FORAGING ECOLOGY OF GREEN TURTLES (*Chelonia mydas*) ON THE TEXAS COAST, AS DETERMINED BY STABLE ISOTOPE ANALYSIS

A Thesis

by

CATHERINE CONCETTA THERESA GORGA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2010

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

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ABSTRACT

Foraging Ecology of Green Turtles (*Chelonia mydas*) on the Texas Coast, as Determined by Stable Isotope Analysis. (August 2010)

Catherine Concetta Theresa Gorga, B.S., Texas A&M University at Galveston

Chair of Advisory Committee: Dr. Andre M. Landry, Jr.

The green turtle, *Chelonia mydas*, is a circumglobal species that exhibits several important developmental or ontogenetic shifts throughout its life history. The first major shift occurs when juvenile turtles migrate from pelagic habitat, where they forage as omnivores, to coastal neritic habitat, where they become primarily herbivores, foraging on algae and seagrass. Anecdotal evidence and gut-content analyses suggest that juvenile green turtles in south Texas bays, such as the lower Laguna Madre and Aransas Bay, undergo an additional ontogenetic shift during this important life history stage.

Evidence from stable isotope analysis (SIA) of scute tissues of green turtles from Texas' lower Laguna Madre and Aransas Bay supports an intermediate stage between this species' shift from pelagic waters to seagrass beds in neritic waters; this additional shift comprises an initial recruitment of post-pelagic juveniles to jetty habitat located on the channel passes Gulf-ward of adjacent bays before subsequently recruiting to seagrass beds in these bays. Examination of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in microlayers of scute tissue from several size classes of green turtles from the lower

Laguna Madre and Aransas Bay was used to confirm the occurrence of two ontogenetic shifts.

Smaller green turtles (< 35 cm SCL) exhibited more depleted $\delta^{13}C$ signatures and more enriched $\delta^{15}N$ signatures, consistent with jetty habitat, compared to those of larger counterparts (> 45 cm SCL) that displayed enriched $\delta^{13}C$ signatures and depleted ^{15}N signatures, consistent with seagrass habitat. Changes in the isotopic composition between these size classes indicate distinct shifts in diet. Post-pelagic juveniles first recruit to jetty habitat and forage primarily on algae, before subsequently shifting to seagrass beds and foraging primarily on seagrass. These findings indicate the use of a characteristic sequence of distinct habitats by multiple life history stages of green turtles in Texas bays, a conclusion with broad management implications for this endangered species.

ACKNOWLDGEMENTS

There are many people I wish to thank. First and foremost is Dr. Andre M. Landry, who so readily accepted me into his lab and did not in the slightest hesitate before taking me on as a graduate student. No matter what snags or difficulties were encountered during this process, Dr. Landry always had the most positive of attitudes.

I would like to thank Dr. Kimberly J. Reich, whose expertise in the field of SIA truly enabled and facilitated my completion of this thesis. Dr. Reich went well beyond the call of duty, as many late night calls and weekend meetings would attest.

I wish to thank Dr. William Neill and Dr. Jay Rooker for agreeing to serve on my committee. It was in Dr. Rooker's Marine Ecology class that I first learned of SIA and its many amazing uses, so one could say that was where this all began. Although Dr. Neill and I met face-to-face only toward the end of this process, he was always willing to offer advice and suggestions.

Thank you to Dr. Tasha Metz and her extraordinary turtle netting crew, who spent several summers capturing turtles in the hot Texas sun. It is Dr. Metz and the turtle crew whom I have to thank for the many biopsy samples I used in the writing of this thesis.

I wish to thank Dr. Jason Curtis, from the University of Florida's Stable Isotope Lab in the Department of Geology in Gainesville, Fl., for running all of the stable isotope analyses, and for the unstinting use of analytic equipment, such as the ASE and

the carbide end mill. Thank you, also, to Melania Lopez, the UF doctoral student who opened her home to me during my brief stay in Gainesville while analyzing samples.

Thank you to Director Tony Amos and his staff at the Animal Rehabilitation Keep, located at the University of Texas Marine Science Institute in Port Aransas, TX, for providing live stranded green turtles for biopsy.

I wish to thank the Department of Marine Biology of Texas A&M University at Galveston, headed by Dr. John Schwarz, for their financial support in the form of Graduate Assistant Teaching positions and mini-grants.

Finally, a great big thank you goes out to all of my friends and family, who have had to put up with me while I struggled through this experience: to my parents, Loretta Scholl and Tony Gorga, who are so proud to have a daughter with a graduate degree; to my dearest friend, Leslie Swick, who was always willing to lend an ear; and finally, to Barry Rathbun, my best friend and constant companion, who knows more than anyone else what it took for me to finish this thesis.

Thank you to anyone else who helped along the way, including the wonderful staff at Texas A&M University College Station and Texas A&M University at Galveston and the brilliant professors and researchers I have had the privilege to learn under.

Thank you one and all.

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CHAPTER I

INTRODUCTION

All sea turtles that inhabit U.S. waters are listed as either threatened or endangered under the U.S. Endangered Species Act (NMFS and USFWS 1991, 1992a, 1992b 1993, 1998a, 1998b). Efforts to rebuild these populations to historic levels have been ongoing for decades and include armed protection of nesting beaches, prevention of illegal egg harvest, and translocation of nests to more protected environments (Alvarado-Diaz et al. 2001, Eckert and Eckert 1990). Hatchling turtles have also been reared in captivity and released as larger juveniles, a process called "headstarting" (Eckert et al. 1992, Pritchard 1980). Implementation of turtle excluder devices (TEDs) in shrimp trawls has reduced the number of turtles killed in fisheries interactions (Crowder et al. 1995). These efforts, focused on nesting beaches and in-water assemblages, have had positive effects on sea turtle populations, some of which are beginning to increase. However, all sea turtle species remain endangered or threatened despite these conservation efforts. As a result, all state and federal sea turtle recovery plans mandate more information be generated about the ecology of constituent species. Detailed information on habitat use, reproductive capability, foraging ecology, and a host of other physiological and ecological factors is essential for government agencies and conservationists to devise effective recovery plans for sea turtles.

This thesis follows the style of Marine Ecological Progress Series.

The green turtle, *Chelonia mydas*, is considered a threatened species in the U.S, with breeding populations in Florida listed as endangered (NMFS and USFWS 1991). The green turtle population in Texas was once large enough to support a commercial harvest exceeding 230,000 kg/yr during the mid-1800s (Hildebrand 1982, Doughty 1984), although overexploitation by this fishery nearly eliminated green turtles from Texas waters (Hildebrand 1982). Gradual population gains (Rabalais and Rabalais 1980) have occurred; however, these have rendered constituent stocks only a fraction of historic levels. Nonetheless, studies indicate that the lower Laguna Madre is home to one of the largest assemblages of green turtles in the northwestern Gulf of Mexico (Shaver 1990, Landry et al. 1992, 1993, Coyne 1994, Shaver 1994).

Green turtles are a circumglobal species in tropical and subtropical waters, with important nesting beaches in Costa Rica, Surinam, and Ascension Island in the mid-Atlantic (Musick and Limpus 1996). Once emerged from their nests, hatchling green turtles migrate to the open ocean, where they spend approximately 3-5 years (Reich et al. 2007), during which time they reach ~25 – 35 cm straight carapace length (SCL). These are the so-called "lost years" because so much is unknown about this stage in the life cycle. Green turtles are omnivorous during this stage of their life (Musick and Limpus 1996, Reich et al. 2007) and take advantage of ocean currents, flotsam, and *Sargassum* mats for transport, protection and food (Carr and Meylan 1980).

Juvenile green turtles make the first of several ontogenetic shifts in habitat at a size of ~25 – 35 cm SCL (Carr and Ogren 1960), when they migrate to a nearshore neritic environment and begin to feed on benthic macrophytes (Bjorndal 1997),

including seagrass (Bjorndal 1985) and algae (Pritchard 1971). These juveniles remain in nearshore habitat as they grow toward sexual maturity (~30-35 years), and sometimes display strong foraging ground fidelity (Musick and Limpus 1996). As adults, green turtles migrate between nearshore foraging grounds and nesting beaches, which may be thousands of kilometers apart (Carr and Ogren 1960).

It has been suggested that green turtles forage on algae in the absence of seagrass (Hughes 1974), although there are locations where colonies of conspecifics foraging on seagrass exist within kilometers of counterparts foraging on algae (Hirth 1971, Garnett and Murray 1981). Anecdotal evidence suggests that this may be the case in the lower Laguna Madre, where spatial distribution of green turtles seems to be life-stage dependent. Post-pelagic juveniles have been observed at jetties in South Texas, where stomach content analyses revealed their diet consisted primarily of available algae (Renaud et al. 1995). By the time Texas green turtles attain 40 cm SCL (Metz and Landry, unpublished data) they have transitioned to foraging in seagrass beds (Coyne 1994).

Ontogenetic shifts in habitat and diet coincide with changes in growth and other vital rates. Because of this, and because ontogenetic shifts may impact the spatial distribution of green turtles, it is important that these shifts be fully understood. A more comprehensive understanding of ontogenetic shifts, habitat use, and diet of green turtles will allow management decisions to be made that protect whole populations, regardless of habitat choice within the geographic area occupied. To further elucidate the ontogenetic shifts of green turtles, they must be examined across a range of

developmental stages; the green turtle assemblage in Texas' lower Laguna Madre presents just such an opportunity. Here, a second ontogenetic shift may occur in which smaller post-pelagic juvenile turtles first recruit to jetty environments, before making a subsequent shift to seagrass communities in the bay system. Although similar to a pattern seen in the Trident Submarine Basin, Cape Canaveral, FL, where juvenile conspecifics have been observed foraging primarily on algae (Redfoot 1997), a subsequent shift of the kind suspected for green turtles in Texas has not been documented.

One tool in the study of animal foraging ecology is stable isotope analysis (SIA). Analyses of isotopes such as carbon (C) and nitrogen (N) in the tissues of a variety of animals, including, but not limited to, fish, birds, marine mammals, and their prey, have been used to assess trophic dynamics and reconstruct animal diets (Collier and Lyon 1991, Fleming et al. 1993, Vander Zanden et al. 1996). Diet reconstructions can be facilitated by SIA because: 1) natural gradients in stable isotopes can be found in the environment as well as in trophic relationships; and 2) over time, animal tissues come to reflect their diet. Stable isotope values of prey are incorporated into the tissues of consumers in a predictable fashion. However, the rate at which this isotopic incorporation occurs varies between species and between tissues of the same species. For example, the average residence time for $\delta^{13}C$ in the Japanese quail (*Coturnix japonica*) varies from 3 days in the liver to 251 days in bone collagen (Hobson and Clark 1992). Thus, with proper baseline data, one can determine the foraging history of an animal by measuring the isotopic signatures in a given tissue. Another facet that must be

considered is the discrimination factor. As prey is consumed, the heavier isotopes are retained in greater number by the body and lighter isotopes are lost through excretory products, such as urine, bile, and respired CO_2. In this way, the organism becomes isotopically enriched in relation to its diet (Minagawa and Wada 1984, Peterson and Fry 1987). The values at which this enrichment occurs are referred to as discrimination factors and can vary greatly among species (Kelly 2000). Discrimination is thought to be dependent on a number of attributes, including tissue type (Hobson and Clark 1992), age or size of animal (Carleton and Martinez del Rio 2005), growth rate, quality and quantity of proteins in the diet (MacAvoy et al. 2001, Reich et al. 2008), and nutritional stressors, such as pregnancy or lactation (Kurle and Worthy 2000). Because isotopic incorporation rates and discrimination factors vary among tissues and taxa, any ecological study of a free ranging organism (or population) should utilize species- and tissue-specific values, whenever possible (Seminoff et al. 2006).

Typically, the carbon isotopic signature is used to determine the basis of an animal's food web (Hobson et al. 1996). Facilitating this process is the fact that naturally occurring gradients in $\delta^{13}C$ exist in the environment. For instance, in the marine realm, oceanic environments (water > 200 m) are more depleted in $\delta^{13}C$ than neritic environments (water < 200 m). Benthic habitat is typically enriched in $\delta^{13}C$ compared to pelagic, as are benthically based food-webs versus food-webs that are established on phytoplankton. Because these gradients exist, $\delta^{13}C$ can be used to elucidate the source of an animal's carbon. Knowing the source of the carbon provides indications of habitat use and can be helpful in identifying migratory pathways.

The discrimination factor for nitrogen occurs in a step-wise fashion that allows stable nitrogen isotopes to be used to determine trophic position of an organism within a food web (Minagawa and Wada 1984, Gannes et al. 1997). With each step in the food web, $\delta^{15}N$ of the consumer becomes enriched in a predictable fashion, relative to the isotopic signature of the prey it has assimilated. A more enriched $\delta^{15}N$ signature indicates a higher trophic level. For example, Godley et al. (1998) determined that $\delta^{15}N$ was approximately 20‰ for loggerhead turtles foraging in the Mediterranean, while $\delta^{15}N$ for green turtles in the same area was approximately 9‰. Loggerheads are known to forage carnivorously on mollusks and arthropods, and their $\delta^{15}N$ signature reflects their status as secondary and tertiary consumers. The more depleted $\delta^{15}N$ signature of the green turtle reflects the fact that green turtles are primary consumers that forage herbivorously. In the same study, leatherback turtles from the western Atlantic had a $\delta^{15}N$ signature of 14‰; these turtles forage mainly on gelatinous organisms such as jellyfish, and their $\delta^{15}N$ signature was indicative of their placement at an intermediate trophic level.

Conventional methods of studying the diet of an organism, such as gut content analysis, provide 'snapshots' (Peterson and Fry 1987) of an animal's diet; whereas, SIA can indicate long-term trends, as a result of varied assimilation and turnover rates of isotopes in bone, blood, muscle, hair and feathers (Schoeninger and DeNiro 1984, Rau et al. 1992, Bearhop et al. 2002, Kurle 2002). By applying appropriate incorporation and discrimination values, stable isotopes provide a chemical "clock" (Phillips and Eldridge 2006) that allows researchers to track changes in an animal's diet. These analyses can

be especially helpful for studying animals that spend the majority of their lives underwater or in unknown or inaccessible locations and, as such, whose foraging habits are difficult to observe. Also, sample collections for SIA can be relatively non-invasive, an important factor when dealing with an endangered species such as the green turtle.

A search of peer-reviewed literature revealed several studies that utilize stable isotopes to investigate the ecology of sea turtles (Godley et al. 1998, Hatase et al. 2002, Biasatti 2004, Hatase et al. 2006, Cart et al. 2008, Reich et al. 2007, 2008, 2010). Reich et al. (2007) used stable isotopes of carbon and nitrogen in scute tissue to confirm that immature green turtles conform to Carr's hypothesis (1952) of oceanic, omnivorous foraging during the "lost years" and to track the ontogenetic shift of green turtles to seagrass foraging in neritic habitat. Scute tissue is particularly appropriate for isotopic clock studies because it is continuously laid down over the surface of the shell, and it is inert once produced. Due to these properties, stable isotopes analyzed from these tissues provide a history of diet and foraging habitat.

Arthur et al. (2008) used stable isotopes to document green turtle foraging ecology throughout constituent life stages, providing further evidence that sea turtles undergo ontogenetic shifts in habitat and feeding that coincide with developmental stages. Stable isotope studies have been conducted on green turtle populations in northwest Africa (Cardona et al. 2009), the Caribbean (Reich et al. 2007), Mediterranean (Godley et al. 1998), and Pacific Ocean (Arthur et al. 2008), but similar studies are lacking for conspecifics in the Gulf of Mexico, particularly constituent assemblages of the lower Texas coast.

Texas bay systems provide a unique opportunity to study the green turtle over a range of life history stages. Based on earlier studies of the green turtle population in Texas (Shaver 1990, Landry et al. 1992, 1993, Coyne 1994, Shaver 1994), I hypothesized that stable isotope signatures of smaller turtles would indicate primarily algae-foraging, while those of larger counterparts would indicate primarily seagrass-foraging. I also hypothesized that isotopic profiles based on successive microsampled scute layers would reveal the occurrence and timing of any shift from algae to seagrass-based foraging, as well as indicate the size of turtle at which the shift may occur. By providing a more complete understanding of green turtle foraging ecology and habitat use, the results of this study can be used to assess critical habitat for this endangered species.

CHAPTER II

MATERIALS AND METHODS

Turtle Capture and Examination. This stable isotope study was part of a larger Texas Sea Grant College investigation to determine the extent that sea turtles utilize habitats in Texas bay systems, focusing primarily in Aransas Bay and the lower Laguna Madre (Fig. 1). Turtles were captured by entanglement netting in the lower Laguna Madre at two locations adjacent to Port Isabel (Fig. 2a), the Mexiquita Flats area (26° 03.347' N, 97° 11.178' W) near the Brownsville Ship Channel and Laguna Atascosa Wildlife Refuge (26° 10.283' N, 97° 17.197' W). Similar capture efforts in Aransas Bay occurred primarily in the East Flats section of the bay (27° 48.751' N, 97° 7.789' W; Fig. 2b).

Entanglement netting operations followed protocol developed by Landry et al. (1999) to successfully capture 981 turtles from Texas and Louisiana coastal waters (Andre Landry, personal communication). Capture operations for green turtles sampled during the study reported herein took place during April through October of 2007–2009.

All green turtles taken in entanglement nets and a subset consisting of live stranded conspecifics captured and rehabilitated by staff of the Animal Rehabilitation Keep (ARK), located at the University of Texas Marine Science Institute in Port Aransas, were subject to measurement, tagging, and biopsy operations. The latter group consisted of green turtles either stranded in *Sargassum* mats or captured by recreational fishermen, and that spent only a short time in rehabilitation. Morphometric data

including straight and curved carapace length and width were taken. All sea turtles were tagged with an inconel tag on the trailing edge of each front flipper and a Passive Integrated Transponder (PIT) tag inserted subcutaneously into the dorsal musculature of one front flipper.

Biopsy Sampling Protocol. Tissue samples for SIA were taken from the carapace of each turtle. The carapace, the hard keratinized tissue of the "shell", was sampled at the 2nd lateral scute, with an anterior sample taken near the inner edge of the scute and a posterior sample taken near the outer edge of the scute (Fig. 3). This protocol yielded a total of two samples per turtle. All samples were collected with a sterile, 6 mm biopsy punch. Samples were preserved in a 70% ethanol solution and held for subsequent analysis.

Samples of seagrass and algae from each study area were opportunistically collected for SIA, in order to provide a carbon and nitrogen baseline for the diet of the sampled turtles. Seagrass and algae samples were frozen for subsequent SIA.

Sample Preparation and Analysis. Scute, seagrass, and algae samples were cleaned with alcohol and rinsed with deionized water, before being dried in an oven at 60°C for 24 hrs. Seagrass and algae samples were diced prior to lipid extraction. Scute samples were left intact as it was necessary that the samples remain whole for micro-sampling, post-lipid extraction. Lipid extraction took place in a Dionex Accelerated Solvent Extractor (ASE), using petroleum ether as solvent.

Following lipid extraction, seagrass and algae were ground and homogenized using a ceramic mortar and pestle. A carbide end mill was used to microsample scute

tissues. To accomplish this, posterior scute samples were glued to microscope slides with dorsal sides up, representing the oldest retained tissue on the scute. Conversely, anterior scute samples were glued to microscope slides with ventral sides up to allow sampling of the most recently synthesized tissues. A carbide end mill was used to remove successive 50 micron layers of each scute (Fig. 3), beginning with either the oldest retained tissue from the posterior scute sample or the most recent tissue from the anterior scute sample; thus, each scute layer represents particular stages in the life history of each turtle. The 50 micron microsampling size was the minimum size needed to generate enough sample for SIA and has no known biological significance at this time. Approximately 600 micrograms of each scute layer were loaded into precleaned tin capsules for SIA; approximately 1000 micrograms of each seagrass and algae sample were loaded in a similar fashion.

Stable isotope analysis was conducted by Jason Curtis at the University of Florida's Stable Isotope Lab, Department of Geology, Gainesville, FL. All tissue and forage samples were combusted in a COSTECH ECS 4010 elemental analyzer interfaced via a Finnigan-MAT Conflow III device (Finnigan MAT, Breman, Germany) to a Finnigan-MAT DeltaPlus XL (Breman, Germany) isotope ratio mass spectrometer. Stable isotope signatures were expressed in standard delta (δ) notation, where:

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

$R_{sample}/R_{standard}$ refers to the ratio of heavy to light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and standard, respectively. The standard for ^{13}C is the Vienna Pee Dee

Belemnite (VPDB) limestone formation. The standard for ^{15}N is atmospheric N_2. All isotopic signatures are expressed in parts per thousand.

Statistical Analyses. Mean values of $\delta^{13}C$ were plotted against mean values of $\delta^{15}N$ for each size class (< 35, 35 – 45, and > 45 cm SCL), and a one-way analysis of variance (ANOVA) was used to determine if significant differences occurred between the newest tissues of the three size classes. If significant differences did occur, Tukey's Honestly Significant Difference (HSD) was used to compare those differences. Comparisons between the two study sites were also made using a one-way ANOVA. Individual $\delta^{13}C$ and $\delta^{15}N$ profiles were created for each turtle from oldest dorsal layer to newest ventral layer and for each size class. Wilcoxon rank-sum tests were run to detect significant differences between signatures of older and newer tissues. All statistical analyses used an alpha value of 0.05. By applying appropriate discrimination factors to isotopic signatures of forage materials, carbon and nitrogen signatures of scute tissues were used to interpret turtle diets.

CHAPTER III

RESULTS

Capture Data. In total, 44 green turtles, acquired as directed captures in Port Isabel (n = 33, Fig. 4a) and Port Aransas (n = 6, Fig. 4b) and incidental live strandings from ARK (n = 5) in Port Aransas, were analyzed for stable isotopes of C and N (Table 1). Turtles from the ARK were live stranded turtles found during cold-stunning events or exhibiting recent trauma at various locations along the Texas coast between Laguna Madre and Matagorda Bay (Fig. 5); no obviously diseased or ill turtles were biopsied.

Size Composition. Turtles captured in the Port Aransas study site ranged from 24.9 to 57.5 cm SCL and averaged 40.8 cm SCL ± 9.0 SD. Those netted near Port Isabel ranged between 27.4 and 61.5 cm SCL while averaging 39.0 cm SCL ± 6.8 SD. The majority of turtles from the ARK were found stranded in and around the Corpus Christi/Aransas Bay system, thus they were included in the Port Aransas data set. These conspecifics ranged from 22 to 48.8 cm SCL and averaged 31.6 cm SCL ± 7.8 SD.

Based on in-water survey and capture data from previous studies in South Texas (Landry and Metz, unpublished data), turtles were separated into three size classes for analysis: < 35, 35 – 45, and > 45 cm SCL. See Table 2 for mean straight carapace length of each size class. Turtles 35 – 45 cm SCL were the most abundant at Port Isabel, accounting for 56% of the total catch (Fig. 6). Turtles > 45 cm SCL, in comprising only 11% of all captures, were the least abundant (Fig. 6). Turtles < 35 cm SCL accounted for the remaining 33% of Port Isabel captures (Fig. 6). In Port Aransas/ARK, turtles <

35cm SCL were the most abundant, accounting for 50% of the total (Fig. 6). Turtles 35 – 45 cm SCL comprised 30% of the catch (Fig. 6). Turtles > 45 cm SCL were the least abundant, accounting for 20% of the turtles biopsied (Fig. 6).

Stable Isotope Analyses. ***Primary Producers.*** Mean isotopic signatures for potential forage material from Port Isabel ranged from -9.79‰ ± 0.26 SD $\delta^{13}C$ and 5.39‰ ± 0.11 SD $\delta^{15}N$ in *Halodule wrightii* to -5.25‰ ± 0.19 SD $\delta^{13}C$ and 4.64‰ ± 0.09 SD $\delta^{15}N$ in *Syringodium filiforme.* *Thalassia testudinum* had mid values of -9.34‰ ± 0.03 SD $\delta^{13}C$ and 5.26‰ ± 0.05 $\delta^{15}N$. Two species of algae were sampled: *Gelidium*, which had a $\delta^{13}C$ signature of -18.85‰ ± 0.10 SD and a $\delta^{15}N$ signature of 7.84‰ ± 0.07 SD, and *Ulva*, which had a $\delta^{13}C$ signatures of -19.01‰ ± 0.10 SD and a $\delta^{15}N$ signature of 7.22‰ ± 0.24 SD. *Sargassum* values (-17‰ to -16‰ δ13C, 2.5‰ to 2.8‰ δ15N) representing potential prey items for pelagic stage turtles have been modified from Rooker et al. (2006) and included in Fig. 7a

In Port Aransas, *Ruppia maritima* had the most depleted $\delta^{15}N$ signature, 1.17‰ ± 0.14 SD, and a $\delta^{13}C$ signature of -11.03‰ ± 0.11 SD. *H. wrightii* had the most enriched $\delta^{15}N$ signature, 7.20‰ ± 0.18 SD, and the most depleted $\delta^{13}C$ signature, -5.20‰ ± 0.08 SD. *S. filliforme* had a $\delta^{13}C$ signature of -10.82‰ ± 0.14 SD and a $\delta^{15}N$ signature of 6.32‰ ± 0.23 SD. *T. testudinum* had a $\delta^{13}C$ signature of -12.03‰ ± 0.16 SD and a $\delta^{15}N$ signature of 5.59‰ ± 0.11 SD. Of the algae sampled, *Ulva* was enriched in $\delta^{13}C$ and depleted in $\delta^{15}N$ compared to *Gelidium* (-17.08‰ ± 0.03 SD $\delta^{13}C$ and

8.52‰ ± 0.12 SD $\delta^{15}N$ for *Ulva* versus -20.39‰ ± 0.17 SD $\delta^{13}C$ and 10.07‰ ± 0.11 SD $\delta^{15}N$ for *Gelidium*). See Fig. 7b for these values.

Regional Differences. No significant difference was detected between signatures of $\delta^{13}C$ and $\delta^{15}N$ in the newest scute tissues of turtles < 35 cm SCL from Port Aransas and Port Isabel ([p = 0.069 ($\delta^{13}C$), p = 0.733 ($\delta^{15}N$), n = 15, α = .05] Fig. 8). A similar comparison between turtles 35 – 45 cm SCL from Port Aransas and Port Isabel yielded significant difference in signatures of both $\delta^{13}C$ and $\delta^{15}N$ ([p = 0.000 ($\delta^{13}C$), p = 0.000 ($\delta^{15}N$), n = 23] Fig. 8). The newest tissues of turtles > 45 cm SCL from Port Aransas and Port Isabel were significantly different in signatures of $\delta^{13}C$ (p = 0.007, n = 6), but this was not the case for $\delta^{15}N$ signatures ([p = 0.786, n = 6] Fig. 8).

Port Isabel. Mean signatures of $\delta^{13}C$ in scute tissues ranged from -14.58‰ ± 3.61 SD for turtles < 35 cm SCL to -8.50‰ ± 0.64 SD for turtles > 45 cm SCL. The mean $\delta^{13}C$ signature for turtles 35 – 45 cm SCL was -10.62‰ ± 2.21 SD. The lowest mean $\delta^{15}N$ signature, 8.02‰ ± 1.34 SD, was for turtles 35 – 45 cm SCL, while turtles < 35 cm SCL had the highest mean, 9.71‰ ± 1.29 SD. Turtles > 45 cm SCL had the mid value, with a mean $\delta^{15}N$ signature of 8.55‰ ± 0.63 SD.

Signatures of $\delta^{13}C$ and $\delta^{15}N$ in the newest scute tissues were significantly different ([p = 0.000 and p = 0.003 for $\delta^{13}C$ and $\delta^{15}N$, respectively, n = 33, α = .05] Fig. 9) across the three size classes. Tukey's HSD analysis revealed that significant differences existed between the $\delta^{13}C$ signature of the newest tissues of turtles < 35 cm SCL and those turtles 35 – 45 cm SCL and > 45 cm SCL (p = 0.001, n = 30, and p = 0.003, n = 13, respectively) and that significant differences existed between the $\delta^{15}N$

signatures of the newest tissues of turtles < 35 cm SCL and turtles 45 – 45 cm SCL ($p = 0.002$, $n = 30$).

Stable carbon and nitrogen isotopic profiles for turtles < 35 cm SCL, 35 – 45 cm SCL, and > 45 cm SCL, respectively, are shown in Figures 11–13. Neither $\delta^{13}C$ ($p = 0.178$, $n = 33$) or $\delta^{15}N$ ($p = 0.383$, $n = 33$) were significantly different between the oldest and newest tissues sampled for any size class

Port Aransas. Turtles < 35 cm SCL had the lowest mean $\delta^{13}C$ signature, -18.28‰ ± 0.98 SD, followed by turtles 35 – 45 cm SCL (-16.86‰ ± 2.91 SD). Turtles 35 – 45 cm SCL had the highest mean $\delta^{15}N$ signature, 11.12‰ ± 2.76 SD, while the mid value, 10.36‰ ± 2.25 was for turtles < 35 cm SCL. Turtles > 45 cm SCL had the highest mean $\delta^{13}C$ signature, -13.23‰ ± 2.65 SD, and the lowest mean $\delta^{15}N$ signature, 9.66‰ ± 2.37 SD.

Signatures of $\delta^{13}C$ and $\delta^{15}N$ in the newest scute tissues were significantly different ([$p = 0.000$ and $p = 0.025$ for $\delta^{13}C$ and $\delta^{15}N$, respectively, $n = 11$, $\alpha = .05$] Fig. 10) across the three size classes. Tukey's HSD analysis revealed that significant differences existed between the $\delta^{13}C$ signatures of the newest tissues of turtles < 35 cm SCL and turtles 35 – 45 cm SCL versus those of turtles > 45 cm SCL ([$p = 0.000$, $n = 8$ for turtles < 35 cm SCL/35 – 45 cm SCL comparison], [$p = 0.000$, $n = 6$ for turtles 35 – 45 cm SCL/> 45 cm SCL comparison]). $\delta^{15}N$ signatures of the newest tissues were significantly different between turtles 35 – 45 cm SCL and turtles > 45 cm SCL ($p = 0.026$, $n = 6$).

Stable carbon and nitrogen isotopic profiles for turtles < 35 cm SCL, 35 – 45 cm SCL, and > 45 cm SCL, respectively, are shown in Figures 11–13. Signatures of $\delta^{13}C$ between older and newer tissues were not significantly different for any size class ($p = 0.606$, $n = 11$). A similar result was found for signatures of $\delta^{15}N$ between older and newer tissues ($p = 0.519$, $n = 11$).

CHAPTER IV

DISCUSSION

Signatures of $\delta^{13}C$ and $\delta^{15}N$ in newest scute tissues were significantly different among the three size classes of green turtles foraging in both Port Isabel and Port Aransas study sites. In Port Isabel, isotopic signatures indicate that green turtles < 35 cm SCL forage primarily on algae, whereas the carbon and nitrogen signatures of turtles 35 – 45 cm SCL and > 45 cm SCL indicate that these turtles forage primarily on seagrass (Fig. 9). Tukey's analyses validate these conclusions; when mean signatures of scute tissues were compared between the size classes, significant differences were found in the isotopic signatures between turtles < 35 cm SCL versus turtles 35 – 45 cm SCL and turtles > 45 cm SCL, but no significant differences were found between turtles 35 – 45 cm SCL and turtles > 45 cm SCL. Based on the conclusions derived from the mean isotopic signatures, differences were expected between turtles < 35 cm SCL versus turtles 35 – 45 cm SCL and turtles > 45 cm SCL, since turtles < 35 cm SCL had isotopic signatures indicating algae-foraging, while signatures from turtles 35 – 45 cm SCL and turtles > 45 cm SCL indicated seagrass-foraging.

Mean isotopic scute signatures indicate that turtles < 35 cm SCL in Port Aransas forage primarily on algae (Fig. 10) and turtles > 45 cm SCL forage primarily on seagrass (Fig. 10). These results are similar to those for Port Isabel. In contrast to Port Isabel, however, mean isotopic scute values for turtles 35 – 45 cm SCL indicate that turtles of this size in Port Aransas are foraging primarily on algae (Fig. 10). Tukey's analyses

reinforce these conclusions; when means were compared between the size classes, no significant differences were found in the isotopic signatures between turtles < 35 cm SCL and turtles 35 – 45 cm SCL, but significant differences did occur between turtles < 35 cm SCL and turtles 35 – 45 cm SCL versus turtles > 45 cm SCL. Turtles < 35 cm SCL and turtles 35 – 45 cm SCL had signatures that indicated algae-foraging, versus turtles > 45 cm SCL, whose signatures implied seagrass-foraging.

These conclusions regarding diet were realized by applying appropriate discrimination factors to scute tissue signatures and then comparing those values to signatures of potential forage material. In this instance, discrimination factors of +0.17‰ and +2.92‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, the high end of the range reported by Seminoff et al. (2006), were used. Isotopic signatures from the turtles fall within the range dictated by application of these discrimination factors to seagrass and algae signatures reported herein.

Isotopic values of algae and seagrass from Port Isabel and Port Aransas are similar to those from previous studies that have examined isotopic signatures in marine algae (France 1995, Rogers 2003, Wang and Yeh 2003) and seagrass (Benedict et al. 1980, Anderson and Fourqurean 2003, Berlinger and Butler 2006). In general, seagrass is enriched in $\delta^{13}C$ and depleted in $\delta^{15}N$ in comparison to that of algae (Fig. 13).

Similar trends in green turtle foraging strategies are seen between Port Isabel and Port Aransas (Fig. 8). Isotopic signatures of scute tissues of turtles from Port Aransas are, on the whole, more depleted in $\delta^{13}C$ and enriched in $\delta^{15}N$, but this may be an effect of sampling effort. Most of the turtles from Port Aransas were found stranded live along

beaches and jetties of the Corpus Christ/Aransas bay system, where algae is abundant, or in sargassum mats; as such, they are, on average, smaller than their counterparts from Port Isabel that were captured in the seagrass beds. Because of the differences in size, the variation in isotopic signatures was expected. Also, isotopic signatures of potential forage in Port Aransas were more depleted in $\delta^{13}C$ and enriched in $\delta^{15}N$ than those from Port Isabel. For example, $\delta^{13}C$ for algae from the genus *Gelidium* from Port Isabel was -18.85‰ ± 0.07 SD, while $\delta^{13}C$ for the same species from Port Aransas was -20.39‰ ± 0.17 SD. *Syringodium filiforme* (manatee grass) had a $\delta^{15}N$ signature of 4.64‰ ± 0.19 SD in Port Isabel, but a $\delta^{15}N$ signature of 6.32‰ ± 0.23 SD in Port Aransas. Disparity in isotopic signatures of forage materials between sites establishes a different isotopic landscape for each site. Turtles from Port Aransas were foraging on algae and seagrass with more depleted $\delta^{13}C$ and more enriched $\delta^{15}N$ signatures than their counterparts from Port Isabel; thus, isotopic signatures from the turtles follow the same pattern as their forage materials.

When mean signatures of the newest scute tissues were compared between each size class in both sites, no significant differences occurred in either the carbon or nitrogen isotopic values of turtles < 35 cm SCL. $\delta^{13}C$ and $\delta^{15}N$ indicated that turtles of this size in both Port Isabel and Port Aransas are foraging primarily on jetty algae and not in *Sargassum* mats common to both areas. Although similar $\delta^{13}C$ signatures are seen between *Sargassum* (-17‰ to -16‰, Rooker et al. 2006) and values of jetty algae herein (-20.39‰ to -17.08‰), $\delta^{15}N$ signatures of *Sargassum* (2.5‰ to 2.8‰, Rooker et al. 2006) place this potential forage source outside the boundaries described by applying

appropriate discrimination factors to isotopic signatures reported herein for green turtles (8.02‰ to 11.12‰). Observational evidence has identified the algae-laden jetties that protect the navigational channels in South Texas as habitat for small post-pelagic juveniles (Coyne 1994, Shaver 1994, Renaud et al. 1995). The results of this study support not only the use of jetties as green turtle habitat, but also that constituents are definitively foraging on algae at the jetties for a minimum of 2 –3 years, as demonstrated by successive layers of scute possessing signatures indicative of algae and based on growth rates for immature green turtles (Bjorndal et al. 2000).

Although the isotopic signatures among the smallest turtles were not significantly different between the two study sites, significant differences did occur between the mean $\delta^{13}C$ and $\delta^{15}N$ signatures of turtles 35 – 45 cm SCL between the two sampling sites. $\delta^{13}C$ and $\delta^{15}N$ signatures of turtles from Port Isabel indicated that turtles of 35 – 45 cm SCL are foraging primarily on seagrass, but $\delta^{13}C$ and $\delta^{15}N$ signatures of turtles from Port Aransas indicated that turtles of this size are foraging primarily on algae. It is possible that the three turtles from Port Aransas recruited to the seagrass beds at a larger size within the 35 – 45 cm SCL range and have not yet had time to incorporate the seagrass isotopic signature, or that turtles of this size in Port Aransas may be incidentally ingesting epiphytic algae or invertebrates at a higher rate than did their counterparts from Port Isabel. However, these results are more likely an effect of sampling limitation. Of the three turtles sampled in this size class, two were captured from the seagrass beds and one was a live stranded animal retrieved by ARK from the South Jetty. Microsampling of scutes from this size class was also restricted to only 2 or 3 layers per scute. The

inadequacy of the sample size for Port Aransas advocates caution in accepting conclusions based on the data herein. Turtles 35 – 45 cm SCL from Port Isabel, which had a much larger sample size (n = 20) captured from the seagrass beds, follows the more probable trend.

As for turtles > 45 cm SCL, significant differences occurred between the $\delta^{13}C$ signatures, but did not occur between the $\delta^{15}N$ signatures. Results for this size class indicated that larger turtles in both Port Isabel and Port Aransas are foraging primarily on seagrass. This inference is supported by the fact that all turtles from both sites in this size class were captured from seagrass beds.

The changes in isotopic composition between the size classes indicate distinct shifts in diet, similar to that found by Reich et al. (2007) and by Arthur et al. (2008) in green turtles in the Bahamas and Queensland, Australia, respectively. Developmental migrations for juvenile sea turtles are not a new idea (Carr 1952). In the Bahamas, Bjorndal and Bolten (1996) described juvenile green turtles recruiting to adjacent developmental habitat prior to recruitment in seagrass beds for continued growth, as has Redfoot (1997) in Trident Basin, FL. Limpus et al. (2005) found that green turtles in Shoalwater Bay, Australia were segregated by size-class within the bay, with smaller turtles occupying shallower waters around mangroves and rocky intertidal zones, while the larger turtles utilized deeper waters over the seagrass beds. The results from Limpus et al. (2005) were based on capture data and do not indicate differences in foraging strategy, unlike the conclusions from this study based on SIA. Arthur et al. (2008) used SIA to conclude that turtles in Moreton Bay were separating via size class, when smaller

juvenile turtles had more depleted $\delta^{13}C$ signatures than did larger adult turtles. This is indicative of changes in foraging strategy from algal to seagrass-based diets. Based on the isotopic data collected for this study, green turtles in Port Isabel and Port Aransas are segregating on a size class basis.

Although I hypothesized that isotopic signatures between older and more recently laid-down scute tissues would be different, no significant differences were detected in signatures of $\delta^{13}C$ and $\delta^{15}N$ from all size classes of green turtles captured at both study sites. This may be due to the sampling protocol used in this study that required two biopsies of the 2nd lateral scute, with an anterior sample taken near the inner edge of the scute and a posterior sample taken near the outer edge of the scute. However, the oldest retained tissue on a turtle's carapace occurs along the posterior margin of the inner edge of the second lateral scute—*not* along the outer edge that was sampled (Fig. 3). Conversely, the most recently laid-down tissue on a turtle's carapace occurs along the anterior margin of the outer edge of the second lateral scute—*not* the inner edge that was sampled (Fig. 3). Failure of the sampling protocol used in the present study to capture oldest and most recent tissues may mean that these tissues aren't representative of the same amount of life history that other studies have yielded. Reich et al. (2007), by using a protocol that examines a longer period of green turtle life history, were able to determine that significant differences in carbon and nitrogen isotopes occurred between oldest and newest scute tissues of recent recruits to their study site in the Bahamas, indicating a shift from omnivory in the pelagic habitat to herbivory in neritic habitat .

The shorter timescale of scute data presented herein explains the parity between isotopic signatures from oldest and newest tissues. Data from scutes sampled by the present study suggest that smaller turtles (< 35 cm SCL in Port Isabel and < 45 cm SCL in Port Aransas) have already lost the oceanic signature documented by Reich et al (2007), but the algae signature from the jetty habitat remains in their tissues. Conversely, the more limited scale of time represented by scute tissues herein necessitates that the larger turtles (> 45 cm SCL in both sites) have lost the algae signature and retain only the signature representing seagrass.

Individual isotopic profiles of turtles from Port Isabel and Port Aransas study areas (Figs. 11–13) support the conclusion that green turtles in Texas bays undergo an ontogenetic shift in diet and habitat use from algae-laden jetties to seagrass beds. Turtles < 35 cm SCL have carbon and nitrogen signatures that indicate algal foraging, with depleted $\delta^{13}C$ signatures (Fig. 11a) and enriched $\delta^{15}N$ signatures (Fig. 11b). Although the differences between older and younger scute tissues were not significant in this class, the overall trend for the profiles over time is toward enriched $\delta^{13}C$ signatures (Fig. 11a) and depleted $\delta^{15}N$ signatures (Fig. 11b), which indicates that an ontogenetic shift from algae foraging to seagrass foraging is occurring.

A similar trend is seen in individual profiles for turtles 35 – 45 cm SCL from Port Isabel. Overall, results for this size class indicate seagrass foraging. However, two turtles, PI07-7-9w and PI08-8-17w, show depleted carbon (Fig. 12a) and enriched nitrogen (Fig. 12b) signatures in their older tissues, indicative of algae foraging, and enriched carbon (Fig. 12a) and depleted nitrogen (Fig. 12b) in their younger scute

tissues, indicative of seagrass foraging. This shift from depleted to enriched carbon and enriched to depleted nitrogen signatures is similar to that reported by Reich et al. (2007) and Arthur et al. 2008 in green turtles in the Bahamas and Australia, respectively. Both studies documented an upward shift in carbon signatures and a downward shift in nitrogen signatures that were indicative of ontogenetic shifts in individual turtle profiles (Reich et al. 2007) and among size classes (Arthur et al. 2008).

Individual profiles of green turtles from Port Aransas sites (Fig. 12) support the conclusions that constituents are foraging primarily on algae. Unlike the individual profiles from green turtles near Port Isabel, no shifts in diet were seen. This result is likely due to failures in the sampling protocol in terms of limited sample size, diminished microsampling of scute layers, and shorter timescale due to choice of scute sampling locations.

Individual profiles for turtles > 45 cm SCL captured in Port Isabel and Port Aransas study sites (Fig. 13) indicate these turtles forage primarily on seagrass and do not exhibit dietary shifts. Landry and Metz (unpublished data) have determined that 40 cm SCL is the modal size at which green turtles recruit to seagrass beds from the jetty, a trend isotopic data from this study confirm. Based on trajectories from satellite tag data (Landry and Metz, unpublished data), green turtles of the lower Laguna Madre possess strong fidelity to the Laguna Madre as a foraging site, only migrating to laguna systems in Mexico to escape colder waters during winter. The lack of change in isotopic signatures suggests these turtles have been foraging in seagrass beds, in the lower

Laguna Madre or Mexico, for a minimum 3 – 4 years (Bjorndal et al. 2000) and have lost the algae signature seen in smaller counterparts.

Thalassia testudinum (turtle grass), *Syringodium filiforme* (manatee grass), and *Halodule wrightii* (shoal grass) comprised the majority of seagrass randomly sampled from Port Isabel and Port Aransas study areas. Some *Ruppia maritima* (widgeon grass) was also found, only in Port Aransas. Although *H. wrightii* accounts for 46% of the seagrass cover in the lower Laguna Madre, it occurs mainly in fringes along the shorelines of Padre Island and adjacent mainland (Onuf 2002). The two study sites near Port Isabel, Mexiquita Flats and Laguna Atascosa, are dominated by *T. testudinum* (Onuf 2002). *T. testudinum* and *H. wrightii* are also the dominant species in the Aransas Bay system (Kopecky and Dunton 2006). Green turtles have not shown a species-wide preference for any seagrass species. Instead, turtle preferences seem to be site specific and may be a function of seagrass abundance (Ferriera 1968) or selective choice by the turtles (Balzas 1980). In southern Florida and the Caribbean, turtles forage mostly on *T. testudinum* (Bjorndal 1980, Ogden et al. 1983), while in Mosquito Lagoon in northern Florida, turtles foraged mostly on *S. fileforme* (Mendonca 1983). Based on gut content analyses, Coyne (1994) concluded that green turtles in the lower Laguna Madre foraged primarily on *H. wrightii*, but the full diet composition of green turtles in Port Isabel and Port Aransas is unknown at this time. Also unknown is whether the composition of the diet is based on selective choice or density of seagrass species. Detailed percent composition studies of seagrass beds and isotopic mixed model analyses incorporating as

much potential prey sources and forage materials are required to further elucidate green turtle dietary components.

CHAPTER V

CONCLUSION

In conclusion, stable carbon and nitrogen isotopic values from this study provide definitive evidence that the Texas green turtle population goes through a multistage ontogenetic shift. Post-pelagic juveniles first recruit to jetty habitat appear to forage primarily on algae, before subsequently shifting to seagrass beds, once they have attained 35 – 45 cm SCL, a minimum of 2 – 3 years. Once in the seagrass beds, green turtle diet is predominantly composed of seagrass. This is the first multistage ontogenetic shift documented for green turtles in the northern hemisphere.

This study is only the first step in utilizing SIA to gain comprehensive knowledge of the ecology of green turtles in south Texas. Subsequent studies should incorporate as much potential prey items and forage material as possible into mixed model analyses, including invertebrates found along jetties and in seagrass beds, as well as any epibionts found on seagrass blades. While these mixed model analyses could reveal important components of green turtle diet, there is still some uncertainty in their application to the study of herbivorous animals. Thus far, mixed models have been run primarily on carnivorous animals that lack the special adaptations herbivores possess to digest plant material, such as microflora in the hindgut. A controlled feeding study of captive green turtles should first be implemented to evaluate the efficacy of isotopic mixed models to the study of green turtle diet. Ensuing studies should focus on the smallest size class turtles, particularly those found along the jetties, and use scute tissues

with a longer history of dietary data, to track ontogenetic shifts from the pelagic to coastal neritic environments.

The long-term goal of the research herein and all future endeavors is the recovery of Texas green turtles. To that end, managers need as much detailed information as possible about foraging ecology and habitat use in order to focus conservation efforts where such effort will do the most good.

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APPENDIX A

TABLES

Table 1: Number of green turtles biopsied from Port Isabel and Port Aransas, Texas study sites during 2007-2009.

Port Isabel	**Number of turtles**
Mexiquita Flats	
2007	10
2008	8
2009	11
Laguna Atascosa	
2008	2
2009	2
Port Aransas	
East Flats	
2007	5
South Jetty	
2008	1
ARK	
2007	4
2008	1

Table 2: Mean, range, and standard deviation of straight carapace length of each size class of green turtles biopsied from Port Isabel and Port Aransas, Texas study sites.

Port Isabel	**Frequency**	**Mean SCL (cm)**	**Min. SCL (cm)**	**Max. SCL (cm)**	**Std Dev.**
Size 1	10	32.1	27.4	34.1	2.0
Size 2	20	40.4	36.4	44.3	3.0
Size 3	3	53.4	46.5	61.5	7.6
Port Aransas					
Size 1	5	28.1	24.9	29.5	1.9
Size 2	3	38.2	35.0	40.8	2.9
Size 3	3	49.7	45.4	57.5	6.8

APPENDIX B

FIGURES

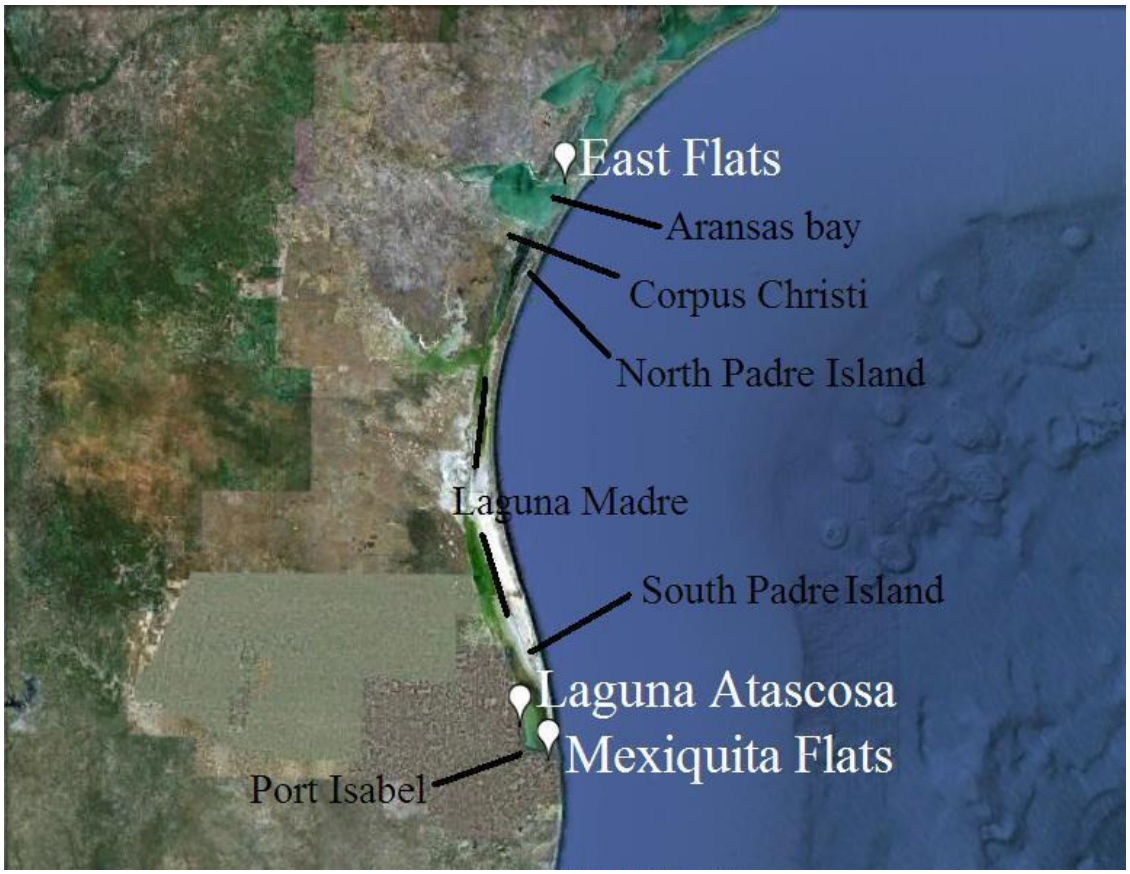

Figure 1: Entanglement netting locations used in the in-water capture of green turtles along the Texas coast during 2007-2009.

Figure 2a: Lower Laguna Madre, Texas netting locations used for in-water capture of green turtles during 2007-2009.

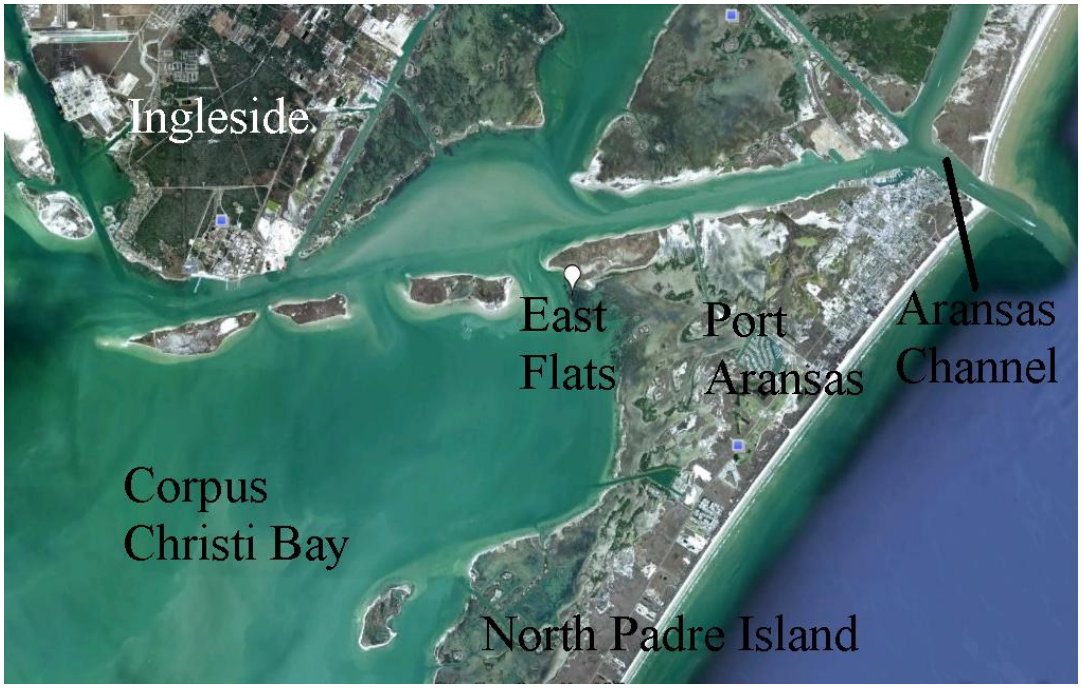

Figure 2b: Aransas Bay, Texas netting location used in the in-water capture of green turtles during 2007-2009.

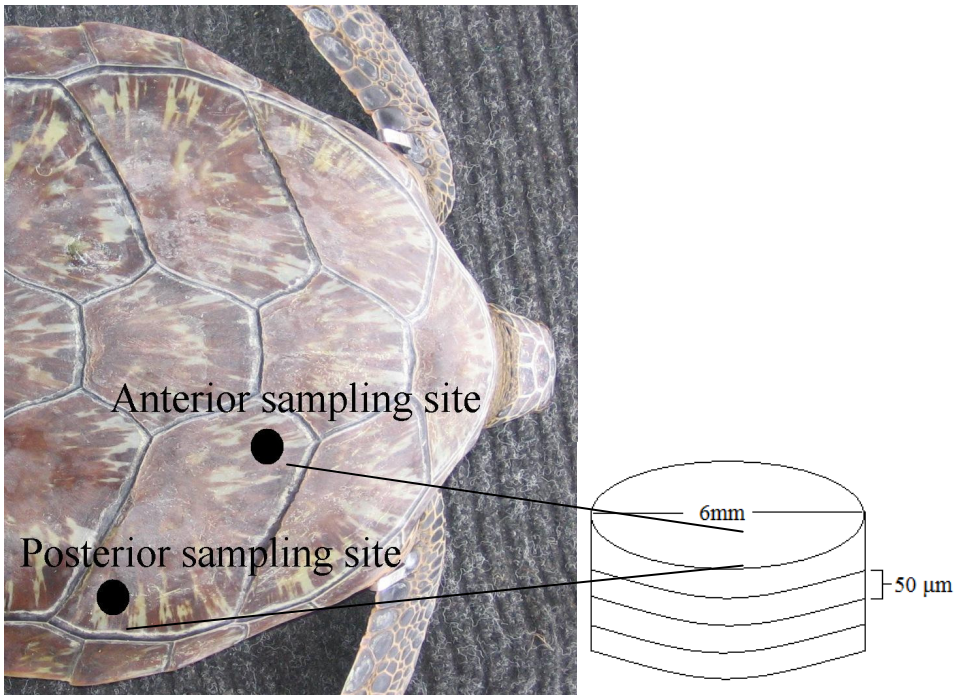

Figure 3: Green turtle carapace biopsy sampling sites with scute microsampling. Two sites were sampled in order to collect successive layers of scute tissue representing oldest retained tissue (from the posterior sampling site) and newest laid down tissue (from the anterior sampling site).

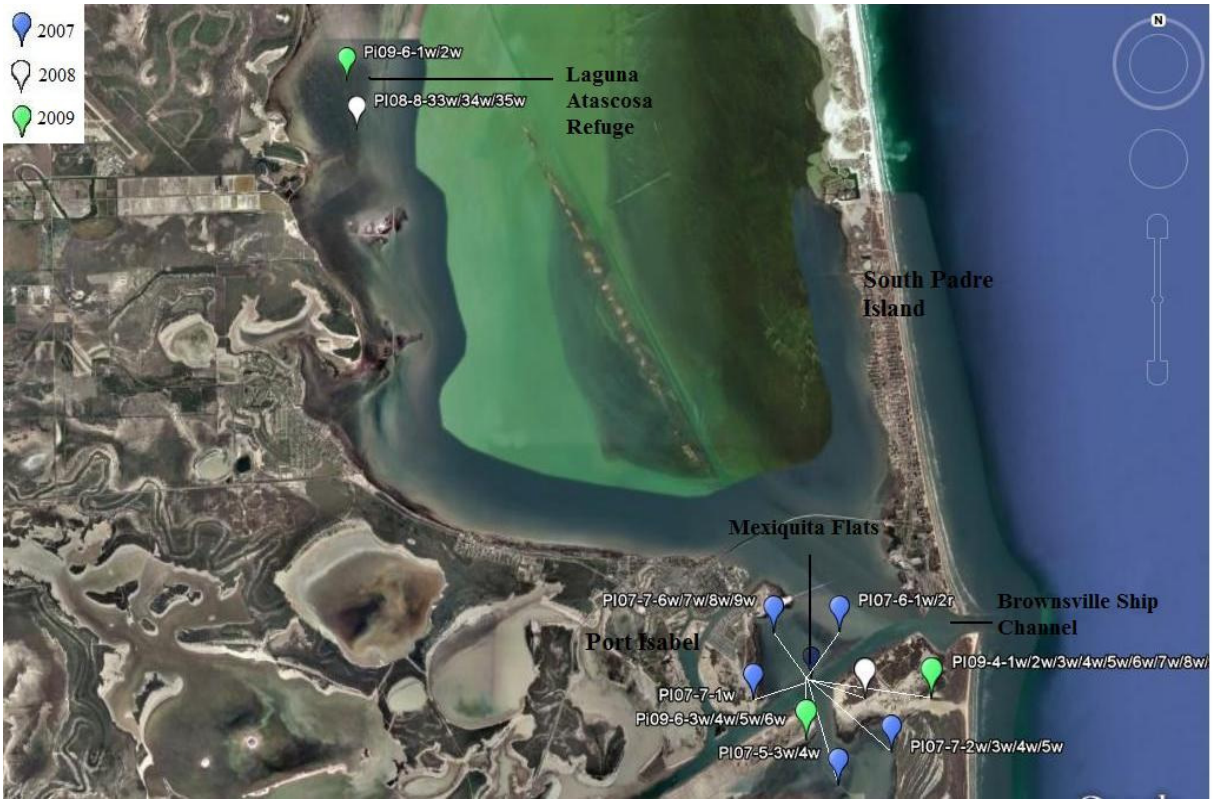

Figure 4a: Location of in-water captures of green turtles from the lower Laguna Madre, Texas during 2007-2009.

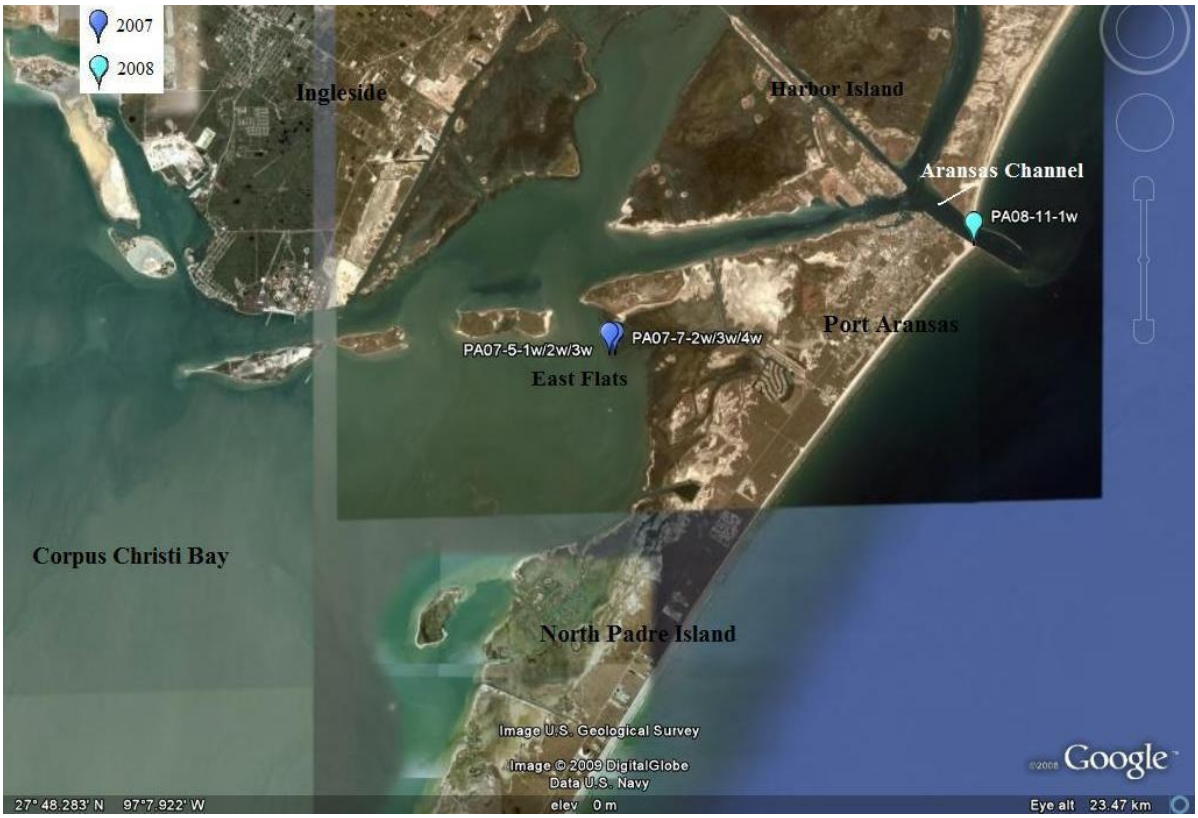

Figure 4b: Locations of in-water captures of green turtles from Texas' Aransas Bay System during 2007-2008.

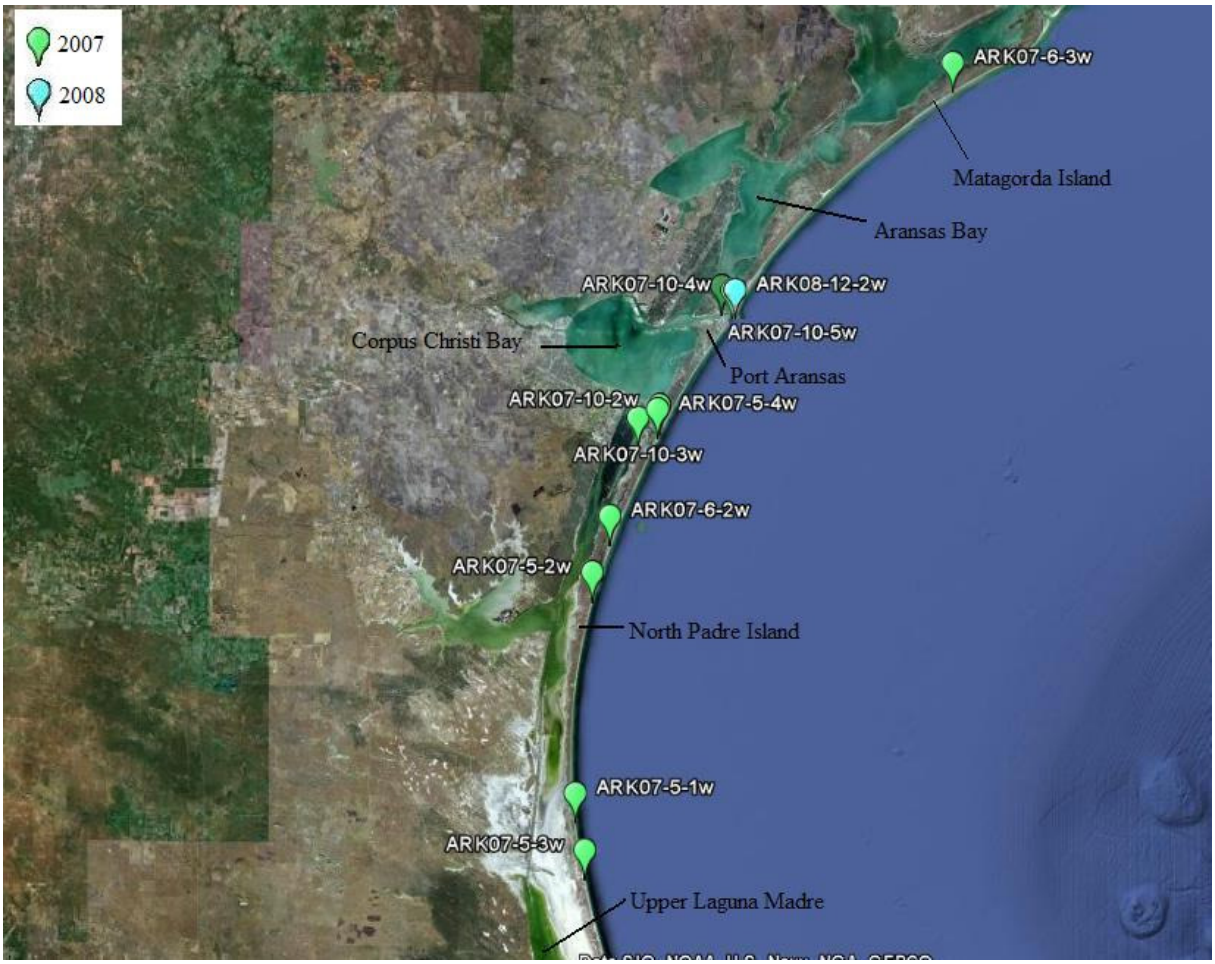

Figure 5: Stranding locations of green turtles made available for biopsy sampling by the Animal Rehabilitation Keep, Port Aransas, Texas, during 2007-2008.

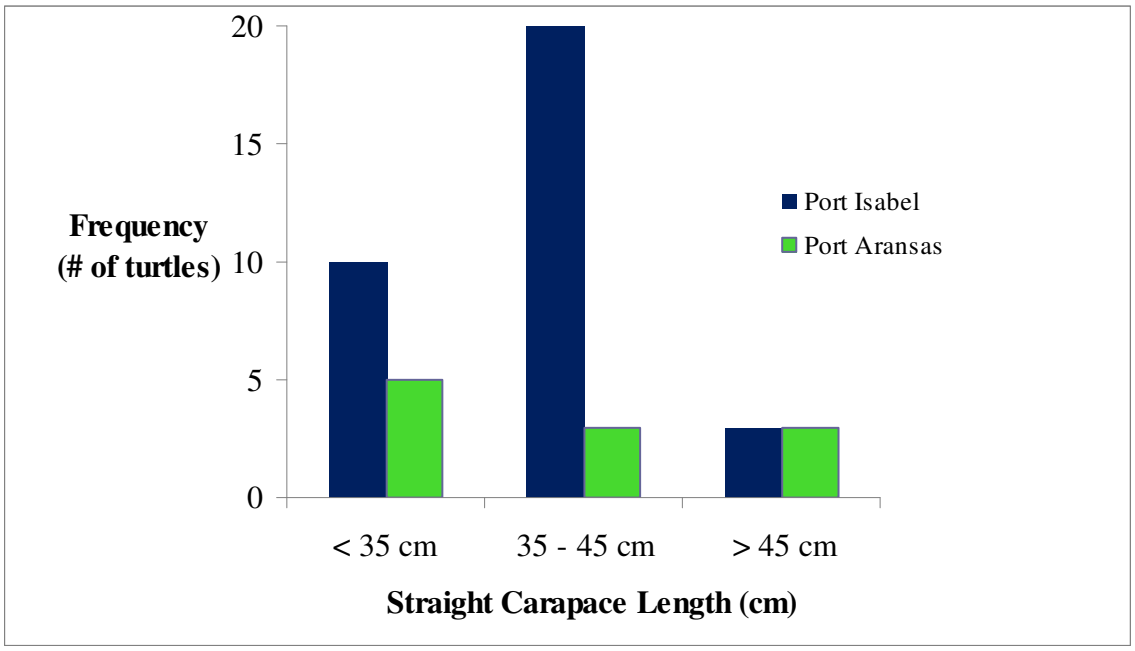

Figure 6: Carapace length frequency (cm) of green turtles captured near Port Isabel and Port Aransas, Texas during 2007-2009.

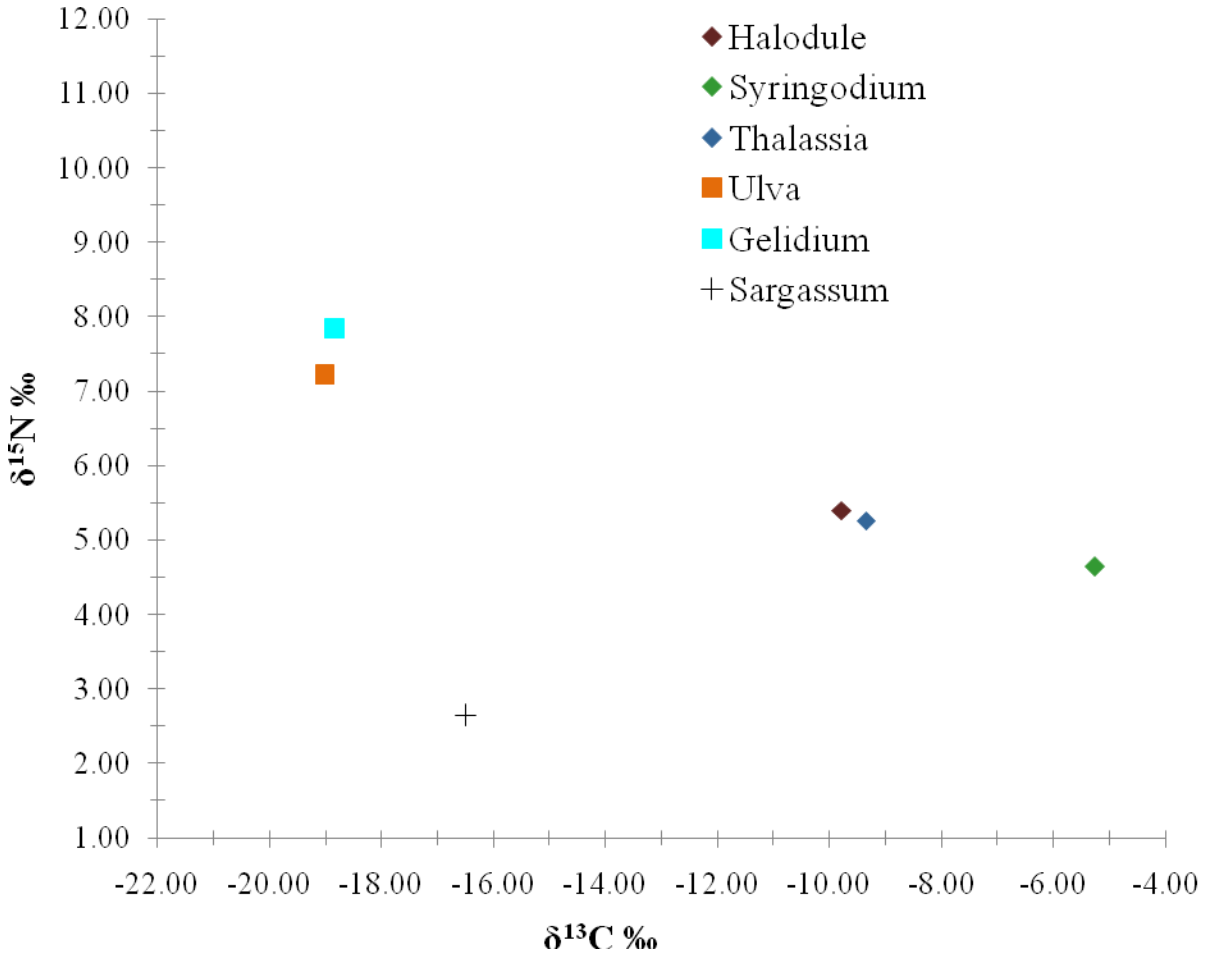

Fig. 7a: Mean isotopic signatures of seagrass and algae sampled from Port Isabel, Texas. *Sargassum* value modified from Rooker et al. (2006)

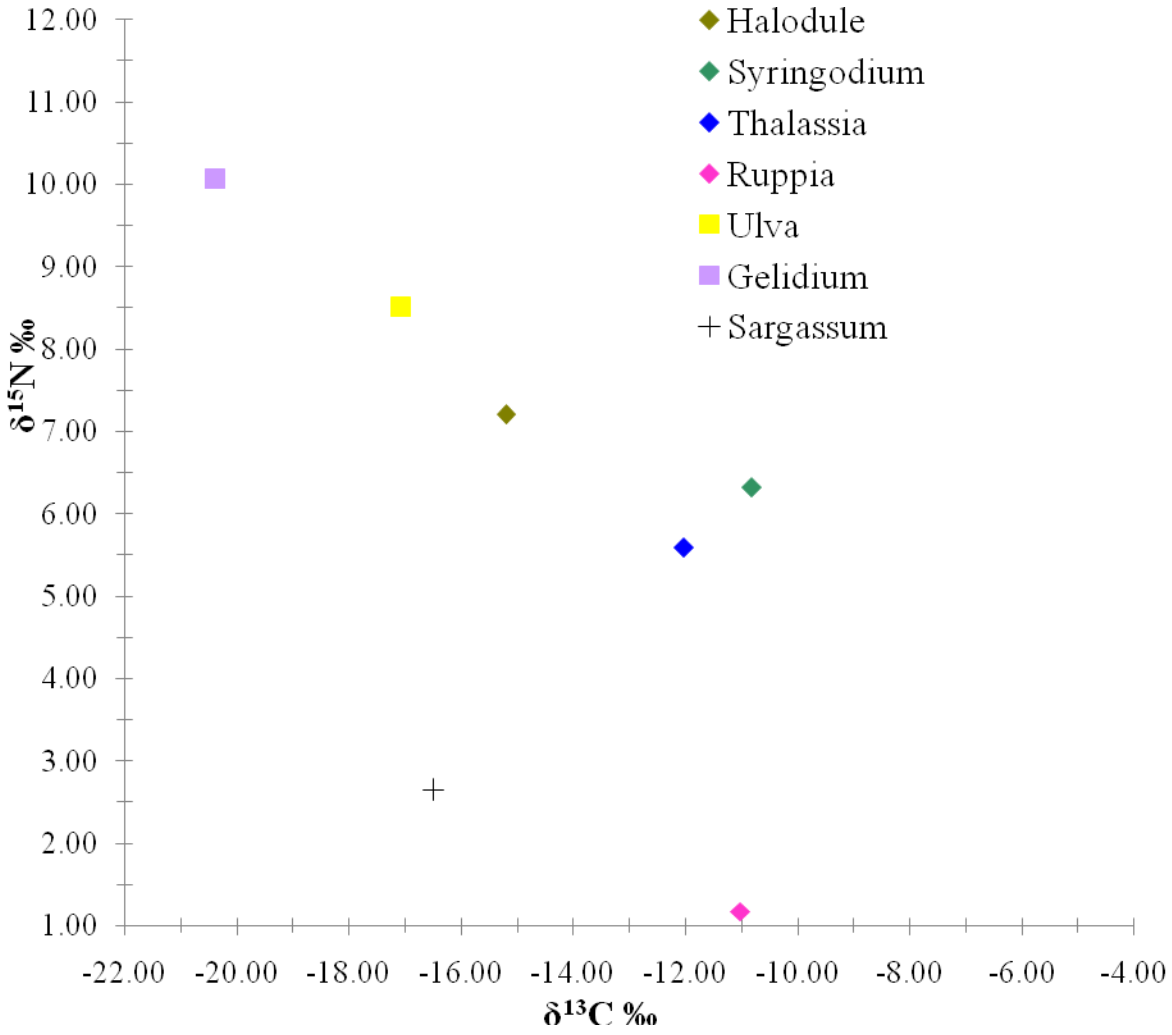

Fig. 7b: Mean isotopic signatures of seagrass and algae sampled from Port Aransas, Texas. *Sargassum* value modified from Rooker et al. (2006)

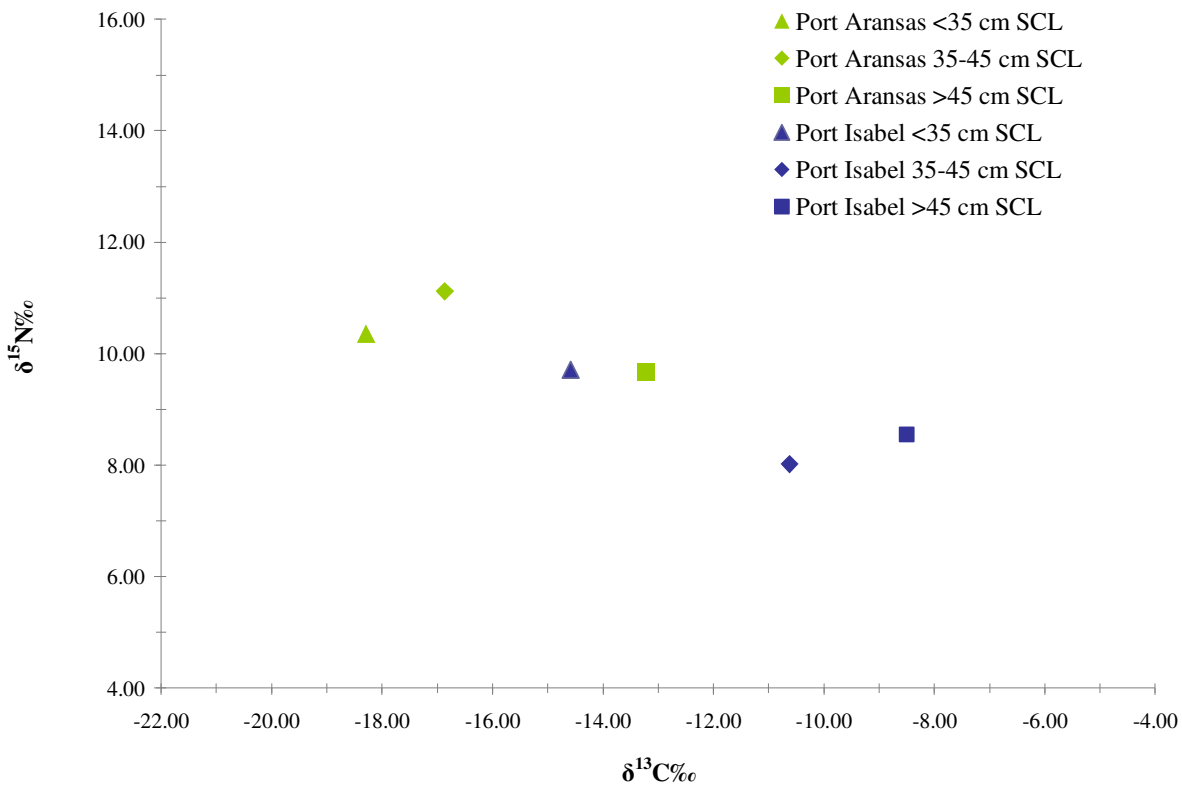

Fig. 8: Mean $\delta^{13}C$ and $\delta^{15}N$ values for all size classes of green turtles from Port Aransas and Port Isabel, Texas.

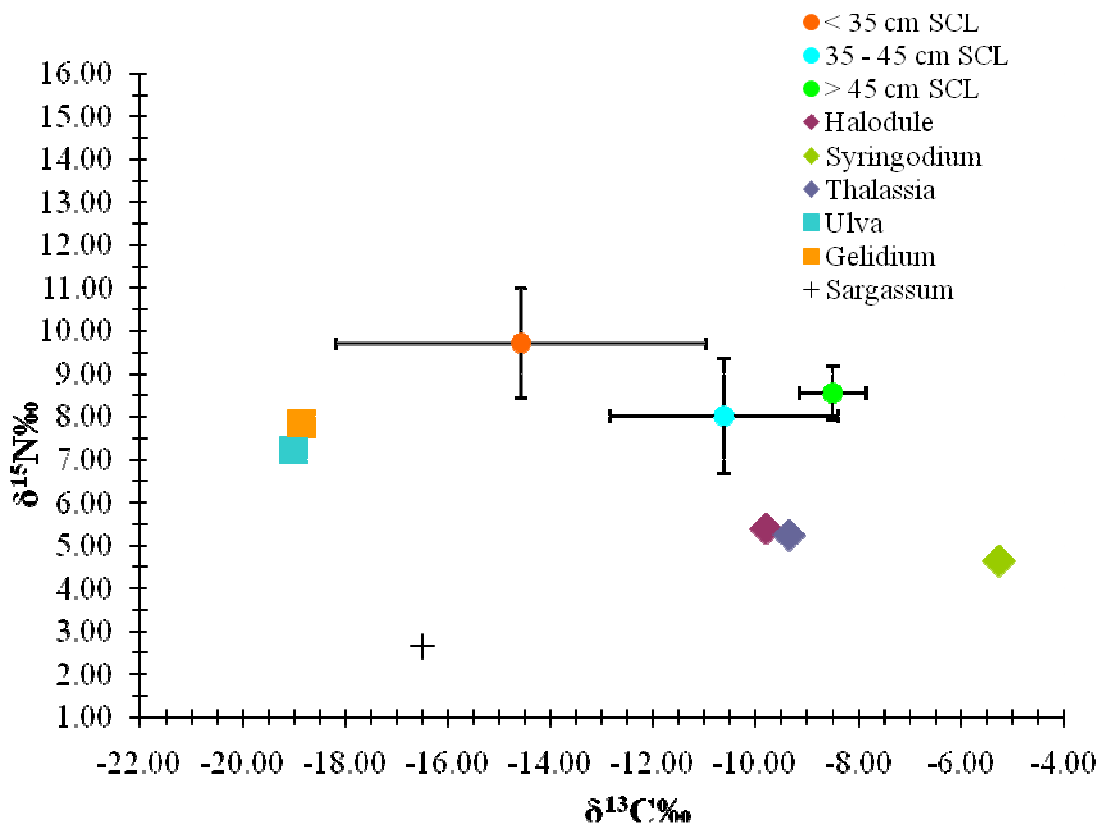

Figure 9: Mean $\delta^{13}C$ and $\delta^{15}N$ (± sd) values for newest tissues of green turtles collected from Port Isabel, Texas sampling sites. ● indicates turtle size class ■ indicates algae species. ◆ indicates seagrass species *Sargassum* value modified from Rooker et al. (2006)

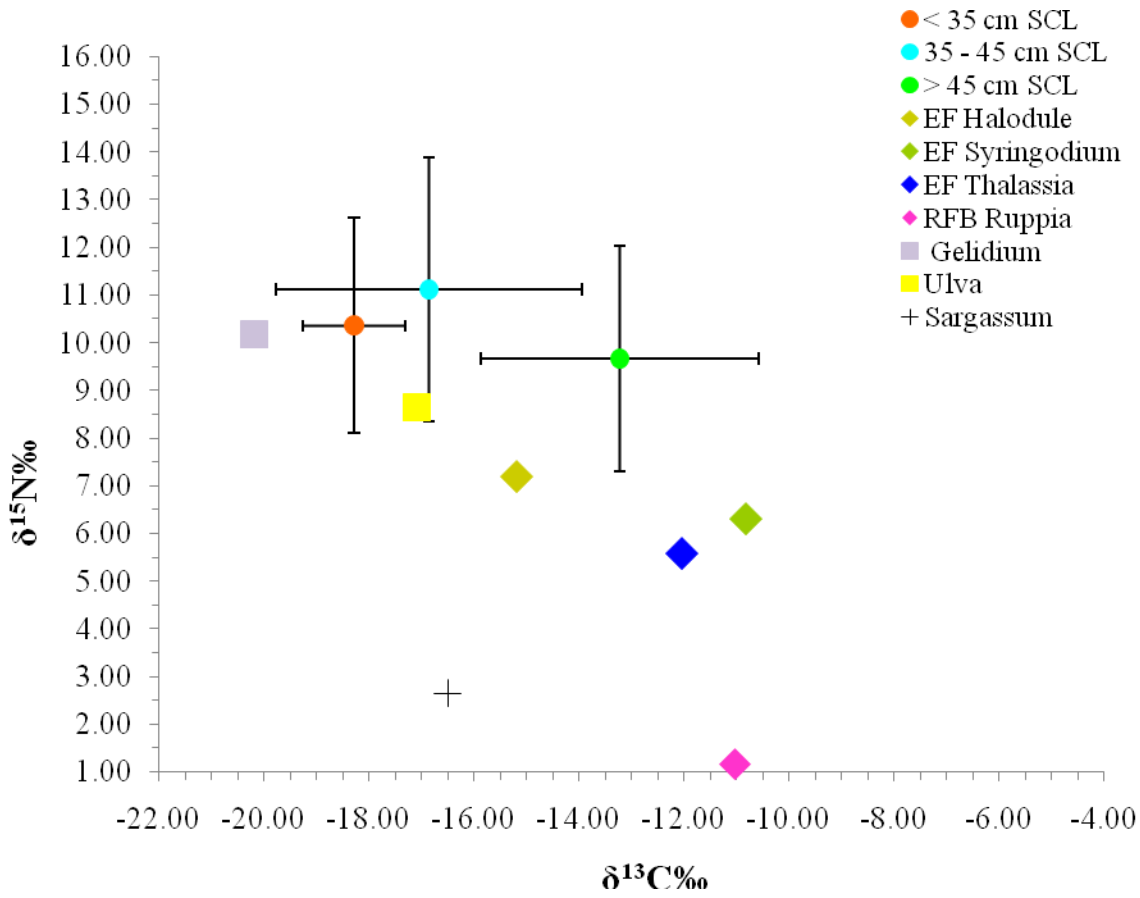

Figure 10: Mean $\delta^{13}C$ and $\delta^{15}N$ (± sd) values for newest tissues of green turtles collected from Port Aransas, Texas sampling sites. ● indicates turtle size class ■ indicates algae species. ◆ indicates seagrass species. *Sargassum* values modified from Rooker et al. (2006)

Fig. 11a

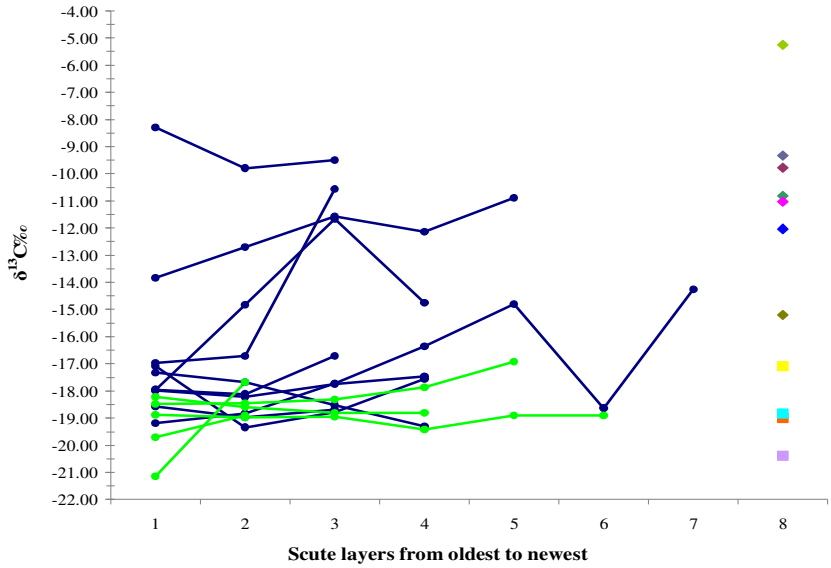

Fig. 11b

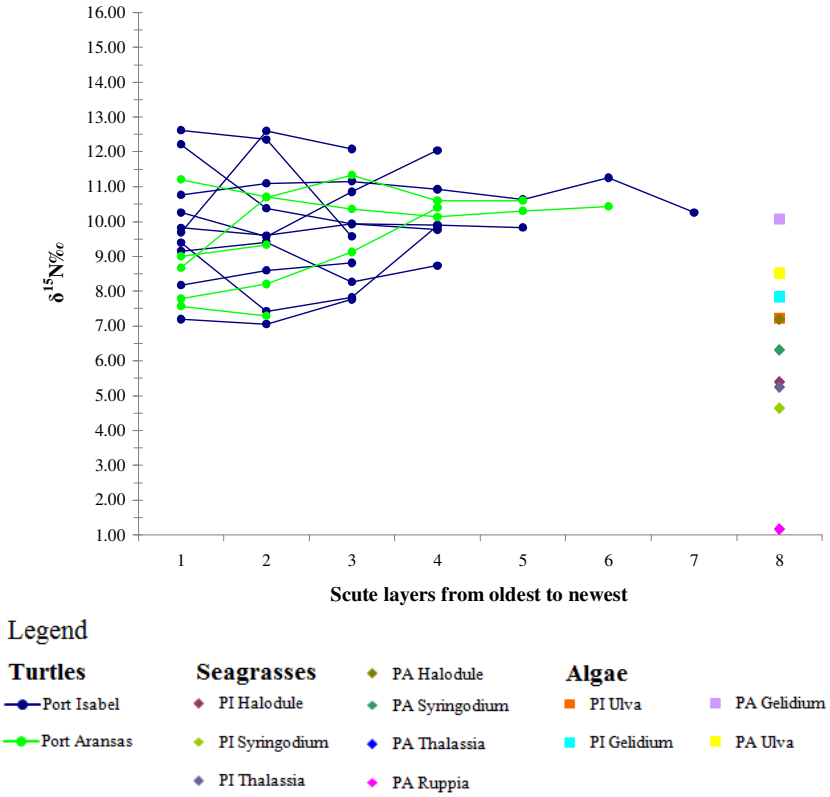

Figure 11 a, b: Stable carbon and nitrogen isotopic profiles for green turtles < 35 cm SCL from Port Isabel and Port Aransas, Texas.

Fig. 12a

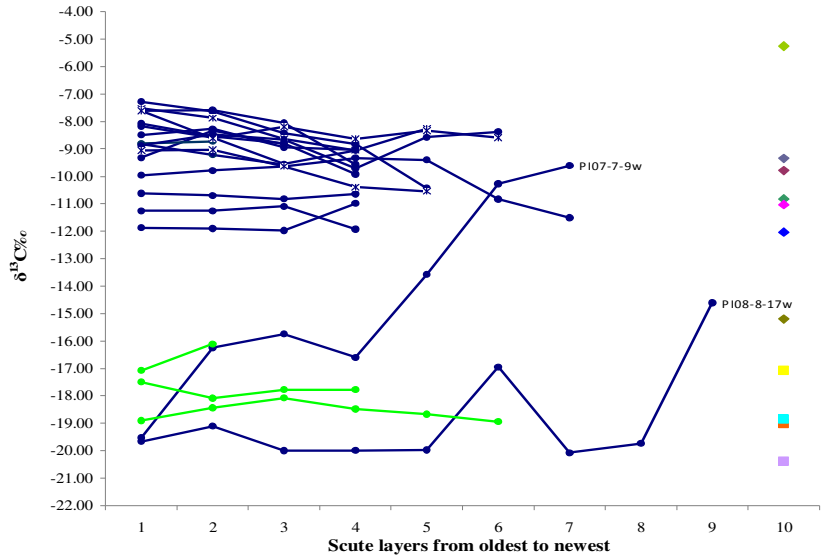

Fig. 12b

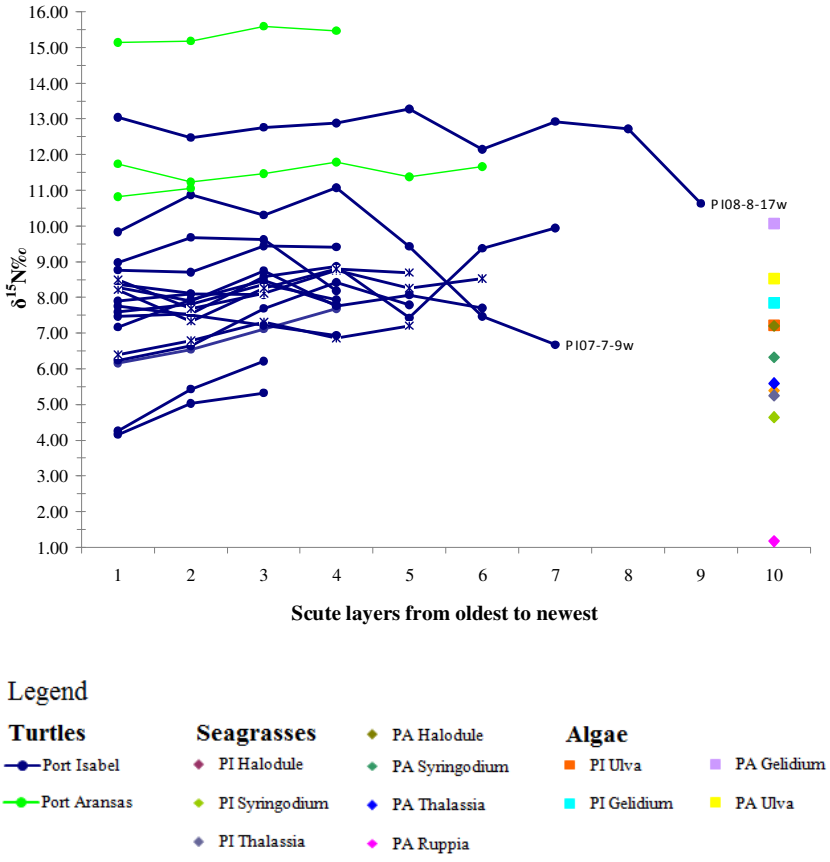

Figure 12 a, b: Stable carbon and nitrogen isotopic profiles for green turtles 35 – 45 cm SCL from Port Isabel and Port Aransas, Texas.

Fig. 13a

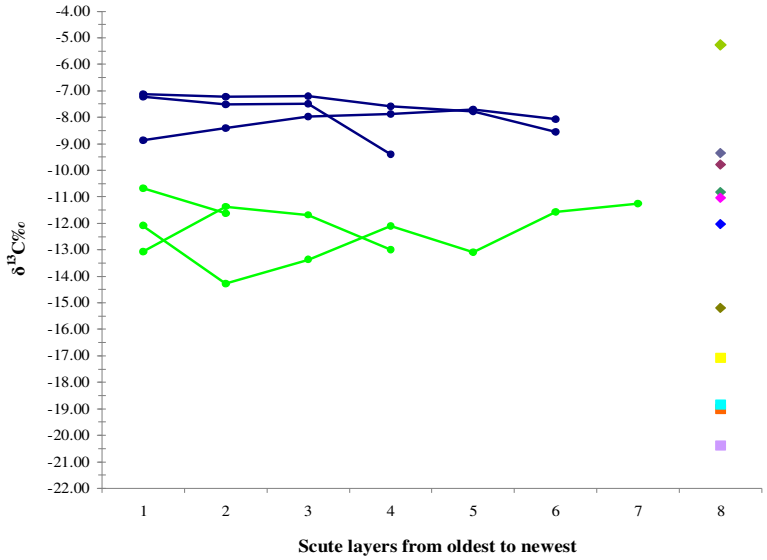

Fig. 13b

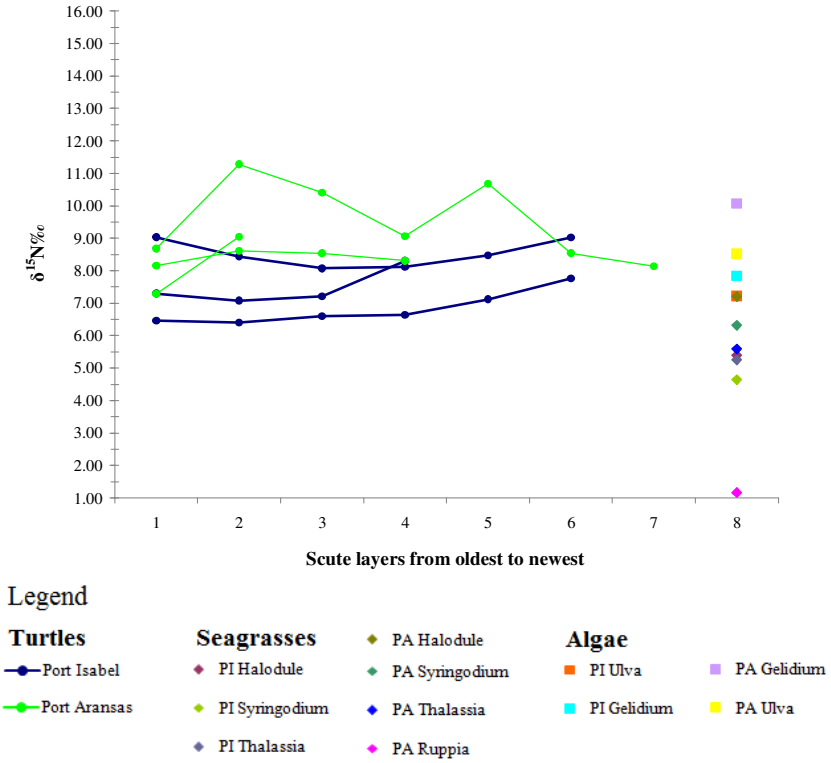

Figure 13 a, b: Stable carbon and nitrogen isotopic profiles for green turtles > 45 cm SCL from Port Isabel and Port Aransas, Texas.

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