits appear to be related to habitat selection preferences as individual size distribution differs between the four sampling regions (CCL: Kruskal-Wallis, $F = 8.937$, $df = 3$, $p = 0.030$; Mass: Kruskal-Wallis, $F = 10.979$, $df = 3$, $p = 0.012$); this could be due to habitat preferences or to distinct dietary items consumed in the atoll regions. The Palmyra Atoll population contains individuals that likely utilize a more variable and complex dietary repertoire including omnivorous prey items.

Distribution of $\delta^{13}C$ signatures did not differ across size classes (Kruskal-Wallis, $p = 0.808$) however $\delta^{15}N$ values did differ across size classes (Kruskal-Wallis, $p = 0.001$); subadults being less enriched in nitrogen than adults (Mann-Whitney U $p < 0.001$) and juveniles (Mann-Whitney U $p < 0.05$). Size class distribution and $\delta^{15}N$ values differ between sampling locations suggesting some spatial structure to the Palmyra foraging grounds.

Using $\delta^{13}C$ and $\delta^{15}N$ ratios to determine the spatial and temporal variation in sea turtle foraging habitat will help fill an important gap in our knowledge of sea turtle ecology and identify critical areas for sea turtle habitat conservation at Palmyra Atoll. This study will also contribute to a better understanding of the variation in ontogenetic diet shifts of juvenile green sea turtles as they recruit to neritic foraging grounds.

CHAPTER ONE

Introduction to Green Turtle Conservation, Ecology & Research Techniques

CHAPTER ONE: Introduction to Green Turtle Conservation, Ecology & Research Techniques

Green Turtle Population Status

Sea turtles inhabit every ocean basin and face a variety of external stressors. Over-harvesting, incidental capture in fishing gear, hunting on nesting beaches, marine pollution and habitat degradation have all led to declines of sea turtle populations (Bjorndal & Jackson 2003). Due to the low nutritional quality of their diet, green turtles (*Chelonia mydas*) exhibit slow growth rates and delayed maturation (Bjorndal 1982, Bjorndal 1985). Nesting green turtles also experience increased interannual variation in remigration intervals and reproductive output (clutch size) compared to non-herbivorous sea turtle species (Broderick et al. 2001); this is likely due to the linkage of the availability of herbivorous dietary items at foraging grounds and environmental factors (Bjorndal 1985, Broderick et al. 2001, Troëng & Chaloupka 2007). Other species feeding in higher trophic levels may not experience such variability due to dietary limitations (Broderick et al. 2001). Low dietary quality, environmental factors, foraging substrate availability and increasing anthropogenic stressors act in concert to increase the vulnerability of green turtles to population decline (Heppell et al. 2003).

Over the past three generations the global green turtle population is thought to have declined by over 70% (Seminoff 2002). Although populations are beginning to increase (Balazs & Chaloupka 2004, Chaloupka et al. 2008) protection is still necessary as some populations continue to be threatened with extinction. Under the Endangered Species Act green turtles are considered threatened throughout their range (Federal Register 1978) with endangered breeding populations in Florida and on the Pacific coast of Mexico (Federal Register 1999, Seminoff et al. 2007). The International Union for Conservation of Nature (IUCN), however, lists green turtles

as globally endangered (Seminoff 2004). With a circumglobal distribution, nesting takes place in more than 80 countries; the largest populations are found in the tropics and to a lesser degree the subtropics (Hirth 1997).

To adequately protect all green turtle life stages and conserve the global population, scientists and managers must understand the biology and ecology of the species. It is recognized that green turtles fulfill significant ecological roles (Bjorndal & Jackson 2003). They help sustain ecological functioning through nutrient transfer to sandy beaches, acting as prey for marine predators (Bjorndal & Jackson 2003) and moderate grazing of seagrass and macroalgae which increases productivity (McNaughton 1985, Moran & Bjorndal 2005) and species richness in nutrient rich ecosystems (Proulx & Mazmuder 1998). However, knowledge of their ecology is incomplete and impedes effective conservation of this species and their ecological role.

Historically, research and conservation efforts have focused on nesting beaches to increase egg and hatchling survival; however sea turtles spend less than 1 % of their lives on nesting grounds (Bjorndal 1999). Nesting surveys examine only two life stages, breeding females and hatchlings, providing limited information on the remaining constituents of a population (Chaloupka & Limpus 2001). Effective stock assessment and conservation planning require demographic data on all life stages (Chaloupka & Limpus 2001) therefore nesting surveys alone are insufficient sources of demographic information. Focusing on foraging populations, with mixed-stage aggregations, will expand the ecological knowledge of characteristics specific to all green turtle life stages.

Green Turtle Ecology

After hatching from nesting beaches immature green turtles enter the pelagic environment (Carr 1987, Musick & Limpus 1997) where they maintain an omnivorous diet supporting high growth rates (Bjorndal 1985, Reich et al. 2007, Boyle and Limpus 2008). Once the carapace reaches a size between 25 and 40 cm (eg. Balazs 1982b, Limpus 1982, Bjorndal & Bolten 1988) juveniles typically recruit to neritic developmental areas. Most juveniles in the Atlantic Ocean recruit at smaller sizes than those in the Pacific (Hirth 1997). In many populations this habitat shift coincides with a dietary shift from omnivory to herbivory (Carr & Ogren 1960). The process of this ontogenetic shift is highly variable. Some populations exhibit direct shifts from omnivory to herbivory (Reich et al. 2007) while others undergo complex, asynchronous shifts in habitat and diet (Arthur et al. 2008, Cardona et al. 2009, 2010).

Once in the neritic habitat most populations consist of individuals surviving on either seagrass or algae (Bjorndal 1985, Bjorndal 1997) with digestive physiology specific to either source (Bjorndal 1980, Bjorndal et al. 1991). However, individuals may supplement their diet with the occasional animal product (Seminoff et al. 2002b). There is recognized regional variation in the foraging behavior of green turtles. Many Pacific Ocean populations have algal dominated diets (e.g. Russell & Balazs 2009) while Atlantic species feed heavily on seagrass (e.g. Bjorndal 1980, Mortimer 1981). In addition, populations in the eastern Pacific have been observed consuming animal products (e.g. Seminoff et al. 2002b).

The variability in the ontogeny of foraging characteristics may be connected to the nutritional content and availability of resources (Balazs 1980). It is therefore useful to understand how food availability and foraging habits differ between populations to determine proximate and ultimate causes for regional differences in parameters such as growth rate or survivorship. In

addition, a more thorough understanding of the inter- and intra-population variability in foraging ecology will better inform managers of the role of the green turtle as a consumer on foraging grounds and help identify the most critical habitats for this species.

Studying Foraging Ecology

Foraging ecology has been difficult to study because the individuals were inaccessible or sampling techniques were insufficient. Traditional methods for studying foraging ecology (i.e. oral or gastric lavage, fecal analysis and direct observation) have many limitations (Bjorndal 1997, Forbes 1999). In addition, they provide evidence for recent foraging habits only, limiting the temporal information researches are able to obtain from samples.

The development of stable isotope analysis has improved accessibility and is an additional technique for understanding wildlife foraging ecology. Sample collection is minimally invasive (for collection methods, see Reich & Seminoff 2010). In addition, different tissue types vary in their metabolic turnover rates allowing for examination of various time-scales of isotopic incorporation (Tieszen et al. 1983). Tissues with rapid isotope turnover (e.g. liver, carbon half-life of 6.4 days) reflect the most recent diet assimilation while tissues with slower isotope turnover (e.g. hair, carbon half-life of 47.5 days) represent longer-term diet assimilation (Hobson & Clark 1992, Arthur et al. 2008). Metabolically inactive, keratinized tissues (e.g. nail or scute), which are continuously laid down, provide long-term data on assimilated diet because the tissue retains the isotopic signature from the time of tissue synthesis (Hobson & Schnell 1998, Reich et al. 2007).

Carbon and nitrogen are the most commonly used elements for stable isotope analysis of foraging ecology. Carbon isotopes are conserved through trophic levels and are therefore used to

infer diet or species-specific prey item consumption (DeNiro & Epstein 1978, Teeri & Schoeller 1979). Distinct isotopic dichotomies exist between certain habitats and may therefore enable detection of habitat changes. For example, inshore neritic areas can be distinguished from oceanic foraging habitats (Fry & Parker 1979) as the former are enriched in C^{13} (Rau et al. 1983). Distinct isotopic dichotomies also exist between pelagic and benthic ecosystems and high and low latitudes (France et al. 1995). Pelagic foraging areas and higher latitudes typically exhibit depleted carbon signatures when compared to benthic foraging areas and lower latitude habitats (Hobson et al. 1994, France et al. 1995, Cherel & Hobson 2007). It may be hard to decipher which dichotomy is driving the isotopic signatures of turtles in areas of overlapping habitat types.

While carbon isotopes are generally conserved through trophic levels and used to interpret habitat and diet, nitrogen isotopes increase as one moves up through the food chain and can therefore be used to infer trophic position (DeNiro & Epstein 1981, Minagawa & Wada 1984, Hobson et al. 1994). Coordinated analyses of both carbon and nitrogen isotopes provide parallel information on diet/habitat and trophic position increasing the ability to resolve complex trophic interactions (Macko et al. 1982).

There are, however, some caveats that need to be considered when interpreting isotopic information. Body condition (e.g. Hobson et al. 1993), body mass (e.g. Carleton & Martinz del Rio 2005), growth rate (e.g. Reich et al. 2008) and tissue type (Tieszen et al. 1983, Reich et al. 2008) are a few examples of biological factors that may be influencing isotope incorporation, assimilation and fractionation. These biological factors may alter the isotopic signature of individual turtles (Martinez del Rio et al. 2005, Reich et al. 2008) and ultimately effect scientific interpretation of this information.

An increasing number of studies have used stable isotope analysis to understand the feeding ecology sea turtles (e.g. Godley et al. 1998, Hatase et al. 2006, Reich et al. 2007, Arthur et al. 2008, Caut et al. 2008, Cardona et al. 2009, Seminoff et al. 2009, Reich et al. 2010, Vander Zanden et al. 2010). These isotopic analyses have again highlighted the variability in foraging ecology requiring further study in additional geographical areas to fully understand intra and interpopulation variability of green turtle foraging habits.

Inshore neritic areas of the central Pacific Ocean provide foraging grounds for adults and developmental areas for post-pelagic juvenile green turtles (Balazs 1982, Balazs 1995, Chaloupka et al. 2004, Sterling et al. accepted). Many central Pacific Ocean stocks are insufficiently studied leading to gaps in knowledge. Focused studies aimed at understanding the foraging ecology of and threats to these populations are required to fill this gap in the near-term (Seminoff et al. 2007). Using stable isotope analysis to better understand the foraging ecology of Pacific populations will also add to the comprehensive understanding of global green turtle ecology and habitat requirements.

Research Summary

As part of the Pacific Remote Islands Marine National Monument, the Palmyra Atoll National Wildlife Refuge (PANWR) (Fig 2.1) serves as a mixed-stage foraging ground for green turtles, supporting juvenile, subadult and adult size classes. Yet little is known about the ecology of this population (Sterling et al. accepted). The Palmyra Atoll population is previously unstudied and scientists are lacking the fundamental ecological knowledge, which hinders effective conservation and management of the atoll. In 2005, the American Museum of Natural History (AMNH) initiated a comprehensive study of the sea turtle populations found around

Palmyra Atoll. To provide adequate conservation efforts, the AMNH sought to understand the biology and connectivity of Palmyra Atoll green turtles. As part of this larger study my thesis aims to identify the mechanism of ontogenetic shifts and describe the stage-specific foraging habits exhibited by this population using stable isotope analysis.

More specifically chapter two will examine dietary changes within juvenile individuals to identify ontogenetic shifts experienced by the Palmyra Atoll population. In addition the synchrony of dietary and habitat shifts will be examined to determine if they concurrently occur. Finally, chapter three will investigate the foraging characteristics of this mixed foraging population of green turtles. This chapter will provide a brief description of current knowledge of green turtle foraging ecology. An analysis of stable carbon and nitrogen isotopic ratios in carapace scute tissues, using statistical tools, will aid in identifying stage-specific differences in foraging behavior and how they relate to microhabitats found around the atoll.

CHAPTER TWO

Dietary Shifts in the Ontogeny of Green Turtles in the Central Pacific

CHPATER TWO: Dietary Shifts in the Ontogeny of Green Turtles in the Central Pacific

ABSTRACT

Juvenile green turtles (*Chelonia mydas*) shift to neritic foraging grounds after a developmental period in the pelagic environment and are believed to experience a corresponding shift from omnivory to herbivory. Recent evidence, however, suggests the ontogeny of these dietary shifts may vary between populations and that some individuals maintain an omnivorous diet after recruitment to neritic habitats. Further support is needed to substantiate if these are widespread phenomena. To investigate the variation in foraging habits of green sea turtles, scute samples were collected from juvenile sea turtles at the Palmyra Atoll National Wildlife Refuge (PANWR), from 2008 to 2010. Palmyra Atoll serves as a major sea turtle foraging ground in the central Pacific Ocean. Successive layers were removed from each scute biopsy sample and analyzed for stable carbon and nitrogen isotopic signature. A linear mixed model indicated enriched $\delta^{13}C$ values in juvenile green turtles that may suggest an ontogenetic shift from pelagic to neritic habitats in Palmyra Atoll turtles. However, $\delta^{15}N$ values of sequential scute layers suggest that Palmyra Atoll turtles may not undergo a direct trophic shift from omnivory to herbivory. In addition, high levels of individual variation of $\delta^{13}C$ and $\delta^{15}N$ signatures in sequential scute layers from individual turtles imply that Palmyra Atoll juveniles may utilize multiple foraging strategies. Maintenance of an omnivorous diet even after recruitment to Palmyra Atoll foraging grounds is one possible explanation for the enriched $\delta^{15}N$ signatures seen in some individuals. Another hypothesis is that increasing $\delta^{15}N$ values may indicate a change in baseline environmental $\delta^{15}N$ values or prey isotopic values between habitats. High individual

variability suggests that this may be a generalist population with individuals maintaining a variable and complex dietary repertoire including omnivorous prey items.

Keywords: green turtle, ontogenetic shift, stable isotope, foraging ecology, Central Pacific

INTRODUCTION

Green turtles (*Chelonia mydas*) are understood to switch to a neritic life stage inhabiting the inshore marine environment after a period of pelagic development following hatching (Carr 1982). It is believed that recruitment to neritic habitats coincides with a direct shift from omnivory to herbivory (Carr & Ogren 1960, Bjorndal 1997, Bolten 2003). Stomach contents of post-hatchling individuals from the southwest Pacific Ocean reveal a pelagic existence (Boyle & Limpus 2008) with further support from stable isotope analyses indicating a shift from a pelagic, omnivorous life stage to a neritic herbivorous existence after recruitment to a foraging ground (Reich et al. 2007, Arthur et al. 2008).

Recent studies, however, indicate populations may vary in the ontogenetic shifts of green turtles when they arrive at neritic foraging grounds. In the Mediterranean (Godley et al. 1998) and Japan (Hatase et al. 2006) green turtle populations exhibit facultative diet shifts with older individuals incorporating animal products into their diet. Evidence from Mauritania (Cardona et al. 2009) and Mediterranean green turtles (Cardona et al. 2010) suggests an asynchronous shift in diet and habitat contradicting previous hypotheses of direct ontogenetic shifts. The variable methods of habitat and dietary shifts may denote differences in foraging mechanisms between and/or among green turtle populations suggesting that direct dietary shifts may not occur in all populations. Further support is needed to substantiate if these are widespread phenomena.

Stable isotope analysis has been used in the marine environment to provide insight into sea turtle feeding ecology (e.g. Godley et al. 1998, Hatase et al. 2006, Wallace et al. 2006, Reich et al. 2007, 2008, Arthur et al. 2008, Caut et al. 2008, Cardona et al. 2009, Seminoff et al. 2009, Wallace et al. 2009, Reich et al. 2010, Vander Zanden et al. 2010, Lemons et al. 2011, Pajuelo et al. 2012). This technique provides a minimally invasive method of understanding foraging ecology of cryptic or protected species, such as the green turtle.

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes change in predictable ways in natural systems allowing for accurate interpretation of isotopic trends (Peterson & Fry 1987). In the process of isotopic fractionation heavier isotopes increase in abundance compared with light isotopes (C^{13}:C^{12} and N^{15}:N^{14} respectively) (Owens 1987, Peterson & Fry 1987). The ratio of heavy to light isotopes is measured and compared between various sources. The customary δ notation indicates the difference (parts per thousand, ‰) between the sample and standard isotopic concentrations (Peterson & Fry 1987).

Nitrogen ratios are conserved throughout a trophic level increasing predictably from autotrophs through the various heterotrophic levels (Minagawa & Wada 1984, Lepoint et al. 2000). Consumers are typically enriched in $\delta^{15}N$ by 3-4‰ relative to their diet as the heavier ^{15}N is preferentially retained during excretion (DeNiro & Epstein 1981, Minagawa & Wada 1984). Nitrogen isotope information can therefore be used to infer trophic position (Minagawa & Wada 1984). Carbon isotope ratios change very little through trophic levels (DeNiro & Epstein 1978). Consumers are enriched by ~1‰ compared to prey (DeNiro & Epstein 1978, Rau et al. 1983). Upper level consumers therefore reflect the carbon signatures found in producers at the base of the food web (Teeri & Schoeller 1979). Delta ^{13}C ratios may also distinguish between inshore neritic and oceanic foraging habitats (Fry & Parker 1979) as inshore neritic habitats are enriched

in C^{13} values (Rau et al. 1983). Consequently, $\delta^{13}C$ signatures are used to infer diet (DeNiro & Epstein 1978) or habitat (Fry & Parker 1979, Rau et al. 1983).

There are some caveats to consider when interpreting isotopic information. Body condition (e.g. Hobson et al. 1993), body mass (e.g. Carleton & Martinz del Rio 2005), growth rate (e.g. Reich et al. 2008), tissue type (Tieszen et al. 1983, Reich et al. 2008) and isotopic routing (Schwarcz 1991) may be factors influencing isotope assimilation and fractionation. For example, growth rate was shown to explain up to 52% of isotopic incorporation in juvenile loggerhead turtles (Reich et al. 2008). Isotopic routing may occur if isotopes are not mixed well with an individuals' isotopic "pool" prior to routing resulting in dietary components preferentially directed to certain tissues. Isotopic routing may vary between individuals. After consideration of these caveats, using isotope analyses of both carbon and nitrogen to understand diet and trophic position may increase the ability to resolve the complex associations between green turtles and their environments (Macko et al. 1982).

In addition to addressing the above caveats, it is also necessary to determine the appropriate tissue type to address research questions (Hobson et al. 1993). Various types of sea turtle tissues including skin, blood plasma, red blood cells and scute have been used to obtain stable carbon and nitrogen isotopic information. The tissue turnover rate will determine the timeline of the information retained in the tissue (Tieszen et al. 1983). Tissues with high turnover rates (eg. plasma) track recently assimilated isotopic signatures while tissues with low turnover rates (e.g. skin) will incorporate information from a longer time period (Reich et al. 2008). Studies of past and present foraging ecology of a species thus use a variety of tissue types and can help elucidate changes in foraging patterns over time.

Scute from the carapace of a sea turtle is keratinized tissue that remains inert after synthesis (Reich et al. 2007). A layer of scute is produced by accretion when underlying epidermis grows causing the outer cells to die and become keratinized (Wilson et al. 2003). Continual production of scute tissue originating from the ventral surface results in older tissue on the dorsal side (outermost layer) and younger tissue on the ventral side (innermost base layer) (Fig. 2.2) (Reich et al. 2007). Comparison of $\delta^{13}C$ and $\delta^{15}N$ signatures of successive scute tissue layers allows for examination of resource use over time as the scute tissue retains isotopic signatures from the time of tissue formation (Reich et al. 2007, Vander Zanden et al. 2010). This time-integrated information (Tieszen et al. 1983) exceeds traditional diet analysis methodologies that may be unable to incorporate similar time scales (i.e. gastric lavage or scat analysis). While there are a variety of tissues that may be used to examine foraging ecology, scute tissue is an effective tissue to use when examining isotope variability in a longer time frame or when studying organisms that are difficult to sample over time.

There is a paucity of data regarding the foraging ecology of many central Pacific green turtle populations. The variability in the method of juvenile foraging development lends itself to further exploration of unstudied populations to provide a better understanding of global green turtle foraging ecology. This study was initiated to provide a more thorough understanding of the ontogeny of foraging behavior of green turtles in the central Pacific Ocean using stable isotope analysis.

Palmyra Atoll, a remote uninhabited atoll in the central Pacific, currently serves as a mixed-stage foraging ground for a previously unstudied green turtle population (Sterling et al. accepted). Little is known about the ecology of this population. Examining the past diet of Palmyra Atoll juveniles will allow us to examine the ontogeny of foraging ecology in a central

Pacific green turtle population. More specifically, examining the $\delta^{13}C$ and $\delta^{15}N$ signatures of sequential layers removed from juvenile scute samples will help elucidate the timing and mechanism of ontogenetic shifts should this population exhibit such shifts.

Time since recruitment is unknown for the sampled juvenile turtles therefore I cannot hypothesize as to when in the temporal history of the turtle or in what layer a diet or habitat switch is expected. However, if Palmyra Atoll juvenile green turtles once consumed omnivorous dietary items in the pelagic, open-ocean environment, older scute tissue produced at a younger turtle age should have enriched (higher) $\delta^{15}N$ values and depleted $\delta^{13}C$ values.

METHODS

Study Site

The study site, Palmyra Atoll National Wildlife Refuge (5.525554°N, 162.045905°W), is a relatively pristine atoll ecosystem located in the central Pacific Ocean (Fig. 2.1). The habitat consists of a diverse assemblage of foraging substrate (Williams et al. 2008) and supports a substantial green turtle population (Sterling et al. accepted).

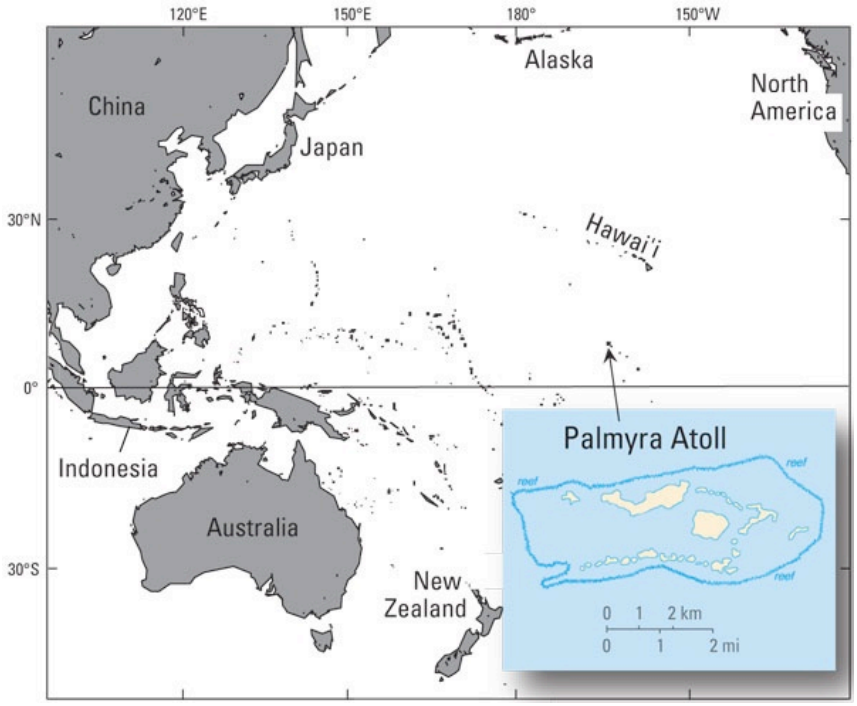

Fig. 2.1. Map of the location of the Palmyra Atoll National Wildlife Refuge in the central Pacific Ocean (USGS 2011)

Sample Collection

Scute sample thickness varies based on the size/age of the turtle, carapace location and the location within the individual scute (medial or lateral, anterior or posterior) (Day et al. 2005). Scute biopsies collected from different areas of the carapace may reflect different times of isotope assimilation throughout an individuals' lifetime (Day et al. 2005). The anterior portion of the scute is the area where new growth forms. There is less accumulated keratinized scute tissue on the anterior portion of the scute making this biopsy thinner and newer than any posterior biopsy. In addition, outer layers of scute, exposed to the elements, are gradually worn away or shed although the rate of scute loss is unknown (Cardona et al. 2009). This external wear on carapace scute tissue causes a medium-term retention time of isotopic information, unlikely to

represent the entire lifetime of an adult individual (Godley et al. 1998); therefore, posterior tissue samples from juvenile individuals were used to examine ontogenetic niche shifts as they would most likely provide the oldest retained information of diet in the Palmyra Atoll green turtles.

Scute biopsies were collected from 18 live-captured juveniles (Appendix A). Sample collection took place over three summer seasons: 2008 (n = 4), 2009 (n = 8) and 2010 (n = 6). Hand-capture techniques, standard turtle rodeo techniques (Limpus & Walter 1980, Limpus & Reed 1985), tangle nets or scoop nets were used to capture the turtles. After cleaning the carapace with ethanol, a 3mm (2008) or 5 mm (2009/2010) sterile biopsy sample was collected. Posterior samples, reflecting older tissue, were collected from the medial edge of the L3/L4 or C2/C3 scutes. Samples were stored and dried until analysis. Body measurements, including curved carapace length (CCL), and tail length (TL) were taken using a flexible measuring tape; straight carapace length (SCL) was measured using rigid metal calipers.

A body condition index (BCI) was calculated using Fulton's K equation (condition index = mass/SCL^3 x 10^4) (Bolger & Connolly 1989, Bjorndal et al. 2000, Labrada-Martagon et al. 2010). In order to provide context for the BCI scores, the mean and range of BCIs for turtles from Palmyra were calculated and compared to body condition categorization criteria developed for green turtles in Queensland, Australia by Flint et al. (2009). Animals with BCIs over 1.20 were considered to be in "very good" condition, those with BCIs between 1.11 – 1.19 were classified as in "good" condition, those between 1.00 – 1.10 were considered "average, and those under 1.00 were considered to be in "poor" condition. Stage classes were determined using CCL as a proxy for age; juveniles included any individual with 30 to 64.9 cm CCL.

Sample Preparation

In the lab, posterior scute samples were dried at 60°C in glass vessels for at least twenty-four hours. Following lipid removal, using a modified methanol/chloroform (2:1) extraction technique (Bligh & Dyer 1959), samples were dried for an additional twenty-four hours. Posterior samples were glued with the ventral side (most recently deposited layer) attached to a glass slide allowing for layer removal starting from the dorsal side (oldest tissue) of the carapace biopsy; successively younger tissue was removed with each layer (Fig. 2.2).

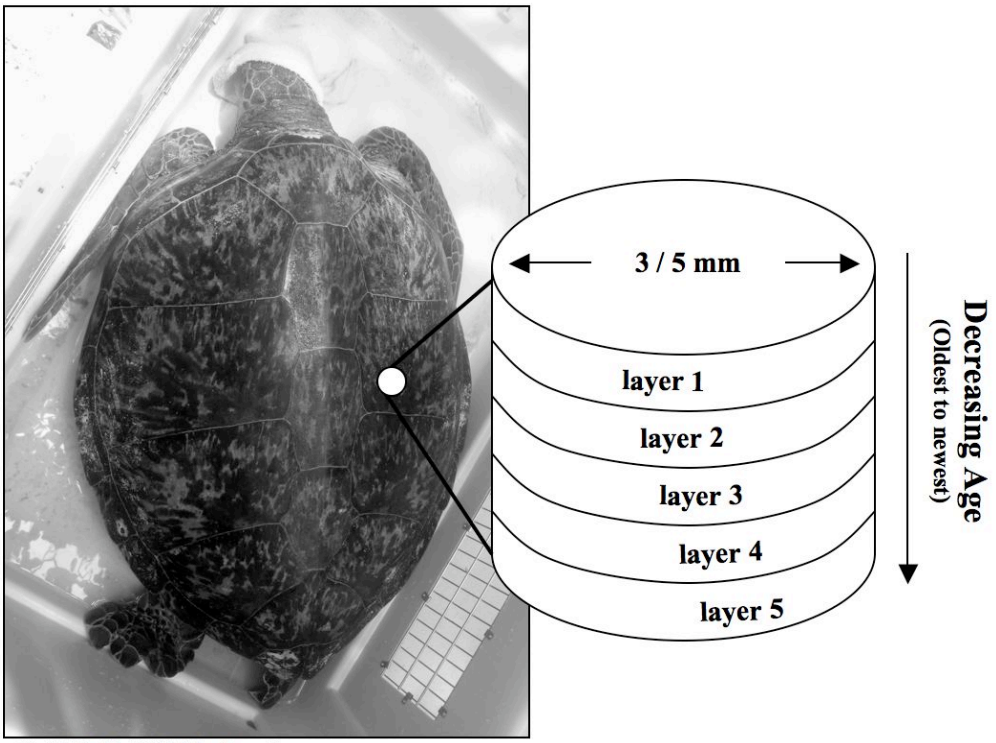

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Fig. 2.2. Example of posterior sampling sites on green sea turtles at Palmyra Atoll. The outermost layer (layer 1) of the posterior scute sample was the oldest tissue sampled. Tissue age decreased to the newest, innermost ventral layer (layer 5 in the image). The number of layers

varied between individual samples and depended on the thickness of the scute tissue sample. Each layer was approximately 50 μm in depth. Image adapted from Reich et al. Biol. Lett. 2007

A minimum of 0.10 mg of ground sample was required for stable isotope analysis. A layer thickness of 50 μm allowed for retrieval of the required sampled amount (Reich et al. 2007); therefore, successive 50 μm layers were removed from each posterior scute sample using a Merchantek Micromill sampling system or a manual Sherline Model 2010 Carbide Endmill (Cat # 970.557L/Model #: 970.557B) outfitted with a 1.16" Endmill Drill Bit (Cat # 402-0625). The number of layers removed per sample was directly related to the original sample thickness, which varies between individuals. The number of scute tissue layers removed per individual varied from 2 to 10 layers equivalent to a biopsy thickness of 100 μm to 500 μm respectively. Each finely ground layer was weighed after removal, sealed in tin capsules and shipped to Boston University Stable Isotope Laboratory (BUSIL) for isotopic analysis.

Stable Isotope Analysis

Continuous flow measurements of C^{13} and N^{15} isotopes were done using a GVI Isotope ratio mass spectrometer and Eurovector elemental analyzer at Boston University Stable Isotope Laboratory. Each sample was flash combusted in the Eurovector CN analyzer after which the combustion products were separated chromatographically and introduced into the GVI Isotope ratio mass spectrometer.

Stable isotope abundances were reported in δX values (X representing either C^{13} or N^{15}). The R_{sample} and $R_{standard}$ notation represents the ratio of heavy isotope to light isotope ($^{13}C/^{12}C$ and

$^{15}N/^{14}N$). Delta X values are given as parts per thousand (‰) differences from the international standards, usually referred to as per mil, and are calculated by the following formula:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

The results were compared to secondary gas standards calibrated to international standards obtained from the National Bureau of Standards (BUSIL); $R_{standard}$ for ^{13}C was PeeDee belemnite and $R_{standard}$ for 15N was atmospheric nitrogen. Laboratory standards were run after every 15 samples; any anomalous results were rerun. For well-ground organic material an external precision of 0.2 per mil is required, however, 0.1 per mil was typical for both carbon and nitrogen (BUSIL).

Statistical Analysis

Isotope and curved carapace length (CCL) data were examined for normality and homoscedasticity using a Shapiro-Wilk test and Levene test respectively. Assumptions of normality and homoscedasticity were not met in all cases therefore nonparametric analyses were used. A Spearman's rank correlation coefficient was used to determine the correlation between $\delta^{13}C$ and $\delta^{15}N$ ratios in juveniles to test for asynchrony in habitat utilization and diet shifts. If there was no correlation between $\delta^{13}C$ and $\delta^{15}N$ ratios it is unlikely that diet shifts occur simultaneously with habitat shifts (Cardona et al. 2009). A Spearman's rank correlation was also used to examine the relationship between isotopic signatures, turtle size (CCL) and body condition. In addition, a linear mixed model (LMM) was used to investigate the effect of scute layer (fixed effect) and turtle ID (random effect) on $\delta^{13}C$ and $\delta^{15}N$ values. Sampling techniques

caused the sample size (*i.e.* # of layers) to vary between individuals and resulted in some missing data. The LMM is the statistical method capable of addressing the study questions in spite of these data limitations (West et al. 2007). Statistical analyses were carried out using PASW Statistics 18.0.3 (2010) and/or JMP 8.0.2 (2009). Statistical significance was determined at the level of 0.05 and a confidence interval of 95% was used. Results are reported as mean ± standard deviation (SD).

RESULTS

A total of 18 captured juveniles were used for this study. Curved carapace length (CCL) of sampled individuals ranged from 44.50 - 60.10 cm (mean ± SD = 54.59 ± 5.07 cm) and weights ranged from 10.20 – 44.00 kg (20.55 ± 7.14 kg). All turtles exhibited "very good" (> 1.20) body condition index (BCI) except one turtle with "average" BCI. A Spearman's correlation coefficient showed no correlation between $\delta^{13}C$ values and BCI ($r = -0.158$, $p = 0.107$) and a marginal positive correlation between $\delta^{15}N$ signatures and BCI ($r = 0.191$, $p = 0.051$). In addition, there was no clear relationship between $\delta^{13}C$ and $\delta^{15}N$ values in juvenile posterior samples ($r = 0.145$, $p = 0.141$). No correlation was found between CCL and $\delta^{15}N$ values ($r = -0.169$, $p = 0.083$); however, there was a strong positive correlation between CCL and $\delta^{13}C$ ($r = 0.321$, $p < 0.001$) with $\delta^{13}C$ enrichment as CCL increased.

The linear mixed model (LMM) results suggested that carbon signatures significantly varied by scute layer ($df = 9$, $F = 2.073$, $p = 0.042$). Overall, carbon values became more depleted over time by approximately -0.2‰; however there is a high degree of variability and not all animals follow this trend. Isotope variation across the scute layers of an individual ($Z = 6.268$, $p < 0.001$) accounted for approximately 49% of the total $\delta^{13}C$ variance while isotopic variation

between individuals ($Z = 2.476$, $p = 0.013$) accounted for the remaining 51% of $\delta^{13}C$ variance (Fig. 2.3a).

Nitrogen signatures also exhibited a significant decline in $\delta^{15}N$ signatures from layer 1 to 10 ($F = 2.604$, $df = 9$, $p = 0.011$). A high degree of variation in $\delta^{15}N$ values within ($Z = 6.237$, $p < 0.001$) and among turtles ($Z = 2.462$, $p = 0.021$) existed. Between-turtle variation explained approximately 54% of the variance in ^{15}N values while isotopic variation within the individuals explained the remaining 46% of $\delta^{15}N$ variance (Fig. 2.3b).

Two groups emerge when examining patterns in $\delta^{13}C$ values for layer 1, which represents the oldest tissue sampled from the scute (Fig. 2.3a). For layer 1, seven individuals have layer 1 $\delta^{13}C$ values greater than -14.6‰ (hereinafter referred to as the "enriched group") while six individuals have layer 1 $\delta^{13}C$ values less than -16.9‰ (hereinafter referred to as the "depleted group"). The enriched and depleted groups are separated by a gap in $\delta^{13}C$ values between -14.6‰ and -16.9‰. The depleted group is composed of smaller individuals, with 5 of the 6 individuals with ≤ 56 cm CCL. The enriched group is composed of larger individuals, with 5 of the 7 individuals with > 56 cm CCL. A trend of first layer $\delta^{13}C$ enrichment with increasing CCL (smaller size, depleted $\delta^{13}C$ and larger size, more enriched $\delta^{13}C$) is substantiated by a positive correlation between CCL and $\delta^{13}C$ values. ($r = 0.321$, $p < 0.001$).

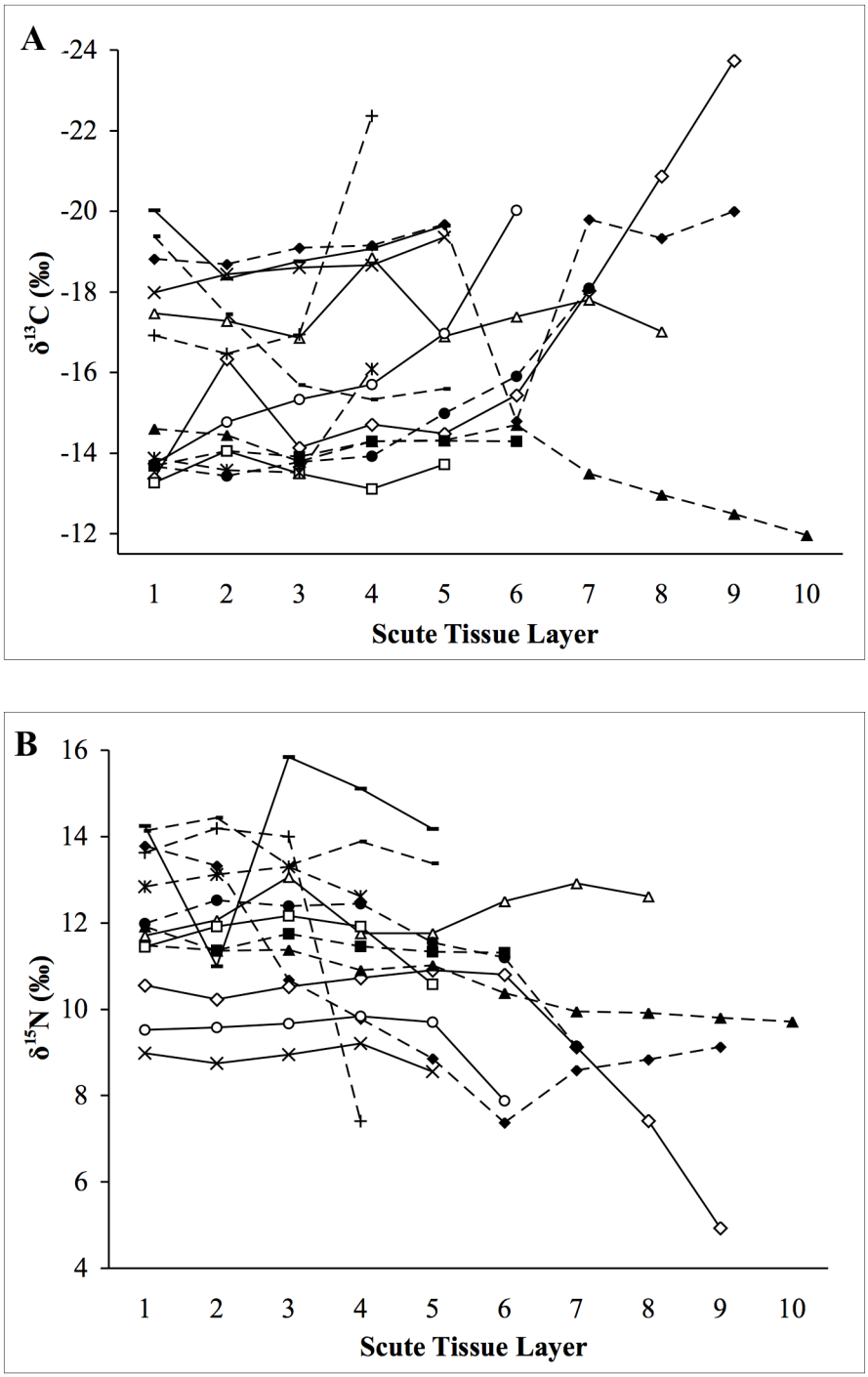

Fig. 2.3. Successive scute layer (A) $\delta^{13}C$ and (B) $\delta^{15}N$ values from live captured juvenile green turtles sampled from 2008 to 2010 at Palmyra Atoll. The combination of layer symbol and line style is unique for each individual turtle and is consistent across figures. Turtle age increases

across the x-axis with layer 1 representing the youngest turtle age (oldest tissue sampled). The numbers of layers vary between individuals and depend on the thickness of the sample. Samples with missing values were not reported in the figures to improve figure clarity although they were included in the analyses.

DISCUSSION

Habitat

Well-understood gradients of stable carbon isotopes ($\delta^{13}C$) exist in the marine environment allowing for interpretation of habitat change based on the carbon signature of an individual's tissue. These $\delta^{13}C$ gradients range from depleted $\delta^{13}C$ environments (oceanic, pelagic and high latitude) and/or food webs to enriched $\delta^{13}C$ environments (neritic, offshore, benthic and low latitude) and/or food webs (Goericke & Fry 1994, Hobson et al. 1994, France 1995). As in other studies (e.g. Reich et al. 2010) the existence of multiple $\delta^{13}C$ gradients can confound the interpretation of $\delta^{13}C$ values.

Post-pelagic turtles that have recruited from oceanic to neritic foraging grounds would be expected to exhibit increasing $\delta^{13}C$ enrichment over the temporal history of the turtle. This enrichment would result from a shift from offshore foraging locations to inshore foraging locations (Hobson et al. 1994). A correlation between CCL and $\delta^{13}C$ values for the oldest scute tissue (layer 1) may suggest that as carapace size increases juvenile turtles shift in either their habitat utilization (pelagic vs. neritic) or feeding location. However, the linear mixed model (LMM) results show a significant decreasing $\delta^{13}C$ trend over time suggesting that Palmyra Atoll juveniles forage on substrate that is slightly depleted in carbon.

It is possible that the Palmyra Atoll turtles may have recruited to the neritic habitats of the atoll in the very recent past and the enriched isotopic signatures of this environment have not yet been incorporated into their scute tissue. As previously mentioned, scute tissue may not represent the entire lifetime of an individual due to carapace wear (Godley et al. 1998, Cardona et al. 2009). The exact rate of external carapace wear is unknown and likely varies between individuals. It may also be possible that the sampled turtles have not recently recruited and therefore the depleted signature of their early pelagic life is no longer contained in the scute tissue. However, recently recruited Caribbean juvenile green turtles (> 36 cm SCL) retained the isotopic signature of an omnivorous diet in the pelagic open ocean in the outermost scute tissue layer (Reich et al. 2007).

This population likely employs multiple foraging strategies. Sampled juvenile green turtles exhibit a high degree of individual variation in the trending of carbon signatures over time. Half of the juvenile turtles exhibit a decrease in $\delta^{13}C$ signatures of at least 1.5‰ between layers one and ten. This may suggest that these animals have not recently recruited and the tissue timescale of our samples does not include their early life history. In contrast, the other half of the sampled turtles exhibits no change or an enriched carbon signature over time; enriched carbon signatures likely suggest a shift from pelagic to neritic environments.

The carapace size of sampled juvenile turtles varied ranged from 44.50 to 60.10 cm CCL with 60.10 cm being on the larger end of the juvenile categorization. It is therefore likely that layer 1 of individuals from the enriched group (e.g. 60.10 cm CCL, the largest individual sampled) do not represent the same time in history as layer 1 of smaller individuals from the depleted (e.g. 44.50 cm CCL, the smallest individual sampled). Individuals from the enriched group may have deposited layer 1 tissue after recruitment to a developmental area or neritic

habitat while layer 1 of individuals from the depleted group may have been deposited as the turtle was entering the pelagic environment after hatching. However, previous studies indicate that growth rates remain relatively the same for turtles 40 to 60 cm CCL (Zug et al. 2002) and that recruitment to Hawaiian neritic grounds occurs by 35 cm CCL (Balazs 1980). Additional study is required to identify if turtles at Palmyra Atoll exhibit these same features in growth and recruitment before my hypothesis can be accepted.

Finally, the depleted and enriched groups may have obtained these isotopic signatures from distinct geographic regions. The individuals may have originated in different hatching areas and/or pelagic habitats (Lahanas et al. 1998). Ongoing studies in genetic analyses are currently underway and will help to determine Palmyra's juvenile turtle geographic origination. Additionally, satellite telemetry or the use of other elemental stable isotopes may help decipher the original habitat from which Palmyra Atoll juveniles have recruited.

Trophic Position

The stable nitrogen isotope ($\delta^{15}N$) values of sequential scute layers suggest that Palmyra Atoll juveniles undergo a trophic shift from omnivory to herbivory, as there is a significant decline in nitrogen signatures over time. However, the high degree of individual variation suggests that the dietary shift of this population may be complex. Eight sampled juvenile individuals exhibit a nitrogen decline of at least 1.5‰ between layers one and ten. The remaining ten individuals sampled either exhibit no change or have increasing nitrogen values between layers one and ten.

Those Palmyra Atoll turtles with increasing nitrogen values may supplement a primarily herbivorous diet with higher quality foods during transitional periods resulting in enriched $\delta^{15}N$

values. Another hypothesis is that the increasing $\delta^{15}N$ values of some individuals indicate a change in baseline environmental $\delta^{15}N$ values or prey isotopic values between habitats. For instance, prey items such as filter feeders living in anoxic conditions of the lagoons may have modified nitrogen characteristics. Additional studies of baseline values and prey items from Palmyra Atoll are needed before either potential explanation can be confirmed.

A rapid decline in $\delta^{15}N$ values between layers may suggest a direct shift in trophic position (Reich et al. 2007). Although most of the individuals with declining nitrogen values showed a gradual decline there are two individuals that follow the pattern of a direct shift. The remaining 16 individuals, however, exhibit relatively constant or steadily decreasing $\delta^{15}N$ values over time as opposed to a drastic jump between trophic level positions at any one point. It is possible these turtles are gradually integrating more plant products into their diet over time. As the proportion of plant items in the diet increases compared to other dietary items the $\delta^{15}N$ signature should decrease becoming less enriched as a result of the consumer feeding increasingly on lower trophic level prey. As a whole population, these juveniles exhibited a statistically significant decrease in nitrogen values over time, totaling 2.95‰, suggesting that this population likely experiences a shift in trophic position.

There is a marginal correlation between body condition index (BCI) and $\delta^{15}N$ values. There was no similar correlation between BCI and $\delta^{13}C$ suggesting that the correlation between nitrogen and body condition may be related to the type of prey being consumed and not based on the location of the prey items prior to consumption. If there was a correlation between carbon and body condition one would interpret this as being related to the environmental conditions, *i.e.* where the turtle is feeding, oceanic vs. neritic.

Synchrony of Diet and Habitat Shift

The absence of a correlation between $\delta^{13}C$ (habitat and prey indicator) and $\delta^{15}N$ (trophic position indicator) may support either an asynchronous shift in diet and habitat in Palmyra Atoll juvenile green turtles or the existence of asynchronous assimilation rates, which can occur in growing animals (Reich et al. 2008). Isotopic routing (Schwarcz 1991) may also lead to asynchronous assimilation rates, if individuals are routing dietary components differently.

In tropical areas where algae are dominant, nutrient limitations may require green turtles to reach a larger size before recruitment (Thayer et al. 1984). Turf algae and red and green coralline algae are the dominant cover in Palmyra Atoll nearshore environments (McFadden et al. 2010). Nutritional limitations of these food sources may cause post-pelagic juveniles to enter a transitional periods where they inhabit developmental areas (Bolten 2003, Arthur et al. 2008) before recruiting to "permanent" foraging grounds. These developmental areas may coincide with adult foraging grounds however they may also be geographically distinct (Musick & Limpus 1997, Godley et al. 2003, Meylan et al. 2011). There may also be transitional periods in the type of prey consumed rather than the location of foraging suggesting that some turtles around the atoll could be in a transitional dietary state. This population likely makes use of a more complex foraging repertoire than has been described for other green turtle populations (e.g. Reich et al. 2007). This complexity can easily be seen by the high individual variation in $\delta^{13}C$ and $\delta^{15}N$ trending over time (Fig. 2.3). Juveniles recruiting to Palmyra Atoll likely utilize multiple foraging strategies, prior to and possibly after recruitment, in attempts to maximize survivorship.

Conclusion

There is a gap in the understanding of juvenile green turtle ecology because of their limited availability during the lost years between hatching and recruitment to neritic foraging grounds. Juvenile green turtles may recruit to Palmyra Atoll after hatching from disparate areas around the Pacific Ocean. This potential for diverse recruitment highlights the importance of Palmyra as a green turtle foraging ground in the central Pacific. Development of appropriate conservation measures requires a fundamental understanding of the foraging characteristics and habitat usage of all Palmyra Atoll green turtle stage classes. Having a better understanding of Palmyra Atoll green turtle population characteristics may also lead to a better understand of other foraging aggregations found around the Pacific in similar remote island or atoll locations. Using stable isotope analysis to examine the past foraging habits of Palmyra Atoll green turtles will grant us a better understanding of the ontogeny of foraging ecology in these turtles and help us understand the context for current foraging behavior.

The Palmyra Atoll juveniles do exhibit general trending in $\delta^{13}C$ and $\delta^{15}N$ values over time, however there is also a high degree of individual variation. This suggests that Palmyra Atoll juveniles employ a wide variety of foraging strategies and as a whole comprise a more generalist population consuming the necessary products to increase survival. Future study of the survivorship of turtles with each foraging strategy (*e.g.* benthic vs. pelagic, herbivore vs. omnivore) will allow for examination of direct effects on survival.

There is increasing awareness that green turtles are not necessarily obligate herbivores (Mortimer 1982) as they were once believed to be. Based on stable isotopic signatures it is apparent that Palmyra Atoll juveniles ingest a mixed diet, possibly supplementing their herbivorous diet with animal products even after recruitment to neritic foraging grounds. This

phenomenon has been seen in other populations (Godley et al. 1998, Seminoff et al 2002b, Hatase et al. 2006, Amorocho & Reina 2007). Understanding the nutritional demands needed for growing sea turtles may help understand the maintenance of an omnivorous diet and in what instances this may beneficial. This will also add to our understanding of green turtle population dynamics and may result in essential alterations to current recovery plans to include more variable foraging substrate and critical habitat.

ACKNOWLEDGMENTS

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CHAPTER THREE

Description of Green Turtle (*Chelonia mydas*) Foraging Ecology in the Central Pacific Using Stable Isotope Analysis

CHAPTER THREE: Description of Green Turtle (*Chelonia mydas*) Foraging Ecology in the Central Pacific Using Stable Isotope Analysis

ABSTRACT:

Green turtles (*Chelonia mydas*) are thought to be herbivorous after recruitment to foraging grounds; however, recent studies suggest that green turtles may supplement their diet with animal products after recruitment to neritic foraging grounds. Further study is needed to substantiate if this is a widespread phenomenon and to determine if variation exists between individuals and populations. Stage-specific and spatial variations in foraging habits of green turtles at the Palmyra Atoll National Wildlife Refuge in the Central Pacific were studied using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic tools. Live-captured green turtles (n = 75) were sampled from 4 regions around the atoll between 2008 and 2010 (mean curved carapace length = 73.55 cm). Stage classes were determined using the curved carapace length (CCL) as a proxy for age and were categorically coded as juvenile (≥ 64.9 cm CCL), sub-adult (65.0 – 84.9 cm CCL) or adult (85.0 ≤ cm CCL). A total of 21 juveniles, 28 subadults and 26 adults were sampled. Anterior scute biopsies representing the most recently deposited (*i.e.* younger) tissue were removed to allow for examination of $\delta^{13}C$ and $\delta^{15}N$ values from the most recent foraging history of the turtles.

An analysis of anterior scute samples indicate no significant differences in $\delta^{13}C$ values existed, suggesting that all three stage classes were foraging in the same environment. Significant differences in $\delta^{15}N$ were found between stage-classes (Kruskal-Wallis, $p = 0.001$) with subadults depleted in $\delta^{15}N$ compared to juveniles (Mann-Whitney U, $p = 0.018$) and adults (Mann-Whitney U, $p < 0.001$), suggesting that subadults may forage at different trophic levels.

These stage-specific habits may be related to habitat selection preferences as turtles captured in each of the four sampling areas exhibited different mean CCL (Kruskal-Wallis, $F = 8.937$, $df = 3$, $p = 0.030$) and mass measurements (Kruskal-Wallis, $F = 10.979$, $df = 3$, $p = 0.012$) measurements; size class distribution also differed between the four sampling regions (Kruskal-Wallis, $p = 0.023$). This population contains individuals that likely utilize a more variable and complex dietary repertoire including omnivorous prey items.

Keywords: Palmyra Atoll, green sea turtles, carbon, nitrogen, discrimination factors

INTRODUCTION

After hatching from nesting beaches, immature green turtles (*Chelonia mydas*) enter the open ocean where they spend a variable amount of time subsisting on both plant and animal products (Carr & Ogren 1960). After this pelagic period, termed the "lost years" (Carr 1987), juvenile green turtles recruit to nearshore, neritic habitats where they predominantly forage on either algae or seagrass until reaching maturity (Bjorndal 1980, Musick & Limpus 1997).

Regional variations in green turtle foraging habits exist largely because of local availability of resources (Bjorndal 1980, Garnett et al. 1985, Carrion-Cortez et al. 2010). Turtle foraging selectivity or preference also influences regional green turtle foraging habits (Balazs 1980, Bjorndal 1985, Lopez-Mendilaharsu et al. 2008, Arthur & Balazs 2008). For example, in the Hawaiian archipelago there are over 400 algal species, however only nine are dominant items in the green turtle diet (Balazs 1980). Usually these preferences relate to nutrient requirements as essential nutrient concentrations vary between algal species (Balazs 1980). While green turtles are considered to be the only truly herbivorous sea turtle species, predominately feeding on

either seagrass (Mortimer 1981, Arthur & Balazs 2008) or on algae (Balazs 1980, Bjorndal 1991, Seminoff et al. 2002b), recent studies have suggested that some green turtle populations may continue an omnivorous diet even into adulthood supplementing their herbivorous diet with animal products in nearshore environments (e.g. Seminoff et al. 2002b, Amorocho & Reina 2007). Further study is needed to substantiate if this is a widespread phenomenon.

Green turtle foraging aggregations are often composed of mixed stage-classes, with immature post-pelagic juveniles foraging among mature adults (Bolten 2003). Green turtles may however, exhibit transition periods specifically utilizing unique habitat as developmental areas during juvenile years (Bolten 2003, Arthur et al. 2008). Mixed-stage aggregations and/or coinciding juvenile developmental areas with adult foraging grounds may result in niche partitioning (Arthur et al. 2008, Bresette et al. 2010). This is likely to happen in areas with diverse habitat characteristics and may cause defined stage-specific spatial structure of foraging habitats with juveniles segregated from adults (Bresette et al. 2010). For example, in the Hawaiian Islands, juveniles frequent foraging areas much to shallow for adults to use (Balazs 1980). Understanding spatial structure and diet composition of the green turtle is necessary to identify the most important resources and critical foraging habitats for each green turtle stage class as foraging ecology is directly linked to nutrition, health, growth rates and reproductive output (Bjorndal et al. 1982, Arthur & Balazs 2008).

Historically, many aspects of sea turtle foraging ecology have been difficult to study either because individuals were inaccessible or sampling techniques were insufficient. Traditional techniques such as direct observation or stomach content analysis are only able to provide insight on recent dietary history and do not elucidate differences in nutritional content and quality of diet items. Assimilation of nutrients may also vary between consumer species;

however, this cannot be determined via traditional techniques leaving questions as to the difference between what an organism eats and what nutrients are assimilated (Levey & Martinez del Rio 2001). These inefficiencies have led to the gap in knowledge regarding a significant portion of the green turtle life history that currently exists.

The development of stable isotope analysis has allowed for a more inclusive examination of green turtle foraging ecology. This minimally invasive technique has become a tremendously important tool for studying foraging ecology (Godley et al. 1998, Hatase et al. 2006, Reich et al. 2007, Arthur et al. 2008, Caut et a. 2008, Cardona et al. 2009, Seminoff et al. 2009, Reich et al. 2010, Vander Zanden et al. 2010). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are commonly used for dietary inference. Heavier isotopes increase in abundance compared with light isotopes in a process called isotopic fractionation (Owens 1987, Peterson & Fry 1987). The ratio of heavy to light isotopes – measured in parts per thousand differences from a standard – is given by δ values (Peterson & Fry 1987).

Consumer $\delta^{13}C$ values may be used to infer diet if isotopic signatures differ between prey items (DeNiro & Epstein 1978). Delta ^{15}N ratios are generally conserved throughout a trophic level increasing predictably from autotrophs through the various heterotrophic levels (Minagawa & Wada 1984, Lepoint et al. 2000) with consumers enriched in nitrogen by 3 - 4‰ relative to their diet (DeNiro & Epstein 1981). However, species-specific discrimination factors are more readily available than they were in prior decades and their application results in more accurate analyses of trophic position (Minagawa & Wada 1984, Caut et al. 2008, Caut et al. 2008b).

Many central Pacific Ocean green turtle populations remained unstudied; however, ecological and conservation consequences can arise from population-level variability in resource use. Additional research is necessary to fully understand the variability of foraging ecology in

the central Pacific Ocean. Looking at stage-specific resource and habitat use within a population may ultimately allow for a better understanding of the overall ecology and behavior of a species.

As part of the Pacific Remote Islands Marine National Monument, the Palmyra Atoll National Wildlife Refuge (PANWR) (Fig 3.1) serves as a mixed-stage foraging ground for green turtles, yet little is known about the ecology of this previously unstudied population (Sterling et al. accepted). Scientists are lacking fundamental ecological knowledge of the Palmyra Atoll green turtle population, which not only hinders effective conservation and management around Palmyra but also contributes to the lack of understanding of the central Pacific region as a whole. Specifically, an understanding of the habitat requirements for this population may impact how future Palmyra management plans are developed. Gathering information on stage-specific habitat and resource requirements for the Palmyra Atoll green turtle population will result in more effective and comprehensive conservation measures.

Stable isotope analyses may facilitate an understanding of the spatial and temporal variation in foraging habits. It may also help identify the optimal foraging habitat at Palmyra Atoll which is required for effective sea turtle conservation (Hamann et al. 2010). Analysis of recently assimilated isotopes found in newly developed scute tissue (hereafter referred to as "younger scute tissue") of Palmyra Atoll green turtles can provide information on the latest feeding habits of all stage classes. Isotopic ratios in younger tissues can allow for inferences on dietary composition of consumers and allow for interpretation of stage-specific foraging habits.

Green turtles are considered the only herbivorous sea turtle species, although it is possible that they are facultative herbivores (Hatase et al. 2006), consuming animal materials when it is necessary to maximize growth rate and reproductive output. An indication of consumption of animal materials may be an enriched $\delta^{15}N$ ratio in adults, compared to juveniles

(Hatase et al. 2006). The current study aims to establish if this Palmyra Atoll green turtle population follows the ontogentic shift typical of those turtles foraging on seagrasses in the Caribbean (Reich et al. 2007), or if this population exhibits more variable foraging characteristics as seen in many other populations around the globe (Seminoff et al. 2002b, Amorocho & Reina 2007). If the Palmyra Atoll green turtles follow the "typical" ontogenetic shift we expect to see enriched nitrogen values in lower stage classes with a decreasing isotopic enrichment with turtle age.

METHODS

Study Site

This study took place at Palmyra Atoll National Wildlife Refuge (5.525554°N, 162.045905°W) found in the Line Islands of the central Pacific Ocean (Fig 3.1), approximately 1000 miles south of the Hawaiian archipelago. The atoll outer reef ecosystems have remained in a relatively pristine state while the lagoons were heavily modified during World War II. In 2001 Palmyra was purchased by the Nature Conservancy and declared a National Wildlife Refuge (Braun et al. 2008). It is currently uninhabited, with the exception of limited short-term visits by scientific research and U.S. Fish & Wildlife Service (USFWS) refuge management teams.

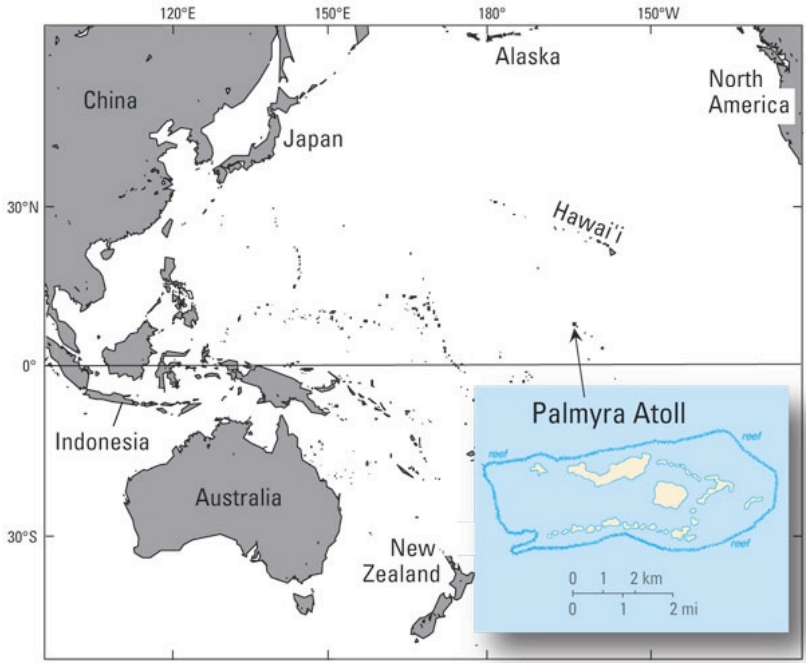

Fig. 3.1. Map of the location of the Palmyra Atoll National Wildlife Refuge in the central Pacific (USGS 2011)

The atoll is made up of a series of islands and connected islets with deep lagoons in the interior and shallow reef flats surrounding the landmass (Fig 3.2); turtles are frequently observed in both areas. Many reef structures also exhibit differences in relative coral communities (Williams et al. 2008) providing a variety of habitats for sea turtle foraging. High levels of coral and crustose coralline algae (reef building organisms) dominate the forereef areas surrounding Palmyra Atoll (Braun et al. 2008). The shallow water reef flats and sandy shallow water lagoons are primarily composed of turf and macroalgae and higher densities of sea turtles are observed utilizing this habitat (McFadden et al. 2010).

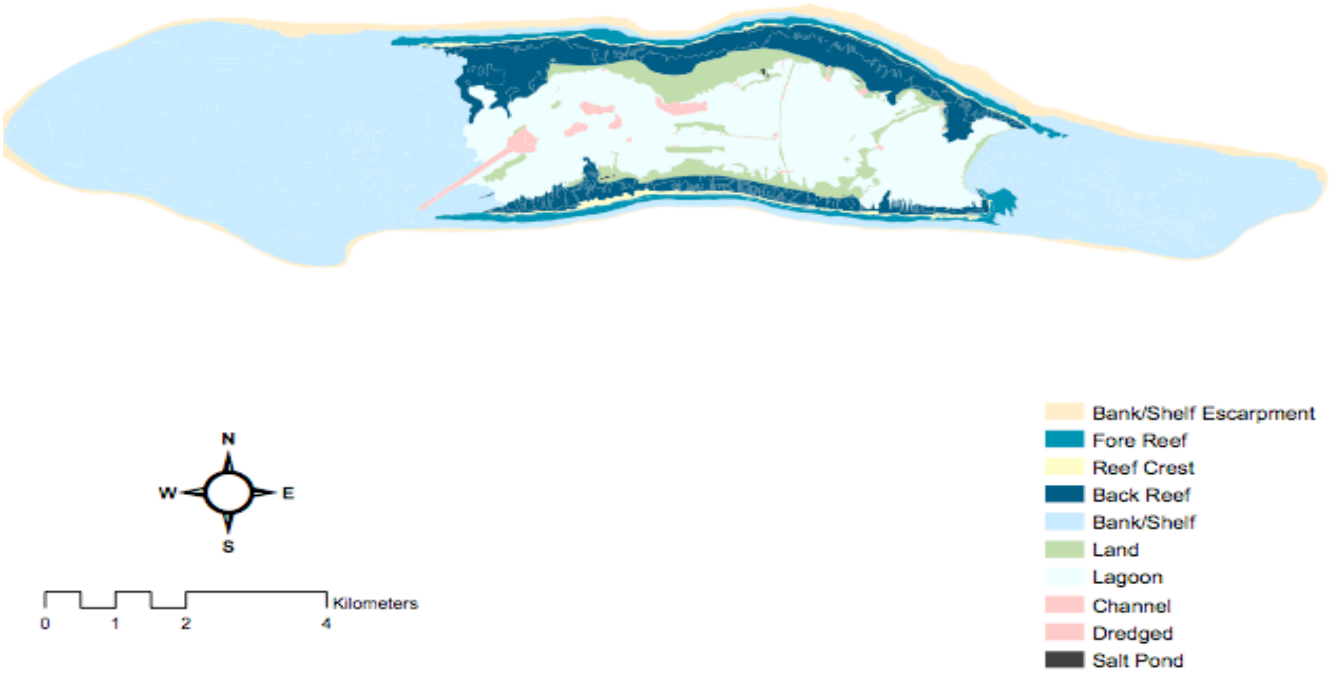

Fig. 3.2. Map of Palmyra Atoll habitat types. Created in ESRI ArcMAP 9.3.1.3000; data collected from the National Oceanic and Atmospheric Administration's (NOAA) Center for Coastal Monitoring and Assessment (CCMA) (Battista 2010)

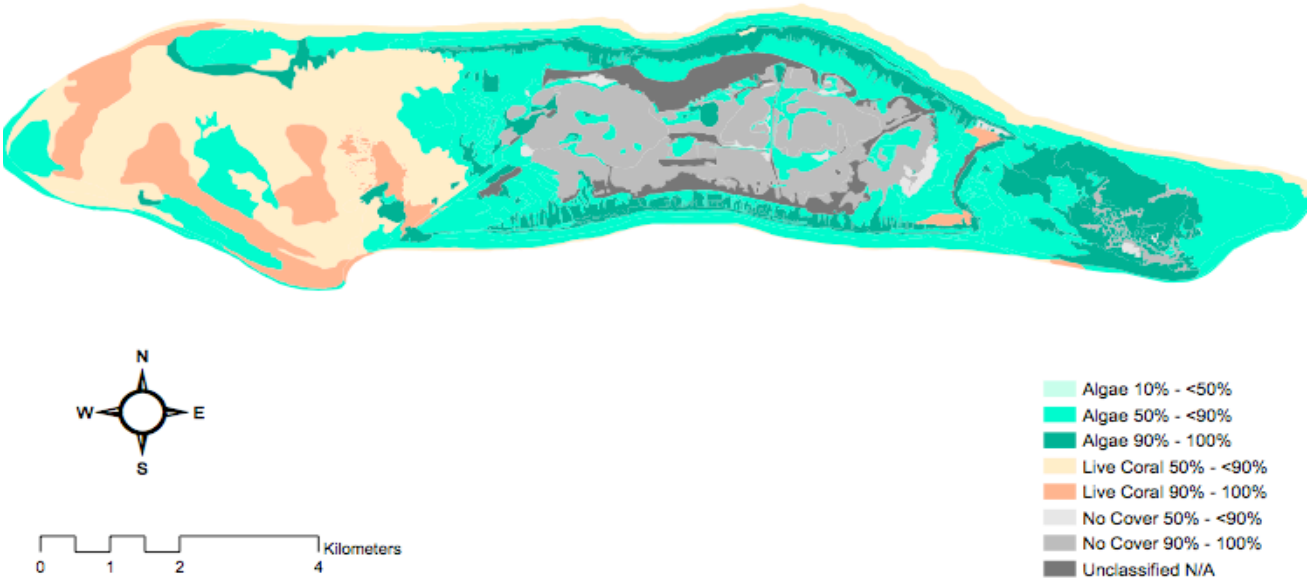

Fig. 3.3. Map of Palmyra Atoll bottom cover. Color shading denotes percentage of cover (green for algae, yellow/orange for coral and grey for no cover or unclassified cover). Created in ESRI ArcMAP 9.3.1.3000; data collected from NOAA's CCMA (Battista 2010)

Sample Collection and Preparation

Live-captured green turtles were sampled from four atoll areas: the northern reef flats, eastern flats and lagoon (hereinafter referred to as eastern lagoon), southern reef flats and western flats and lagoon (hereinafter referred to as western lagoon). These sampling areas were selected because they represent variable habitat characteristics. Animals were captured using three techniques: hand-capture techniques, standard turtle rodeo techniques (Limpus & Reed 1985) or using tangle or scoop nets. Sampling took place in the summer season over three consecutive years (2008-2010).

Scute Samples

After cleaning the carapace with ethanol, a 3mm (2008) or 5 mm (2009/2010) sterile biopsy sample was removed from each captured individual. Following Reich et al. (2007) we collected anterior scute samples (representing younger tissue) from either the L2/L3 or marginal scutes. Scute samples were stored dry at room temperature until analysis (Appendix B).

Body measurements, including curved carapace length (CCL), and tail length (TL) were taken using a flexible measuring tape (cm); straight carapace length (SCL) was measured using rigid metal calipers (cm). A body condition index (BCI) was calculated using Fulton's K equation (condition index = mass/SCL^3 x 10^4) (Bolger & Connolly 1989, Bjorndal et al. 2000, Labrada-Martagon et al. 2010). In order to provide context for the BCI scores, the mean and range of BCIs for turtles from Palmyra were calculated and compared to body condition categorization criteria developed for green turtles in Queensland, Australia by Flint et al. (2009). Animals with BCIs over 1.20 were considered to be in "very good" condition, those with BCIs between 1.11 – 1.19 were classified as in "good" condition, those between 1.00 – 1.10 were

considered “average, and those under 1.00 were considered to be in “poor” condition. Stage classes were determined using CCL as a proxy for age and were categorically coded as juvenile (≥ 64.9 cm CCL), sub-adult (65.0 – 84.9 cm CCL) or adult (85.0 ≤ cm CCL) (Chaloupka & Limpus 2005).

Scute samples were processed in the Ecology, Evolution and Environmental Biology Laboratory at Columbia University prior to stable isotope analysis at Boston University Stable Isotope Lab (BUSIL). Prior to preparation samples were dried at 60°C in glass vessels or on aluminum foil for at least twenty-four hours. Because of the small size of scute biopsies a modified methanol/chloroform (2:1) extraction protocol from Bligh & Dyer (1959) was performed on whole scutes; ground scute was not lipid extracted due to their prohibitively small sample sizes. Samples were then dried at 60°C for an additional twenty-four hours. Each scute sample was then stored in a glass vial at room temperate until weighing.

The most recently deposited scute tissue obtained from the samples is found along the ventral side of the scute, closest to the animal. This sample was used to compare recently incorporated dietary items across stage classes. A Merchantek micromill system or a Sherline Model 2010 Carbide Endmill (Cat # 970.557L/Model #: 970.557B) outfitted with a 1.16" Endmill Drill Bit (Cat # 402-0625) were used to remove a 50 μm layer from the ventral side of each sample. Prior to layer removal each scute sample was glued with the dorsal side (outermost layer) attached to the slide allowing for removal of the most recently deposited tissue layer for analysis. Each layer was removed by grinding down to a preset depth; the resulting tissue powder was collected by a scalpel and transferred into tin capsules. Each tin capsule contained one tissue layer. The tin capsules were then sealed (Costech Analysis) and weighed using a Mettler Toledo

XS105 Dual Range Balance or Mettler Toledo XP6 Microbalance. A minimum of 1 mg of ground scute is needed for each C^{13} or N^{15} analysis sample.

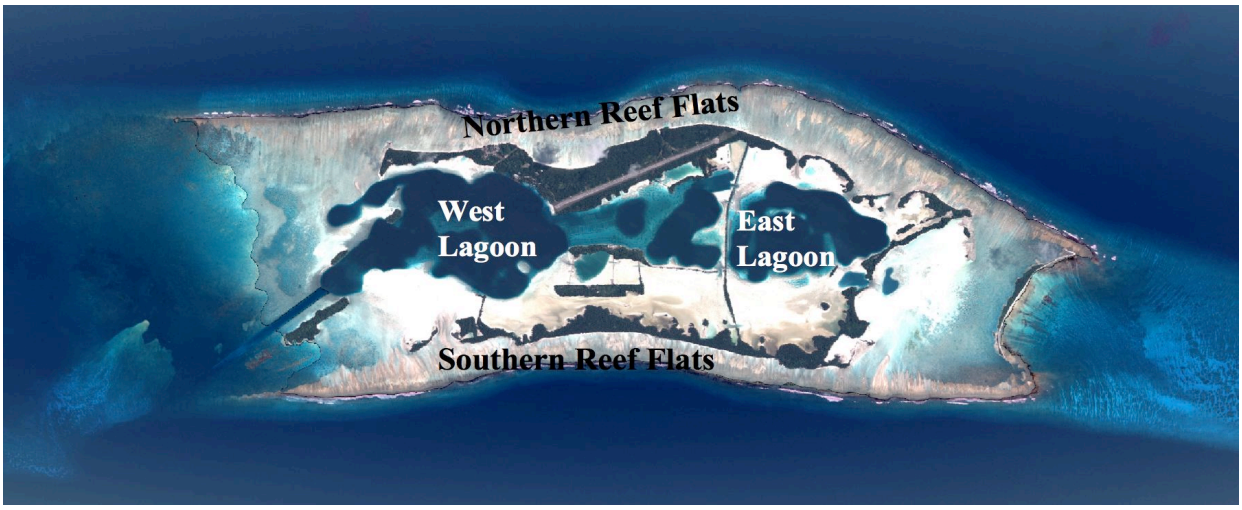

Fig. 3.4. Geographical areas of capture, northern and southern reef flats (in black) and west and east lagoons (in white) used for spatial analysis (NOAA IKONOS 1 meter multispectral image 2001) at Palmyra Atoll

Prey Samples

Prey samples were opportunistically collected in the areas where turtles were captured. Samples were taken from macroalgae, turf algae, crustose coralline algae and invertebrates (Appendix D). Mean carbon and nitrogen values were determined using both actual isotopic analyses of algae from Palmyra Atoll and supplemented with isotopic concentrations from the literature when sample sizes were small (Appendix D, F).

Prey samples were put in a plant press and dried, and then stored in a paper envelope until analysis. Samples rooted to coral had any remaining substrate carefully removed prior to analysis. Samples were dried at 60°C in paper envelopes and ground to a fine powder using a mortar and pestle. Lipids were then removed following the same modified methanol/chloroform

(2:1) extraction technique (Bligh & Dyer 1959) used on scute samples. After lipid removal, the samples were dried and weighed into tin capsules with 2 mg per capsule the amount of substrate necessary for stable isotope analysis. One to two drops of 10% hydrochloric acid (HCL) were applied to the ground sample in each tin capsule to decalcify samples and samples were dried at 60°C for 24 hours.

Stable Isotope Analysis

Continuous flow measurements of C^{13} and N^{15} isotopes were done using a GVI Isotope ratio mass spectrometer and Eurovector elemental analyzer at Boston University Stable Isotope Laboratory (BUSIL). Each sample was flash combusted in the Eurovector CN analyzer after which the combustion products were separated chromatographically and introduced into the GVI Isotope ratio mass spectrometer. Laboratory standards were run after every 15 samples; any anomalous results were rerun.

Stable isotope abundances were reported in δX values (X representing either C^{13} or N^{15}). The R_{sample} and $R_{standard}$ notation represents the ratio of heavy isotope to light isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$. Delta X values are given as parts per thousand (‰) differences from the international standards, usually referred to as per mil, and are calculated by the following formula:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

The results were compared to secondary gas standards calibrated to international standards obtained from the National Bureau of Standards (BUSIL); $R_{standard}$ for ^{13}C was PeeDee belemnite and $R_{standard}$ for 15N was atmospheric nitrogen. Laboratory standards were run after every 15 samples; any anomalous results were rerun. For well-ground organic material an

external precision of 0.2 per mil is required, however, 0.1 per mil is typical for both carbon and nitrogen (BUSIL).

IsoSource

Isotopic ratios of the consumer and consumer food items were compared to assess relative contribution of each food item to overall diet using isotopic mixing models (Phillips & Gregg 2001). Mean $\delta^{13}C$ and $\delta^{15}N$ values were determined for each food source group (e.g. sponge, red algae, green algae and brown algae). The mixing model requires that isotopic values of all prey be significantly different from each other. Accurate interpretation of mixing model results depends upon the accuracy of the diet to tissue discrimination factors used.

Currently discrimination factors for green turtle scute tissue do not exist (Appendix C); however, discrimination factors for green turtle soft tissue (Seminoff et al. 2006) and juvenile loggerhead (9.0 – 13.14 cm straight carapace length (SCL)) scute tissue have been determined (Reich et al. 2008). Green turtle soft tissue diet-tissue discrimination factors range from 0.22 to 2.92‰ nitrogen enrichment and 0 to 1 ‰ carbon enrichment (Seminoff et al. 2006). Loggerhead scute tissues are enriched in carbon by 1.77 (± 0.58) and are depleted in nitrogen by – 0.64 (± 0.09) compared to their prey (Reich et al. 2008).

To compensate for these limitations iterations of IsoSource were run using both green turtle soft tissue - diet discrimination factors (Seminoff et al. 2006) and loggerhead scute tissue - diet discrimination (Reich et al. 2008). Iterations included both the Palmyra prey data signatures and combined (prey and literature) isotopic signature data.

Statistical Analysis

The data were examined for normality and homoscedasticity using a Shapiro-Wilk test and Levene test, respectively. Assumptions of normality and homoscedasticity were not met in all cases and therefore nonparametric analyses were used. The Kruskal-Wallis analysis of variance test was used to examine the isotopic signatures between sampling locations as well as the distribution of CCL and mass between the four sampling regions. A Spearman's Rank Order correlation was used to examine relationships between body measurements and isotopic signatures. Statistical analyses were carried out using PASW Statistics 18.0.3 (2010) and/or JMP 8.0.2 (2009). A significance level of 0.05 and confidence interval of 95 % were considered statistically significant. Results are reported as mean ± standard deviation (SD).

RESULTS

A total of 75 samples were analyzed to describe the foraging behavior of the Palmyra Atoll green turtles (Fig. 3.5). Body measurements ranged from 44.5 - 113.6 cm curved carapace length (CCL) (mean ± SD = 73.55 ± 15.56 cm) with weights between 10.2 - 146.3 kg (52.05 ± 30.49 kg). Based on CCL categorizations previously described, adult individuals comprised 28% (n = 21) of the samples while subadults comprised 37.3% (n = 28) and 34.7 % (n = 26) of the individuals were classified as juveniles (Fig. 3.5). Body condition did not significantly differ between stage classes or sampling locations (Kruskal-Wallis, $p > 0.05$).

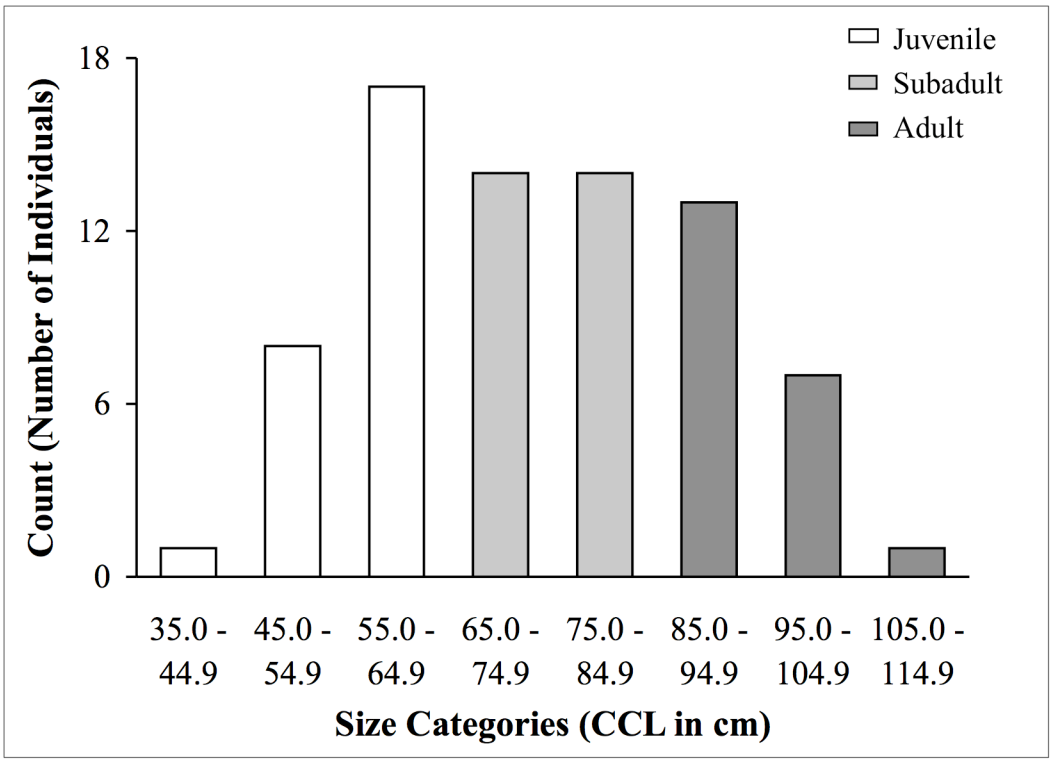

Fig. 3.5. Number of Palmyra Atoll green turtles sampled, per size category, from 2008 to 2010 and used in this study. Size categories are based on curved carapace length (CCL) measured in centimeters

Morphometric measurements (*i.e.* CCL, SCL and TL) were all significantly correlated ($p \leq 0.005$); therefore in order to avoid unnecessary issues with covariance, CCL was chosen to examine the effect of turtle size on isotopic signatures. There was no correlation between $\delta^{13}C$ values and CCL (Spearman's correlation, $R = 0.049$, $n = 75$, $p = 0.679$), however there was a weak, but significant correlation between $\delta^{15}N$ and CCL (Spearman's Correlation, $R = 0.227$, $n = 75$, $p = 0.052$). There was no significant difference in $\delta^{13}C$ isotopic signatures across the sampled stage classes (Kruskal-Wallis test, $p = 0.808$) (Fig. 3.6a). However, differences in $\delta^{15}N$ signatures did exist (Kruskal-Wallis Test, $p = 0.001$), with adults significantly enriched

compared to subadults (Mann-Whitney U, $p < 0.001$), but not differing from juveniles (Mann-Whitney U, $p > 0.05$) (Fig. 3.6b, Table 3.1). Juveniles also were significantly enriched in $\delta^{15}N$ signatures when compared to subadults ($p = 0.018$).

Table 3.1. Mean stage-specific morphometric measurements (± SD) and mean scute tissue isotopic signatures (± SD) from different stage classes of green turtles sampled from Palmyra Atoll between 2008 and 2010. Morphometric measurements include curved carapace length (CCL), straight carapace length (SCL) and weight (kg). Sea turtle stage classes were defined as juvenile (≥ 64.9 cm CCL), sub-adult (65.0 – 84.9 cm CCL) or adult (85.0 ≤ cm CCL)

	$\bar{X}$ **CCL (cm)**	$\bar{X}$ **SCL (cm)**	$\bar{X}$ **Weight (kg)**	$\bar{X}$ **$\delta^{13}C$ (‰)**	$\bar{X}$ **$\delta^{15}N$ (‰)**
Juveniles (n = 26)	57.05 (5.53)	53.98 (5.59)	23.94 (10.99)	-16.18 (2.38)	11.37 (1.64)
Subadults (n = 28)	74.07 (5.55)	69.40 (5.40)	47.65 (11.88)	-15.67 (1.42)	11.04 (1.36)
Adults (n = 21)	93.38 (7.13)	88.10 (6.63)	92.83 (17.40)	-15.71 (1.81)	12.41 (1.40)

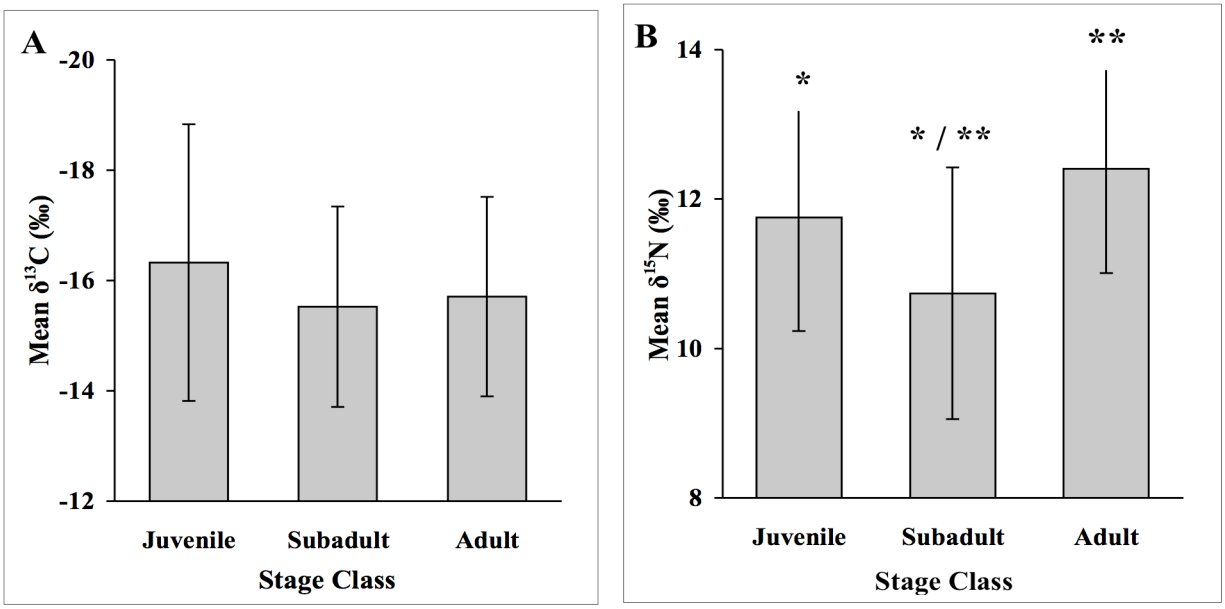

Fig. 3.6. Mean (A) carbon and (B) nitrogen isotopic values of Palmyra Atoll green turtle scute tissue sampled between 2008 and 2010. Sea turtle stage classes were defined as juvenile (≥ 64.9 cm CCL), subadult (65.0 – 84.9 cm CCL) or adult (85.0 ≤ cm CCL). Error bars ± 1 standard deviation. Significance was assessed using a Kruskal-Wallis test and is denoted by a */**. Stage classes with the * are significantly different (juvenile and subadult) and stage classes with ** are significantly different (subadult and adult)

Prey Isotopic Values Description

Prey items were grouped into five categories: chlorophyta (n = 22), phaeophyta (n = 3), rhodophyta (n = 12), blue-green algae (n = 3) and invertebrates (n = 6); invertebrates included sponges and tunicates. Mean isotopic values for prey group ranged from -9.54‰ to -14.55‰ for $\delta^{13}C$ and 7.15‰ to 9.04‰ for $\delta^{15}N$ (Fig. 3.7). When compared to mean values taken from the literature (Table 3.2) (see Appendix F for literature values) Palmyra Atoll macroalgal carbon and nitrogen isotopic values were on average more enriched.

Table 3.2. Prey isotopic values of samples collected from Palmyra and those obtained from the literature (Appendix F). Palmyra samples were collected in the summer season of 2008, 2009 and 2010. Prey groups included green algae (chlorophyta), brown algae (phaeophyta), red algae (rhodophyta) and invertebrates. Mean ±SD scute isotope signature for all sampled green turtles at Palmyra Atoll also included for comparison

Turtle/Prey	**$\delta^{13}C$ (‰)**		**$\delta^{15}N$ (‰)**	
Categorization	**Mean**	**±SD**	**Mean**	**±SD**
Values from the Literature				
Chlorophyta	-17.33	6.70	4.89	2.15
Phaeophyta	-11.88	5.20	5.99	3.35
Rhodophyta	-20.79	4.86	6.40	0.96
Palmyra Values				
Green Turtle (n = 75)	-15.77	2.01	11.46	1.71
Blue-green algae (n = 3)	-14.55	4.30	8.07	1.10
Chlorophyta (n = 22)	-12.18	4.51	7.15	5.27
Phaeophyta (n = 3)	-9.64	2.49	9.04	0.58
Rhodophyta (n = 12)	-13.20	3.53	7.17	1.28
Invertebrate (n = 6)	-11.69	5.02	9.52	0.29

Sampling Area

A Kruskal-Wallis test indicated that sea turtles captured in each of the four sampling areas exhibited statistically different mean CCL ($F = 8.937$, $df = 3$, $p = 0.030$) and weight ($F =$

10.979, df = 3, p = 0.012) measurements. The west lagoon had the largest mean turtle CCL (± SD) of all the regions at 81.61 ± 16.86 cm (range of 44.5 to 102.4 cm). Green turtles sampled from the east lagoon showed a bimodal size distribution that significantly varied from those turtles sampled in the western lagoon ($p < 0.0114$) and ranged in size from 52.4 to 113.6 cm CCL (mean ± SD = 68.51 ± 17.96 cm). The southern and northern reef flats had similarly sized turtles (mean ± SD of southern = 71.67 ± 12.64 and of northern = 72.46 ± 12.62); subadults far outnumbered other size classes captured in these regions.

Stage-class composition also differed between the four sampling areas (Fig. 3.7, Table 3.3). The majority of juveniles sampled were from the eastern lagoon while adults were most common in the west lagoon (Pearsons $X^2 = 18.562$, df = 6, p = 0.005). Adult and juvenile nitrogen values did not differ between sampling areas; however, subadult nitrogen values did differ between sampling areas ($X^2 = 3.909$, df = 3, p = 0.0093). Student's t-tests revealed that subadults sampled from the southern reef flats had significantly depleted nitrogen values compared to northern reef flat subadults (p = 0.0085).

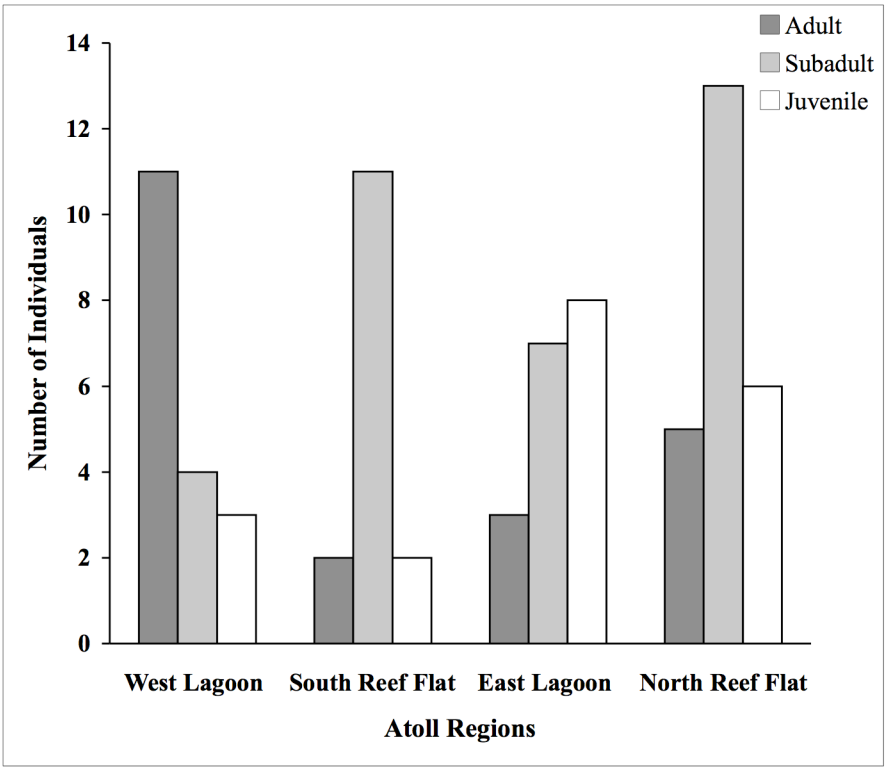

Fig. 3.7. Number of green turtles sampled per region at Palmyra Atoll from 2008 - 2010. Stage class designations were defined as juvenile (≥ 64.9 cm CCL), subadult (65.0 – 84.9 cm CCL) and adult (85.0 ≤ cm CCL)

Table 3.3. Number of Palmyra Atoll green turtle individuals from each stage class sampled per atoll region from 2008 to 2010

	West Lagoon	South Reef Flat	East Lagoon	North Reef Flat
Adult	11	2	3	5
Subadult	4	11	7	13
Juvenile	3	2	8	6

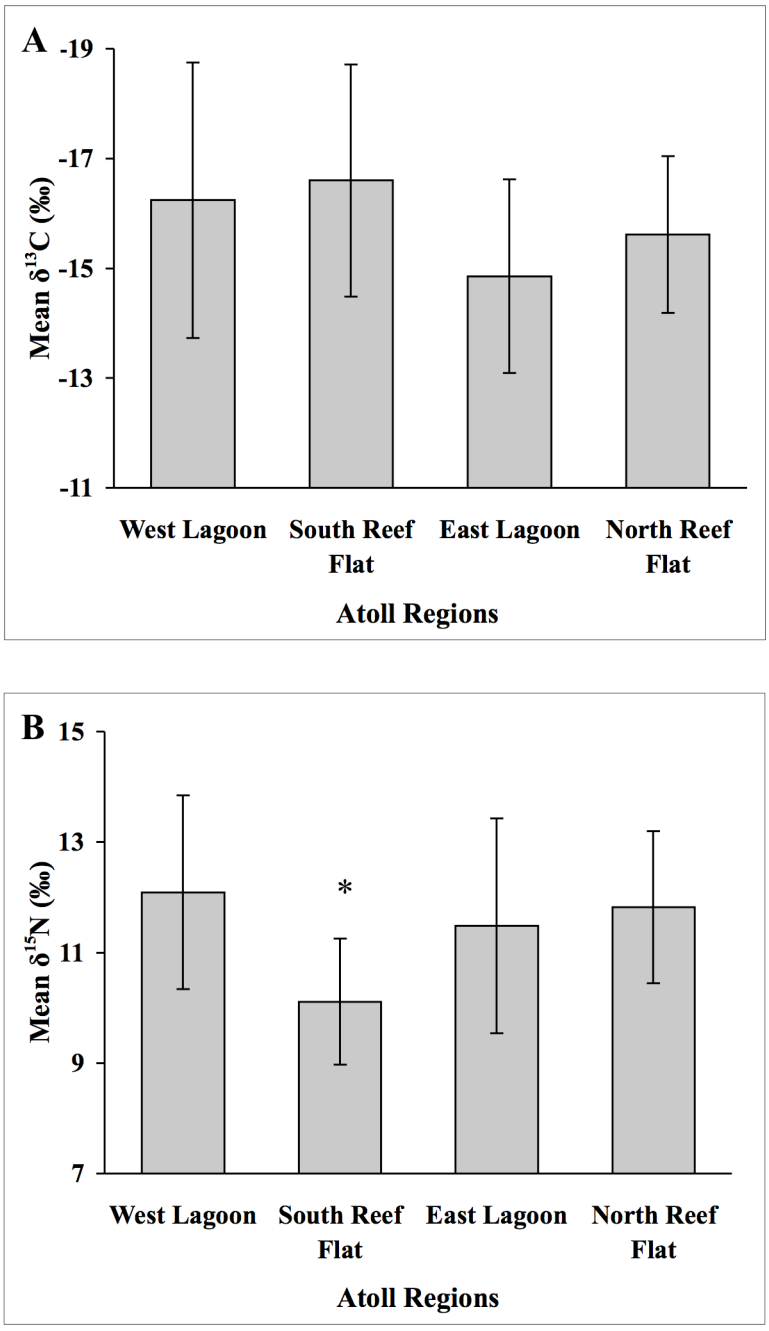

Fig. 3.8. Mean (A) carbon and (B) nitrogen isotopic values for green turtles captured in each atoll region at Palmyra Atoll from 2008 to 2010. Error bars ± 1 standard deviation. Significance was determined using a Kruskal- Wallis test and is denoted by a *

Mean nitrogen signatures for captured sea turtles differed between the four sampling areas (Kruskal-Wallis, $p = 0.001$). Mann-Whitney U tests showed that individuals sampled from the southern reef flat had significantly lower $\delta^{15}N$ signatures than those sampled from the west lagoon, east lagoon and northern reef flat areas ($p \leq 0.005$) (Fig. 3.8b). No differences in mean $\delta^{13}C$ values were found between regions (Kruskal-Wallis, $p = 0.146$) (Fig. 3.8a).

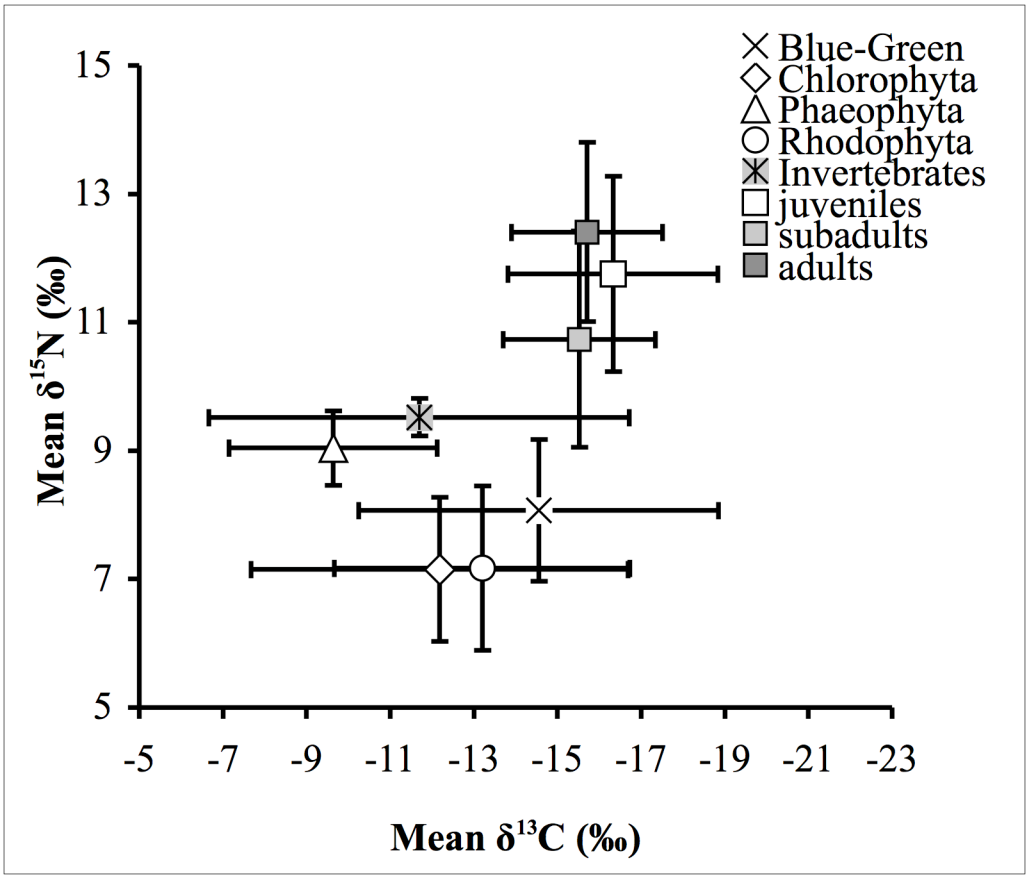

Fig. 3.9. Mean $\delta^{13}C$ and $\delta^{15}N$ values for prey groupings and stage classes for green turtles at Palmyra Atoll sampled between 2008 and 2010. Stage classes were categorically coded based on curved carapace length (CCL) and defined as juvenile ($\geq$ 64.9 CCL), subadult (65.0 – 84.9 CCL) and adult (85.0 $\leq$ CCL). X and Y-error bars are one standard deviation

When comparing isotopic signature of prey versus consumer (turtle), each green turtle was more enriched in nitrogen than the prey groupings (Fig 3.9). In contrast, the green turtles exhibit depleted carbon isotopic signatures compared to prey groups. Phaeophyta is more enriched in nitrogen then chlorophyta and rhodophyta. Phaeophyta falls within one standard deviation of both blue-green algae and invertebrates, being only slightly depleted compared to invertebrates. Chlorophyta and rhodophyta exhibit the most depleted in nitrogen isotopic values.

Analysis of the proportional contribution of the prey groups to the diet of each stage classes using a mixing model, IsoSource, had unfavorable results. In fact, no result could be produced from our isotopic signatures (both Palmyra and combined prey data) using either green turtle soft tissue - diet discrimination factors (Seminoff et al. 2006) or loggerhead scute tissue - diet discrimination (Reich et al. 2008). This is likely related to the fact that all prey groups fall within one standard deviation for carbon of each other (Fig 3.9). Additionally, while a Kruskal-Wallis test identified significant differences in $\delta^{15}N$ signatures between stage classes, subadults were depleted compared to both adults and juveniles, stage classes also fall within one standard deviation of each other in $\delta^{15}N$ (Fig 3.9).

DISCUSSION

Spatial Variation of Isotopic Values

This study indicates that individuals from the southern reef flat have significantly depleted nitrogen levels and slightly depleted carbon values compared to the other three regions (north flats, east and west lagoons). The nitrogen values of adult and juvenile turtles do not differ significantly between the sampling areas while southern reef subadults showed significant nitrogen depletion. Subadult samples, therefore, are likely driving the nitrogen depletion seen

when analyzing the overall trends in sea turtle sampled from the southern reef flat region. The same patterns of nitrogen and carbon depletion were not found in subadult turtles along the northern reef flats. One possible explanation is that the very low sample size of both juvenile (n = 2) and adult sea turtles (n = 2) in the southern reef region are not sufficient to adequately characterize the nitrogen signatures of different stage classes. Alternatively, it is also possible that there is variation in the availability of disparate dietary items between the sampling areas. It is unknown whether the source of this isotopic difference between sampling regions is caused by the stage class composition (suggesting stage-specific dietary preferences) or whether the areas provide prey items differing in nitrogen content. A more thorough sampling of prey items from each turtle sampling area may provide insight on this discrepancy. Additionally, a focus on prey sampling in forereef habitat adjacent to shallow water reefs may give insight into prey availability between habitat types.

Stage-class composition also differs between the nearshore reefs and lagoon areas, with the majority of subadults sampled in the reef flats. Algal reef surveys at Palmyra indicate increased algal species richness along the junction between the reef flats and forereef break where higher levels of wave action likely provide nutrients for higher algal species diversity (McFadden et al. 2010). The northern and southern reef flats, which are presumably more productive areas due to their access of nutrient flow and higher oxygenation caused by wave action (Leigh et al. 1987), are where most of the subadults were sampled.

Fewer juveniles and adults were captured from the southern reef flat than any other sampling area while the largest number of juvenile captures was from the eastern lagoon. The eastern lagoon area likely provides a more sheltered habitat, often a habitat preferred by juvenile

green turtles (Bresette et al. 2010, Carrion-Cortez et al. 2010). This finding may suggest stage-specific spatial habitat preferences to the Palmyra Atoll foraging habitat.

Adult green turtles were the most commonly sampled stage class in the western lagoon area; the rate of adult capture among the four regions was also highest here. Increased availability of varying types of foraging substrate (*i.e.* some shallow water reef rubble, some coral heads, lagoons) in the western lagoon may result in turtles in this sampling area consuming more animal products in attempts to decrease foraging effort (Bjorndal et al. 1991). It is unknown whether adult turtles choose enriched nitrogen prey in the western lagoon because of availability or preference. The existence of a dredged channel in the western lagoon, which allows for large predatory sharks to have access to this region, could be another possible explanation for the lack of juveniles sampled in the western lagoon. Often juveniles are found in more sheltered habitats and avoid deeper channels due to predation risk (e.g. Bresette et al. 2010).

Stage-class sample sizes were low in many of the sampling regions. Low sample sizes may lead to inaccurate estimation of stage composition. Therefore the stage-class composition should be regarded as a general description rather than a statistical fact.

Stage-Specific Isotopic Signatures

Juveniles have the most depleted carbon values of the three stage classes. This may be an indication of their recent shift from a pelagic environment if prey items consumed were primarily planktonic. Green turtles recruit to neritic areas in the Pacific Ocean at approximately 35 - 40 cm SCL (Hirth 1997, Seminoff et al. 2003). However, juvenile carbon signatures are not significantly different from the other stage classes which all have carbon values between -15.5‰

and -16.5‰. This carbon signature range is indicative of a diet predominately composed of macroalgae, which is a $\delta^{13}C$ depleted food source compared to seagrass (Arthur et al. 2008).

Subadults are depleted in nitrogen compared to both juveniles and adults. Adults have the most enriched nitrogen values suggesting that they are possibly feeding at a higher trophic level than both subadults and juveniles (Minagawa & Wada 1984, Aruthr et al. 2008). Ingested proteins are the main source of ^{15}N while carbon comes from proteins, lipids and carbohydrates. Therefore, protein turnover and dietary protein quantity and quality may all affect nitrogen isotopic incorporation (Robbins et al. 2005, Martinez del Rio et al. 2009, Robbins et al. 2010).

It was to be expected that juveniles would have enriched nitrogen values as they have recently recruited from an environment where they are thought to forage on animal and plant items. However, the existence of enriched values in adults was unexpected and may be indicative that at least some individuals utilize an omnivorous diet. There have been other populations that exhibit facultative herbivory (Hatase et al. 2006). It is possible that Palmyra Atoll turtles supplement their herbivorous diet with higher trophic level dietary items. All potential prey samples were not collected in this study (*e.g.* planktonic) therefore this cannot be substantiated at this time. The variation in stage-class nitrogen isotopes suggests that this population contains individuals that likely utilize a more variable and complex dietary repertoire including omnivorous prey items.

When compared to a population of green turtles sampled from Australia (Arthur et al. 2008) the Palmyra Atoll population exhibits more depleted carbon signatures and more enriched nitrogen signatures for all stage classes. While comparison between regions can potentially be problematic because of different baseline environmental isotopic values and potentially different prey sources, some inferences may be made. For example, it is interesting to note that in

Australia there is a relatively large difference in carbon signatures of immature juvenile turtles compared with subadult and adult turtles (Arthur et al. 2008). The Australian subadults exhibit enriched carbon values compared to adult green turtles (Arthur et al. 2008) a similarity with our Palmyra Atoll turtles. It is possible that the adult turtles were predominantly foraging in areas with high macroalgal concentrations which would lead to depleted carbon signatures compare to turtles feeding on seagrasses (Arthur et al. 2008).

Differences between the Australian population isotopic signatures (Arthur et al. 2008) and Palmyra green turtle isotopic signatures may be the result of one or multiple interacting factors. It could be that baseline environmental conditions are causing differing baseline isotopic signatures between the two regions (Pajuelo et al. 2012). It is possible, if the turtle has recently recruited to Palmyra, that the carbon signature of the neritic area is not yet incorporated into the turtle's carapace. It may also be possible that turtles exhibit slight variation in carbon signatures once in the neritic area due to individual differences in preferential routing of dietary components (Schwarcz 1991).

Tissue - Diet Discrimination

Body condition (e.g. Hobson et al. 1993), body mass (e.g. Carleton & Martinz del Rio 2005), growth rate (e.g. Reich et al. 2008), tissue type (Tieszen et al. 1983, Reich et al. 2008) and species (Tieszen et al. 1983) may be influencing isotope assimilation and fractionation. Therefore using reported discrimination factors to interpret foraging habits should be done while maintaining a certain degree of skepticism. This complexity of factors may be one of the reasons I was not successful in using mixing models to predict the proportion of different prey items in the diet of sea turtles. Stable carbon isotopic ratios reported for green turtle soft tissues are

enriched (via discrimination) by approximately 0 - 1‰ greater than that of their prey (Seminoff et al. 2006) while nitrogen may be enriched 0.22 – 2.92 ‰ (Seminoff et al. 2006) per trophic level. It is possible that the sea turtles at Palmyra have varying physiological challenges that impact their assimilation of resources into tissue. This would result in different discrimination factors than those found in the literature. It is important to note that discrimination factors reported by Seminoff et al. (2006) were for whole blood, red blood cells, blood plasma and epidermis, all of which have more rapid turnover rates than sea turtle scute tissue. The carbon diet-tissue discrimination of juvenile loggerhead scute tissue, 1.77‰ (Reich et al. 2008), is higher than that reported for the soft tissues of green turtles (Appendix C). However, the nitrogen diet-tissue discrimination of juvenile loggerhead scute tissue, - 0.64 ‰ (Reich et al. 2008) suggesting a depleted turtle value when compared to diet.

Discrimination factors for loggerhead turtle scute tissue reported by Reich et al. (2008) may be more appropriately applied to Palmyra Atoll green turtles even though they are different species, as the tissue studied is the same. As of yet, there have been no reported values for the discrimination factors of green turtle scute tissue. In contrast to reported discrimination factors for green turtles (Seminoff et al. 2006) and loggerhead turtles (Reich et al. 2008) Palmyra Atoll green turtles exhibit depleted carbon signatures when compared to prey sampled from around the atoll; however, the prey $\delta^{13}C$ values are more enriched than expected for macroalgal species (Arthur et al. 2008). Palmyra green turtles exhibit enriched nitrogen values compared to the sampled dietary items. Palmyra nitrogen tissue-diet differences are similar to values reported for green turtle soft tissue (Seminoff et al. 2006). Additional laboratory study with controlled feeding trials is necessary to refine dietary inferences using green turtle scute tissue.

One or multiple factors may be leading to the lack of producible results from the mixing model using either green turtle soft tissue - diet discrimination factors (Seminoff et al. 2006) or loggerhead scute tissue -diet discrimination (Reich et al. 2008). Dietary isotopes are often allocated to tissues differently between individuals and species in a process termed "isotopic routing" (Schwarcz 1991); this can lead to different isotopic signatures. Differences in growth rate, age, and dietary components may also likely exist between the studied populations (Seminoff et al 2006, Reich et al. 2008, this study), which will affect tissue-diet discrimination.

In addition, all potential Palmyra prey items were likely not collected, as there are 19 previously reported sponge species (Knapp & Bell 2010) and 19 macroalgal species (Bruan et al. 2008). Pelagic phytoplankton, zooplankton and corals were not sampled. Therefore the isotopic signatures of all potential prey items are not represented. Additionally, Palmyra prey $\delta^{13}C$ values are more enriched than expected (Arthur et al. 2008) suggesting that the collection or pre-analysis methodologies may be flawed. Prey values collected from the literature, used to supplement Palmyra values, did not affect the results of IsoSource. The prey groups also have overlapping isotopic signatures (Fig 3.9) and are not significantly different from each other; which limits the utility for a mixing model. Incorporation of elemental concentrations (% C and % N) of the prey items into mixing models may resolve the lack of a significant difference in isotopic signatures of Palmyra prey items and is necessary when comparing diets composed of plant and animal matter (Phillips & Koch 2002, Pearson et al. 2003, Lemons et al. 2011).

Conservation Implications

Palmyra Atoll provides a complex environment with multiple foraging substrates for green turtles. The complexity of this environment is reflected in the complex dietary and

foraging habits of this green turtle population. The possible inclusion of animal products in the diet of older individuals suggests that the switch from omnivory to herbivory may in fact be facultative as has been seen in other populations (*e.g.* Hatase et al. 2006). This may be caused by the abundance of animal foraging substrate available for green turtle consumption.

The habitat diversity found at Palmyra Atoll may also lead to microhabitat selection as reflected in the variation in foraging characteristics exhibited by the stage-classes. While this study did not specifically examine habitat usage, some level of habitat preferences seemed identifiable among different stage-classes throughout the atoll's sampling regions. Understanding the variability in the foraging strategies within and between green turtle populations is extremely important, as green turtles spend the majority in of their lifetime in these habitats (Musick & Limpus 1997, Bolten 2003). Examination of individual amino acids instead of bulk isotopic data may allow for more accurate and detailed descriptions of dietary preferences and spatial distribution in this foraging population (Seminoff et al. 2012).

The need for better understanding of regional variation in foraging ecology of green turtles, due to geographically variable dietary characteristics, has been recognized in previous studies (e.g. Lopez-Mendilaharsu et al. 2005). With green turtle populations threatened by extinction globally, understanding the diversity of foraging strategies employed is extremely important as these characteristics are often linked to reproductive output and growth rates. The current study helps fill the gap in knowledge that currently exists on central Pacific green turtles outside of the well-documented populations such as the Hawaiian archipelago populations. The Palmyra Atoll foraging ground is likely an important area for green turtles and thus a better understanding of the foraging characteristics of turtles in this area will afford greater protection of habitats suitable for the various stages and foraging characteristics.

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Appendix A

Appendix A. Posterior scute $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for green turtles sampled from Palmyra Atoll National Wildlife Refuge between 2008 and 2010. Turtles were sampled from four atoll regions: western lagoon, southern reef flats, eastern lagoon and northern reef flats. Stage classes sampled were categorically coded as juvenile (J, ≥ 64.9 cm CCL), subadult (SA, 65.0 – 84.9 cm CCL) or adult (A, 85.0 ≤ cm CCL). Morphological measurements taken include weight, curved carapace length (CCL), straight carapace length (SCL), tail length (TL). Body condition index (BCI) was calculated using Fulton's K equation (condition index = mass/SCL^3 x 10^4). In addition, the number of layers removed per sample is included

Year	Turtle ID	BCI	Wt (kg)	CCL (cm)	SCL Full (cm)	TL (cm)	$\delta^{13}C$ 1	$\delta^{13}C$ 2	$\delta^{13}C$ 3	$\delta^{13}C$ 4	$\delta^{13}C$ 5	$\delta^{13}C$ 6	$\delta^{13}C$ 7	$\delta^{13}C$ 8	$\delta^{13}C$ 9	$\delta^{13}C$ 10	# layers
2008	GD5	1.52	10.2	44.5	40.6	7.5	-18.82	-18.69	-19.09	-19.15	-19.68	-14.8	-19.8	-19.33	-20		9
	GE2	1.55	17	50.4	47.9	8	-19.38	-17.45	-15.69	-15.33	-15.59						5
	GD10	1.25	15.4	52.8	49.7	10.2	-13.3	-13.91	-13.91		-14.8	-15.13	-15.61	-15.76	-17.9		8
	GD1	1.18	23	60	57.9	9	-13.38	-16.34	-14.14	-14.71	-14.48	-15.44	-18.03	-20.87	-23.74		9
2009	GG1	1.46	11.8	45.5	43.2	6.2	-17.14	-16.26									2
	GH1	2.22	19.2	47	44.2	5	-20.03	-18.33	-18.76	-19.07	-19.64						5
	GG6	1.61	21.3	53.8	51	8.3	-13.87	-13.58	-13.52	-16.08							4
	GD24	1.37	20.2	56	52.8	8.9	-17.98	-18.44	-18.6	-18.66	-19.35						5
	GB15	1.32	23.4	58.5	56.2	10.3	-14.6	-14.45	-13.79	-14.29	-14.32	-14.7	-13.49	-12.97	-12.49	-11.96	10
	GF1	1.00	17.2	59	55.7	9.5	-17.47	-17.28	-16.86	-18.86	-16.9	-17.38	-17.8	-17.01			8
	GG7	1.43	25.2	59.3	56.1	9.3		-15.07	-15.23	-14.79	-15.93						4
	GD19	2.47	44	60	56.3	9.2	-13.73	-14.77	-15.33	-15.7	-16.97	-20.02					6
2010	GG15	1.59	18	52.4	48.4	6	-13.88	-14.2	-16.74								3
	GG30	1.36	18	53.8	50.9	8	-16.92	-16.47	-16.95	-22.36							4
	GB23	1.33	18	54.3	51.3	10	-13.68	-14.05	-13.91	-14.29	-14.31	-14.29					6
	GB22	1.25	20	56.8	54.3	9.1		-17.16	-17.69	-17.93	-18.81	-19.06					3
	GG31	1.42	24	58.5	55.3	10	-13.67	-13.43	-13.78	-13.92	-14.99	-15.91	-18.09				7
	GG22	1.30	24	60.1	57	10.2	-13.27	-14.06	-13.49	-13.11	-13.72						5

Year	Turtle ID	BCI	Wt (kg)	CCL (cm)	SCL Full (cm)	TL (cm)	$\delta^{15}N$ 1	$\delta^{15}N$ 2	$\delta^{15}N$ 3	$\delta^{15}N$ 4	$\delta^{15}N$ 5	$\delta^{15}N$ 6	$\delta^{15}N$ 7	$\delta^{15}N$ 8	$\delta^{15}N$ 9	$\delta^{15}N$ 10	# layers
2008	GD5	1.52	10.2	44.5	40.6	7.5	13.79	13.32	10.68	9.78	8.86	7.37	8.59	8.84	9.13		9
	GE2	1.55	17	50.4	47.9	8	14.13	14.44	13.34	13.89	13.38						5
	GD10	1.25	15.4	52.8	49.7	10.2	10.17	10.28	9.98		9.51	10.27	10.12	10.47	8.98		8
	GD1	1.18	23	60	57.9	9	10.56	10.23	10.52	10.72	10.91	10.8	9.13	7.42	4.93		9
2009	GG1	1.46	11.8	45.5	43.2	6.2	13.64	13.61									2
	GH1	2.22	19.2	47	44.2	5	14.25	10.99	15.84	15.11	14.18						5
	GG6	1.61	21.3	53.8	51	8.3	12.84	13.12	13.3	12.62							4
	GD24	1.37	20.2	56	52.8	8.9	8.98	8.75	8.95	9.21	8.55						5
	GB15	1.32	23.4	58.5	56.2	10.3	11.92	11.35	11.38	10.9	11.02	10.38	9.95	9.92	9.8	9.71	10
	GF1	1.00	17.2	59	55.7	9.5	11.7	12.06	13.07	11.76	11.76	12.5	12.92	12.61			8
	GG7	1.43	25.2	59.3	56.1	9.3		13.47	13.16	13.11	12.38						4
	GD19	2.47	44	60	56.3	9.2	9.52	9.58	9.67	9.84	9.7	7.88					6
2010	GG15	1.59	18	52.4	48.4	6	12.4	12	10.68								3
	GG30	1.36	18	53.8	50.9	8	13.63	14.19	14	7.41							4
	GB23	1.33	18	54.3	51.3	10	11.48	11.37	11.75	11.46	11.33	11.31					6
	GB22	1.25	20	56.8	54.3	9.1		10.15	8.33			8.49					3
	GG31	1.42	24	58.5	55.3	10	11.99	12.52	12.39	12.45	11.55	11.2	9.14				7
	GG22	1.30	24	60.1	57	10.2	11.44	11.92	12.17	11.92	10.58						5

Appendix B

Appendix B. Anterior scute $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for green turtles sampled from Palmyra Atoll National Wildlife Refuge between 2008 and 2010. Turtles were sampled from four atoll regions: western lagoon, southern reef flats, eastern lagoon and northern reef flats. Stage classes sampled were categorically coded as juvenile (J, ≥ 64.9 cm CCL), subadult (SA, 65.0 – 84.9 cm CCL) or adult (A, 85.0 ≤ cm CCL). Morphological measurements taken include weight, curved carapace length (CCL), straight carapace length (SCL), tail length (TL). Body condition index (BCI) was calculated using Fulton's K equation (condition index = mass/SCL^3 x 10^4)

Year	Turtle ID	BCI	Weight (kg)	CCL (cm)	SCL (cm)	TL (cm)	Stage Class	Palmyra Zone Code	$\delta^{13}C$	$\delta^{15}N$
2008	GD5	1.52	10.2	44.5	40.6	7.5	J	West	-20.64	8.33
	GD15	1.29	12.6	49	46	7	J	West	-20.74	15.33
	GE2	1.55	17	50.4	47.9	8	J	West	-20.81	11.26
	GD10	1.25	15.4	52.8	49.7	10.2	J	South	-16.49	9.78
	GD1	1.18	23	60	57.9	9	J	South	-15.63	10.95
	GD7	1.33	36.2	61.3	64.8	14.5	SA	South	-14.86	8.73
	GD11	1.39	27	62	57.9	10.5	SA	South	-17.1	9.74
	GD16	1.55	30.8	63.5	58.4	11.5	SA	South	-19.52	9.7
	GE1	1.60	36.8	65	61.3	13	SA	South	-15.63	10.54
	GD6	1.47	37.2	67.5	63.3	12	SA	South	-17.59	10.64
	GA6	1.38	36.4	68.5	64.2	12	SA	South	-12.54	10.25
	GA4	1.55	40.6	69.5	64	13	SA	South	-15.01	9.69
	GD2	1.40	43.2	76	67.5	14	SA	South	-18.3	7.82
	GD8	1.31	49.4	76.8	72.3	13.5	SA	South	-15.16	9.98
	GD14	1.51	59.6	78.7	73.3	18	SA	South	-16.15	10.3
	GD9	1.27	52.8	81	74.6	14	SA	South	-17.6	10.1
	GA8	1.17	66.9	85.5	83	19	A	North	-16.02	10.05

Year	Turtle ID	BCI	Weight (kg)	CCL (cm)	SCL (cm)	Tail Length (cm)	Stage Class	Palmyra Zone Code	$\delta^{13}C$	$\delta^{15}N$
2009	GG1	1.46	11.8	45.5	43.2	6.2	J	North	-19.4	11.74
	GD24	1.37	20.2	56	52.8	8.9	J	North	-17.8	11.07
	GB15	1.32	23.4	58.5	56.2	10.3	J	North	-14.32	12.38
	GF1	1.00	17.2	59	55.7	9.5	J	North	-13.85	11.73
	GG7	1.43	25.2	59.3	56.1	9.3	J	North	-14.99	13.53
	GD19	2.47	44	60	56.3	9.2	J	North	-15.73	10.5
	GD18	3.06	62.5	64	58.9	10	SA	North	-14.69	9.69
	GD17	2.78	67	66	62.2	11.5	SA	North	-15.36	10.31
	GB14	0.69	16.6	67	62.3	13.5	SA	North	-13	10.53
	GI1	1.35	35.8	67.5	64.2	5	SA	North	-13.18	12.15
	GG5	1.54	43.1	70	65.4	12.2	SA	North	-15.57	10.72
	GB9	1.29	41.1	71.3	68.3	15	SA	North	-14.84	11.13
	GD25	1.33	43.2	74	68.7	14.5	SA	North	-16.54	10.73
	GI10	1.36	48.1	76.1	70.8		SA	North	-15.65	11.18
	GI11	1.35	54.9	78.3	74		SA	North	-17.01	13.86
	GI8	1.34	53.8	78.6	73.7		SA	North	-16.23	12.38
	GD26	1.31	55.6	79	75.2	17	SA	North	-17.49	14.34
	GI4	1.31	57.4	80.7	76	13	SA	North	-15.5	12.94
	GD23	1.38	59.9	81.5	75.7	16.5	SA	North	-16.11	10.27
	GB16	1.58	80	85	79.7	33.5	A	North	-15.79	12.03
	GB10	1.27	74.4	88.7	83.7	49.9	A	North	-14.72	13.04
	GH2	1.33	84.6	91.5	86	42	A	North	-15.87	13.42
	GG8	1.39	107.3	96	91.7	35	A	North	-15.06	13.93
	GD22	1.27	97.5	96.2	91.5	50.5	A	South	-21.33	10.29
	GD20	1.36	101	96.5	90.5	43.5	A	South	-16.09	13.16
	GI12	1.41	108.2	96.9	91.6		A	West	-16.57	14.23
	GI3	1.27	97.4	98	91.6	18.5	A	East	-14.55	10.98
	GG3	1.03	99.8	102.5	99	25.8	A	East	-15.07	13.23
	GI7	1.25	146.3	113.6	105.5	30	A	East	-16.57	13.43

Year	Turtle ID	BCI	Weight (kg)	CCL (cm)	SCL (cm)	Tail Length (cm)	Stage Class	Palmyra Zone Code	$\delta^{13}C$	$\delta^{15}N$
2010	GG15	1.59	18	52.4	48.4	6	J	East	-15.63	12.13
	GG30	1.36	18	53.8	50.9	8	J	East	-16.73	13.64
	GB23	1.33	18	54.3	51.3	10	J	East	-13.35	11.78
	GG13	1.24	18	54.9	52.5	8.6	J	East	-13.62	11.96
	GG17	1.39	21	56.5	53.3	4.9	J	East	-13.56	12.08
	GB22	1.25	20	56.8	54.3	9.1	J	East	-17.13	10.37
	GG31	1.42	24	58.5	55.3	10	J	East	-14.02	12.47
	GG22	1.30	24	60.1	57	10.2	J	East	-15.79	12.29
	GB25	1.30	24	61.4	57	9.8	SA	East	-16.65	9.48
	GG19	1.26	27	64.2	59.8	9.5	SA	East	-13.89	13.42
	GG29	1.48	34	64.7	61.2	12	SA	East	-13.76	11.64
	GG16	1.35	33	68	62.5	10	SA	East	-15.87	11.61
	GD29	1.35	37	69	65	13.8	SA	East	-15.24	9.78
	GG27	1.48	45	72.2	67.2	15	SA	East	-9.81	5.36
	GB30	1.38	41	72.3	66.8	13	SA	East	-16.11	11.04
	GI19	1.54	64	77	74.6	14	SA	West	-16.97	12.08
	GB26	1.35	55	78	74.2	17.5	SA	West	-14.29	10.63
	GD30	1.35	64	81	78	34	SA	West	-16.17	10.56
	GB27	1.38	60	81.5	75.8	12.5	SA	West	-13.96	12.49
	GI14	1.77	89	85.3	79.5	40.5	A	West	-15	12.4
	GI18	1.62	87	85.8	81.2	34	A	West	-15.93	14.45
	GB28	1.26	74	86.5	83.8	30	A	West	-15.69	13.55
	GB24/ GB10	1.31	76	87.2	83.5	37.6	A	West	-17.18	11.04
	GI17	1.48	79	89.4	81.1	29	A	West	-13.68	13.06
	GD31	1.21	79	92	86.7	25.5	A	West	-15.12	11.23
	GG28	1.48	98	93.5	87.1	25	A	West	-14.75	13.29
	GG12	1.41	98	94	88.5	21	A	West	-13.12	12.31
	GG26	1.44	100	94.5	88.5	25	A	West	-13.21	11.63
	GI16	1.18	106	102.4	96.4	23	A	West	-18.53	9.78

Appendix C

Appendix C. Literature sources for loggerhead turtles (*Caretta caretta*), green turtles (*Chelonia mydas*) and leatherback turtles (*Dermochelys coriacea*) discrimination factors. Samples were obtained from hatchling (H) and juvenile (J) turtles. No discrimination factors exist for green turtle scute tissue

Author/Species	Tissue	$\Delta^{13}C$ value (± SD)	$\Delta^{15}N$ value (± SD)	Stage Class
Reich et al. 2008 /	Skin	2.62±0.34	1.65±0.12	H
Caretta caretta		1.11±0.17	1.6±0.07	J
	Scute	-0.86±0.57	0.61±0.16	H
		1.77±0.58	-0.64±0.09	J
	RBC	-0.64±0.73	-0.25±0.30	H
		1.53±0.17	0.16±0.08	J
	Plasma	0.29±0.20	0.32±0.09	H
		-0.38±0.21	1.5±0.17	J
	Whole Blood	0.92±0.34	0.19±0.08	H
		1.11±018	0.14±0.06	J
Seminoff et al. 2006 /	Skin	0.17±0.03	2.80±0.11	J
Chelonia mydas	RBC	-1.11 ± 0.05	0.22±0.03	J
	Plasma	-0.12±0.03	2.92±0.03	J
	Whole Blood	-0.92±0.06	0.57±0.09	J
Seminoff et al. 2009 /	Skin	2.26±0.61	1.85±0.50	J
Dermochelys coriacea	RBC	0.46 ± 0.35	1.49±0.76	J
	Plasma	-0.58±0.53	2.86±0.82	J
	Whole Blood	0.35±0.33	1.98±1.14	J

Appendix D

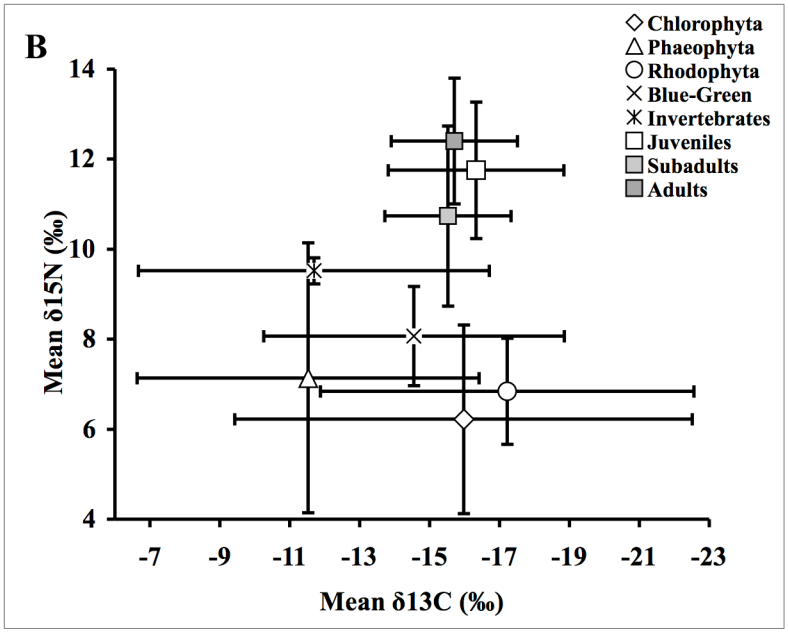
Appendix D. Number of sampled prey items per genus from Palmyra Atoll National Wildlife Refuge from 2008 to 2010

Sample Genus	Number of Samples
Chlorophyta	
Arainavillea	2
Bryopsis	2
Caulerpa	5
Cladophora	3
Codium	1
Dictyosphaeria	2
Halimdea	4
Valonia	2
Phaeophyta	
Acanthophora	1
Dotyophycus	1
Turbinaria	1
Rhodophyta	
Asparagospsis	1
Ceramium	4
Galaxaura	1
Gelidiopsis	2
Geliopsis	1
Hypnea	1
Jania	1
Spirutina	1
Spyridia	1
Blue-green Algae	
Cyanobacteria	3
Invertebrate	
Black Sponge	2
Orange Sponge	1
Purple Tunicates	1
Red Tunicates / Sponge	1
Clay-Colored Sponge	1

Appendix E

Appendix E. (A) Prey isotopic values collected from samples at Palmyra Atoll, those obtained from the literature and combined values. Prey groups included green algae (chlorophyta), brown algae (phaeophyta), red algae (rhodophyta) and invertebrates. (B) Mean $\delta^{13}C$ and $\delta^{15}N$ values for prey groupings and stage classes. Combined values were used for prey groupings. Error bars are ± one standard deviation. All Palmyra atoll prey and green turtle samples were obtained from Palmyra Atoll between 2008 and 2010

A	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
Literature Values				
Chlorophyta	-17.33	6.70	4.89	2.15
Phaeophyta	-11.88	5.20	5.99	3.35
Rhodophyta	-20.79	4.86	6.40	0.96
Palmyra Values				
Chlorophyta	-12.18	4.51	7.15	1.12
Phaeophyta	-9.64	2.49	9.04	0.58
Rhodophyta	-13.2	3.53	7.17	1.28
Blue-Green	-14.55	4.3	8.07	1.1
Invertebrates	-11.69	5.02	9.52	0.29
Combined Values				
Chlorophyta	-15.97	6.54	6.22	2.1
Phaeophyta	-11.52	4.89	7.14	3
Rhodophyta	-17.22	5.34	6.84	1.18



Appendix F

Appendix F. $\delta^{13}C$ and $\delta^{15}N$ values for supplemental prey species from the literature and sampled prey species from Palmyra Atoll. When available both the supplemental and sampled prey isotopic values were included in the prey group mean. Cholorphyta, phaeophyta and rhodopyta group means included the literature values; blue-green algae and invertebrate values are sampled prey only. Means (± standard deviation, SD) were determined for each prey grouping and are shown in grayed boxes

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
Blue-green algae				-14.55	4.30	8.07	1.10
	This Study (2009)			-17.03		8.71	
	This Study (2009)			-9.58		6.80	
	This Study (2009)			-17.03		8.71	
Chlorophyta				-15.97	6.54	6.22	2.10
Avrainavillea	This Study (2009)			-19.92		8.03	
	This Study (2009)			-14.00		6.04	
Bryopsis	This Study (2009)			-16.34		6.74	
	This Study (2009)			-17.34		7.38	
Caulerpa	This Study (2009)			-12.27		5.30	
	This Study (2009)			-11.29		6.16	
	This Study (2009)			-13.90		5.38	
	This Study (2010)			-15.40		7.06	
	This Study (2010)			-14.17		5.31	
	Hyndes & Lavery 2005	*C. gemminata*	nearshore waters, SW Australia	-13.4		7.7	
	Hyndes & Lavery 2005	*C. flexilis*	nearshore waters, SW Australia	-12.6		5.5	
	Raven et al. 2002	*C. cactoides*	Coobowie Bay, SA, Australia	-21.35			
	Raven et al. 2002	*C. flexilis*	Coobowie Bay, SA, Australia	-32.98			
	Raven et al. 2002	*C. microphysa*	W Flower Garden, Gulf of Mexico (winter)	-22.79			

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
	Raven et al. 2002	*C. microphysa*	W Flower Garden, Gulf of Mexico (spring)	-20.3			
	Raven et al. 2002	*C. microphysa*	Sonnier, Gulf of Mexico (summer)	-19.6			
	Raven et al. 2002	*C. microphysa*	Stetson, Gulf of Mexico (fall)	-19.94			
	Raven et al. 2002	*C. microphysa*	Stetson, Gulf of Mexico (winter)	-20.97			
	Raven et al. 2002	*C. microphysa*	E Flower Garden, Gulf of Mexico (winter)	-19.99			
	Raven et al. 2002	*C. obscura*	Stragglers, WA, Australia	-20.33			
	Raven et al. 2002	*C. obscura*	Carnac Island, WA, Australia	-30.18			
	Raven et al. 2002	*C. obscura*	Carnac Island, WA, Australia	-29.59			
	Raven et al. 2002	*C. obscura*	Hamelin Bay, WA, Australia	-31.33			
	Raven et al. 2002	*C. obscura*	Mewstone, WA, Australia	-28.99			
	Raven et al. 2002	*C. obscura*	Mewstone, WA, Australia	-28.59			
	Raven et al. 2002	*C. obscura*	Stragglers, WA, Australia	-30.12			
	Raven et al. 2002	*C. obscura*	Stragglers, WA, Australia	-28.39			
	Raven et al. 2002	*C. obscura*	The Lumps, WA, Australia	-25.92			
	Raven et al. 2002	*C. obscura*	The Lumps, WA, Australia	-31.38			
Cladophora	This Study (2009)			-9.12		8.20	
	This Study (2009)			-9.57		7.44	
	This Study (2010)		Nursery	-15.14		6.64	
	Dailer et al. 2010	*C. sericea*	NW Maui			0.2	
	Vizzini & Mazzola 2003	*Cladophora sp.*	Lake of Sabaudia, Italy	-15.9		5.9	

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
	Lepoint et al. 2000	*C. proliphera*	Gulf of Calvi, Corsica	-17.5		4	
	McClelland et al. 1998	*C. vagabunda*	pond / river, Massachusettes	-15.2		5.4	
	Maberly et al. 1992	*C. sericea*	tentsmuir drift, East coast Scotland	-19.35			
	Maberly et al. 1992	*C. rupestris*	mid-shore, East coast Scotland	-16.66			
	Maberly et al. 1992	*C. rupestris*	sheltered rock pool, East coast Scotland	-13.33			
	Raven et al. 2002	*C. albida*	Filey, England	-10.83			
	Raven et al. 2002	*C. hutchinsonia*	Filey, England	-12.15			
	Raven et al. 2002	*C. rupestris*	Fifeness, England	-14.02			
	Raven et al. 2002	*C. rupestris*	Filey, England	-18.09			
	Raven et al. 2002	*C. rupestris*	East Sands, Scotland	-15			
	Raven et al. 2002	*C. rupestris*	East Sands, Scotland	-15.37			
	Raven et al. 2002	*C. rupestris*	Hemsdale, Scotland	-14.33			
	Raven et al. 2002	*Cladophora sp.*	Gran Canaria	-16.12			
Codium	This Study (2010)		T.H.	-9.82		7.47	
	Kang et al. 2008	*C. arabicum*	macroalgal beds, Samchoek Coast, Korea	-10.3		3.8	
	Lepoint et al. 2000	*C. bursa*	Gulf of Calvi, Corsica	-10.3		3.1	
	Hydes & Lavery 2005	*C. duthaeie*	SW Australia (nearshore waters)	-15.5		4.8	
	Maberly et al. 1992	*C. fragile*	rockpool, East coast Scotland	-15.01			
	Maberly et al. 1992	*C. fragile*	sheltered rockpool, East coast Scotland	-14.08			
	Maberly et al. 1992	*C. fragile*	sheltered rockpool, East coast Scotland	-10.23			

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
	Maberly et al. 1992	*C. fragile*	fife ness, East coast Scotland	-15.38			
	Raven et al. 2002	*C. convulutum*	Brighton Beach / Papatowai Beach, New Zealand	-14.54			
	Raven et al. 2002	*C. fragile*	California	-11.7			
	Raven et al. 2002	*C. fragile*	California	-11.15			
	Raven et al. 2002	*C. fragile*	Brighton Beach / Papatowai Beach, New Zealand	-12.04			
	Raven et al. 2002	*C. fragile*	Brighton Beach / Papatowai Beach, New Zealand	-15.46			
	Raven et al. 2002	*C. fragile*	Helmsdale, Scotland	-12.87			
	Raven et al. 2002	*C. fragile*	St. Andrews, Scotland	-13.26			
	Raven et al. 2002	*C. hubsii*	Catalina Island, CA	-8.17			
	Raven et al. 2002	*Codium sp.*	Fifeness, Scotland	-10.86			
	Raven et al. 2002	*Codium sp.*	Gran Canaria	-9.86			
	Raven et al. 2002	*Codium sp.*	Gran Canaria	-14.47			
	Raven et al. 2002	*Codium sp.*	Hampton Bay, Long Island, NY	-15.76			
	Wang & Yeh 2003	*C. mamillosum*	sublittoral, North Taiwan	-14.2			
Dictyosphaeria	This Study (2010)			-3.21		6.67	
	This Study (2009)			-18.39		6.26	
	Raven et al. 2002	*D. sericea*	Rottnest Island, WA, Australia	-6.33			
Halimeda	This Study (2009)			-11.25		6.66	
	This Study (2009)			-4.86		10.02	
	This Study (2009)			-7.86		8.05	
	This Study (2009)		P.S.	-4.76		8.60	
	Lepoint et al. 2000	*H. tuna*	Gulf of Calvi, Corsica	-19.3		1.3	

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
	Raven et al. 2002	*Halimeda sp.*	Gran Canaria	-11.33			
	Raven et al. 2002	*Halimeda sp.*	Singapore	-6.83			
	Wang & Yeh 2003	*H. macroloba*	tidal pool, South Taiwan	-21.2			
	Wang & Yeh 2003	*H. opuntia*	tidal pool, South Taiwan	-19.7			
Unknown sp	This Study (2009)			-10.75		7.02	
Valonia	This Study (2010)			-11.82		8.06	
	This Study (2009)			-16.84		8.82	
	Raven et al. 2002	*V. clavata*	Gran Canaria	-14.83			
Phaeophyta				-11.52	4.89	7.14	3.00
Acathophora spicifera	This Study (2010)			-12.50		8.58	
	Lin & Fong 2008	*A. spicifera*	Nearshore reef, Opunohu Bay, Moorea			6	
	Wang & Yeh 2003	*A. spicifera*	Upper-tidal pool, East Tiawan	-13.9			
Dotyophycus	This Study (2009)			-7.96		8.86	
Turbinaria	This Study (2010)			-8.46		9.70	
Dictyota	Lepoint et al. 2000	*Dictyota spp.*	Gulf of Calvi, Corsica	-17.4		3.6	
	Umezawa et al. 2002	*Dictyota spp.*	Offshore reef, Ishigaki Island, Japan			2	
	Umezawa et al. 2002	*Dictyota spp.*	Nearshore reef, Ishigaki Island, Japan			8	
	Newell et al. 1995	*D. dicotoma*	Peninsular Malaysia	-19.94		10.36	

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
	Raven et al. 2002	*D. cervicornus*	Stetson, Gulf of Mexico	-15.06			
	Raven et al. 2002	*D. dichotoma*	N of Oban, Scotland	-18.43			
	Raven et al. 2002	*D. dichotoma*	N of Oban, Scotland	-19.68			
	Raven et al. 2002	*D. dichotoma*	Filey, England	-13.06			
	Raven et al. 2002	*D. dichotoma*	Finnoy, Norway	-12.06			
	Raven et al. 2002	*D. menstrualis*	W Flower Gardens, Gulf of Mexico (winter)	-11.06			
	Raven et al. 2002	*D. menstrualis*	Stetson, Gulf of Mexico (spring)	-10.06			
	Raven et al. 2002	*D. menstrualis*	Stetson, Gulf of Mexico (autumn)	-9.06			
	Raven et al. 2002	*D. menstrualis*	Stetson, Gulf of Mexico (winter)	-8.06			
	Raven et al. 2002	*D. pfaffi*	Stetson, Gulf of Mexico (winter)	-7.06			
	Raven et al. 2002	*D. pfaffi*	E Flower Garden, Gulf of Mexico (winter)	-6.06			
	Raven et al. 2002	*D. pulchella*	Stetson, Gulf of Mexico (spring)	-5.06			
	Raven et al. 2002	*D. menstrualis*	E Flower Garden, Gulf of Mexico (winter)	-4.06			
Rhodophyta				-17.72	5.34	6.84	1.18
Asparagospsis	This Study (2009)			-17.09		7.96	
	Raven et al. 2002	*A. armata*	Stricland Bay, WA, Australia	-29.71			
	Raven et al. 2002	*A. armata*	Stricland Bay, WA, Australia	-29.55			
	Raven et al. 2002	*A. taxixformis*	Catalina Island, CA	-28			

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
Ceramium	This Study (2010)			-15.67		9.21	
	This Study (2009)			-14.57		8.17	
	This Study (2009)			-8.91		5.66	
	This Study (2009)			-8.68		6.71	
	Maberly et al. 1992	*C. rubrum*	Rockpool, East coast Scotland	-13.09			
	Maberly et al. 1992	*C. rubrum*	Tentsmuir drift, East coast Scotland	-19.56			
	Raven et al. 2002	*C. rubrum*	Bergen, Norway	-18.29			
	Raven et al. 2002	*C. shuttleworthianum*	Bergen Store Kalsoy, Norway	-19.26			
	Raven et al. 2002	*C. shuttleworthianum*	Flamborough, England	-19.13			
Galaxaura	This Study (2010)			-11.34		7.77	
	Wang & Yeh 2003	*G. marginata*	tidal pool, East Taiwan	-16.2			
Gelidiopsis	This Study (2010)			-10.58		7.80	
	This Study (2009)			-16.40		6.43	
	Raven et al. 2002	*Gelidiopsis sp.*	Stetson, Gulf of Mexico	-19.68			
Geliopsis	This Study (2009)			-15.22		6.34	
Hypnea	This Study (2009)			-17.42		5.63	
	Dailer et al. 2010	*H. musciformis*	Kahana, West Maui			6.6	
	Dailer et al. 2010	*H. musciformis*	South Maui			6.8	
	Hyndes & Lavery 2005	*Hypnea sp A*	SW Australia (nearshore waters)	-19.8		6.8	
	Hyndes & Lavery 2005	*Hypnea sp B*	SW Australia (nearshore waters)	-19.6		5.4	

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
	Raven et al. 2002	*H. volubilis*	Stetson, Gulf of Mexico (spring)	-19.42			
	Raven et al. 2002	*H. volubilis*	Stetson, Gulf of Mexico (winter)	-21.09			
	Raven et al. 2002	*H. volubilis*	E Flower Garden, Gulf of Mexico (winter)	-18.56			
	Raven et al. 2002	*Hypnea sp.*	Gran Canaria	-21.75			
	Wang & Yeh 2003	*H. spinella*	Sublittoral, North Taiwan	-19.8			
	Wang & Yeh 2003	*H. japonica*	Sublittoral, North Taiwan	-21.2			
Jania	This Study (2009)			-7.49		7.53	
	Raven et al. 2002	*J. micrathandia*	Rottnest Island, WA, Australia	-22.55			
	Raven et al. 2002	*J. rubeus*	S Coast of Devon, UK	-12.57			
Spirutina	This Study (2009)						
Spyridia	This Study (2010)		T.H.				
Gelidium	Kang et al. 2008	*G. amansii*	Macroalgal beds, Samchoek Coast, Korea	-16.8		4.7	
	Raven et al. 2002	*G. latifolium*	Bergen Store Kalsoy, Norway	-15.86			
Invertebrate				-11.69	5.02	9.52	0.29
Black Sponge	This Study (2010)			-4.29		9.39	
	This Study (2009)			-15.89		9.19	

Prey Group	Source	Species	Sampling Location	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
Clay Colored Sponge	This Study (2010)			-8.96		9.72	
Orange Sponge	This Study (2010)			-16.56		9.23	
Purple Tunicates	This Study (2009)			-8.90		9.87	
Red Tunicates/ Sponge	This Study (2010)			-15.55		9.74	

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