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## Using Biomarkers to Assess the Migratory Ecology and Reproduction of the Florida Green Turtle (*Chelonia mydas*)

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*University of Central Florida*



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USING BIOMARKERS TO ASSESS THE MIGRATORY ECOLOGY AND REPRODUCTION OF THE  
FLORIDA GREEN TURTLE (CHELONIA MYDAS)

by

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B.S. University of Central Florida, 2011

A thesis submitted in partial fulfillment of the requirements  
for the degree of Master of Science  
in the Department of Biology  
in the College of Sciences  
at the University of Central Florida  
Orlando, Florida

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Major Professor: Katherine L. Mansfield

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## ABSTRACT

Migratory connectivity between breeding and foraging areas is a vital component of the ecology of a diverse collection of marine vertebrates. Habitat quality, composition, and resource availability at these locations have direct ramifications for individual fitness. The green turtle (*Chelonia mydas*) is a long-lived, highly migratory species of conservation concern. Important green turtle nesting habitat in Florida is protected, but more information is needed to identify foraging habitats and the influence these habitats have on reproduction. Here, I used stable isotope analysis of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  and satellite telemetry validation to determine the number of putative foraging areas used by the breeding aggregation at the Archie Carr National Wildlife Refuge (ACNWR), and the relative contribution of each foraging area. I evaluated the influence of foraging area and other variables on egg size, clutch size, hatching success, and emerging success using model selection frameworks. Isotopic values of skin and eggs were used to build conversion equations between the two tissue types. Results suggest strong migratory connectivity between the ACNWR and the Florida Keys/Florida Bay complex. I found that the influences of foraging area are likely to be more detectable when evaluating female-centric fitness metrics like clutch size and egg size; these influences are more muted in hatching and emerging success, which are strongly influenced by nest incubation conditions. These are the first green turtle-specific tissue conversion equations for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and the first  $\delta^{34}\text{S}$  equation for any marine turtle species. These will allow researchers to have a “common currency” between frequently collected samples to better compare results.

Dedicated to my grandparents, Dixie Maddox, Carol Chabot, and Richard Chabot. Though they have passed on from this world, their ceaseless love lives on in my heart forever.

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# CHAPTER 1: BIOMARKERS REVEAL STRONG MIGRATORY CONNECTIVITY IN THE FLORIDA GREEN TURTLE (CHELONIA MYDAS)

## Introduction

Migration is a fundamental aspect of the life histories of a diverse collection of marine vertebrates, including seabirds, sharks, whales, and sea turtles (Weng et al. 2008; Witteveen et al. 2009; Egevang et al. 2010; Ceriani et al. 2012). Among longer-lived vertebrates, migratory behavior is often exhibited as round-trip seasonal movements between foraging and breeding habitats (Dingle and Drake 2007). Some of these transitions can occur over tens of thousands of kilometers (e.g., short-tailed shearwaters, Skira 1991) with significant individual energetic investment. Knowledge of spatiotemporal patterns of these movements is important for understanding connectivity between these habitats.

Migratory connectivity (*sensu* Webster et al. 2002) between breeding and foraging areas is a vital component of the ecology of these organisms. Habitat quality, composition, and resource availability at both locations have direct ramifications for individual fitness (Marra et al. 1998; Norris et al. 2004; Harrison et al. 2011). Migratory connectivity has been described along a gradient between weak and strong (Webster et al. 2002), representing the endpoints of a continuum of habitat use transition. Here, we modify the paradigm found in Webster et al. (2002) and define these endpoints for “strong connectivity” as single foraging contribution and “weak connectivity” as proportionate foraging contribution. Between these are breeding aggregations comprised of different proportions of individuals from more than one foraging aggregation, or disproportionate foraging contribution (Figure 1). Theoretically, single or



heavily skewed disproportionate foraging contribution systems highlight the importance of one foraging area to the reproductive output of a breeding population. However, small-scale disturbances at these foraging sites could have dramatic impacts on the survival and fecundity of the breeding population (e.g. red knots, Baker et al. 2004). For species of conservation concern, an understanding of the migratory connectivity among subpopulations is critical for adaptive management.

Knowledge of connectivity can be extremely useful for creating predictive models of habitat use; however, there is an obvious need for additional data for a variety of marine species and ocean regions. Direct observations and recapture of organisms along migratory routes is difficult. Satellite telemetry historically represented the only logistically feasible means to assess where an organism travelled and what habitats were encountered. Although this technology dramatically improved over time with increasing affordability and refined accuracy, it is expensive and often precludes access to robust sample sizes. As such, the use of novel approaches to complement satellite telemetry are needed. Alternative and complementary techniques such as stable isotope analysis (SIA) can provide coarse descriptions of habitat use at considerably lower costs, allowing researchers to sample a wider breadth of the population. Stable isotope analysis of sampled tissue provides information on trophic position and geographic foraging distributions. The ratio of heavy to light isotopes of common elements found in nature (e.g., carbon, nitrogen, sulfur) vary across space and time and can be used to characterize habitats of origin (Ben-David & Flaherty 2012). The stable isotope values in consumer tissues reflect those from their diet, and can provide intrinsic markers for identifying

foraging location over various time scales if the organism moves among isotopically distinct locations (Hobson 1999; Rubenstein & Hobson 2004).

Determining spatial linkages to foraging habitats for migrant breeders requires explicit knowledge of isotope ratios in these regions. This may be accomplished using spatially-explicit isoscapes (e.g., McMahon et al. 2013) or through the use of tracking (active or passive) a subsample of individuals to foraging areas to use as “samplers” of the isotope ratios found at these sites. Stable isotope analysis has been successfully applied in a number of marine turtle studies to delineate distinct foraging aggregations of females that utilize the same nesting beach (Hatase et al. 2002; Caut et al. 2008; Ceriani et al. 2012; Seminoff et al. 2012; Vander Zanden et al. 2013). Tissues with slow isotopic turnover rates (e.g., skin, blood, eggs) can be used to assess isotope ratios incorporated at foraging areas before migration to the nesting beach begins (Seminoff et al. 2007; Reich et al. 2008; Ceriani et al. 2014a).

The green turtle (*Chelonia mydas*) is a long-lived, highly migratory species that habitually exhibits nest and foraging site fidelity as adults (Limpus et al. 1992; Broderick et al. 2007). Reproductive females generally display natal philopatry, with nesting aggregations being composed of females from two or more foraging sites (Table 3). Nesting in the northwest Atlantic occurs throughout the greater Caribbean, Gulf of Mexico, and east coast of the United States, with major nesting beaches in Costa Rica, Mexico, Suriname, Venezuela, and Florida, USA (Seminoff et al. 2015), and known adult foraging areas in Nicaragua, the Bahamas, and Florida (Vander Zanden et al. 2013; Schroeder et al. 2008; Bresette et al. 2010). Growing evidence suggests that the portion of the Atlantic green turtle population that nests in east

central Florida is one of the fastest growing globally (Chaloupka et al. 2008; UCF Marine Turtle Research Group unpub data.). This is especially true of the Archie Carr National Wildlife Refuge (ACNWR) rookery, which contains approximately 32% of the green turtle nests laid within the Florida each year (FWC-FWRI 2016). Schroeder et al. (2008) and Bagley (unpub. data) used satellite telemetry to track green turtles (n = 11 and n = 10, respectively) to post-nesting feeding locations. Results of these studies identify foraging areas in the Florida Keys/Florida Bay region, off the southeastern coast of peninsular Florida, and in the Bahamas. The small sample sizes preclude interpretation of the relative importance or contribution of these foraging areas, and may not include all foraging aggregations that are utilized by this rookery.

Green turtles in Florida are currently classified as threatened under both the Endangered Species Act and the IUCN Red List; an increase in nest numbers at east central Florida rookeries (including the ACNWR) may indicate regional population recovery (Seminoff et al. 2015). Although green turtle nesting habitat in Florida is protected, more information is needed to identify foraging habitats and threats to these sites. In this study, we used stable isotope analysis in conjunction with satellite telemetry validation to (1) determine the number of adult green turtle foraging areas used by the breeding population at the ACNWR, and (2) the contribution of each of these areas to the nesting population. These data provide insights for our understanding of the migratory connectivity of this rookery, which is likely representative of the region. This study is the first to link SIA and telemetry to understand green turtle migratory ecology in the northwest Atlantic USA.

## **Methods**

### ***Study Site***

Our study was conducted on the 21-km Brevard county portion of the Archie Carr National Wildlife Refuge, located in Melbourne Beach, Florida, USA (Figure 2). The ACNWR beach is a mosaic of privately- and publicly-owned lands, with minimal armoring and general patterns of fall and winter sand erosion followed by spring and summer accretion. Green turtle nesting numbers from this rookery follow a relatively consistent pattern, with a “high” year (in terms of nest numbers) being followed by a “low” year (UCFMTRG unpublished data). This study takes advantage of this biennial pattern, with sampling of untracked females occurring during the “high” 2013 nesting season and the “low” 2014 season.

### ***Turtle Sampling and Measurement***

Fifty-two untracked nesting female green turtles were sampled in 2013, and 50 in 2014. Additionally, samples were collected from 15 satellite-tracked individuals, nine females and six males between 2013-2015. One of the nesting females satellite-tagged by Schroeder et al. (2008) was observed nesting again in 2013, and was included in this study as a satellite-tracked turtle. Individual untracked females were sampled from June through September of each year following a spatial distribution to approach equal coverage across the study area. Weekly sampling effort followed predicted trends through the nesting season based on the previous 5 years of weekly nest numbers (2013 range: 1-5 females, 2014 range: 1-4 females). Straight

carapace length (SCL) was recorded for each sampled individual. Two Inconel flipper tags were applied per turtle (one to each front flipper) and passive integrated transponder (PIT) tags were inserted subcutaneously in the front right flipper to prevent resampling. Two skin biopsies were obtained from sampled individuals using a sterile 4 mm biopsy punch. In 2013, one shoulder biopsy was obtained from the right shoulder midway between the neck and flipper, and another skin sample was acquired by splitting a rear flipper biopsy. In 2014 and 2015, two shoulder biopsies were obtained from sampled turtles. Similar anatomical sampling locations have been used in loggerhead (*Caretta caretta*) turtles with no significant differences in isotopic signatures between sites (Ceriani unpublished data).

### ***Tissue Storage and Processing***

Samples from 2013 were frozen immediately following collection, then transported to the University of Central Florida and stored in a -20°C non-frost-free freezer. Samples from 2014 were stored in 70% ethanol at room temperature. Barrow et al. (2008) found no significant difference in isotope values preserved in 70% ethanol compared to controls, and Hobson et al. (1997) suggested storage in 70% ethanol as a viable alternative to the most common preservation method, freezing. Connective tissue was removed from skin with a scalpel blade, and then skin was sliced into small pieces. These were placed in a freeze drier for 12 hours. Lipids were removed using petroleum ether as solvent in a soxhlet device for 24 hours.

### **Sample Preparation and Stable Isotope Analysis**

Stable isotope values are typically expressed as a comparison of the heavy to light isotope in question to an international standard.

$$\delta X = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1,000 \quad (1)$$

Where  $\delta$  (delta) is the isotope symbol, X is the heavy isotope of the element in question (e.g.  $^{34}\text{S}$ ), and R is the heavy to light isotopic ratio (e.g.  $^{15}\text{N}:^{14}\text{N}$ ), expressed as parts per thousand (‰). Sample processing and analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  only) followed the methodologies described in Ceriani et al. (2014a), while analysis of  $\delta^{34}\text{S}$  followed methodologies laid out in Tucker et al. (2014). Approximately 0.5 to 1.0 mg of each skin sample was placed in a small tin capsule and sent for analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Nitrogen and carbon isotope and bulk composition were measured by CF-EA-IRMS (Continuous Flow Elemental Analyzer Isotope Ratio Mass Spectrometry) at the University of South Florida College of Marine Science Stable Isotope Biogeochemistry Laboratory on a ThermoFinnigan Delta+XL IRMS, are reported in per mil (‰) notation, and are scaled to VPDB ( $\delta^{13}\text{C}$ ) and AT-Air ( $\delta^{15}\text{N}$ ) (Werner et al. 1999). Secondary references were used to normalize raw measurements to the VPDB ( $\delta^{13}\text{C}$ ) and AT-Air ( $\delta^{15}\text{N}$ ) scales (Werner et al. 2002, Qi et al. 2002, Coplen et al. 2006) and to calibrate elemental N, C and C:N. Measurement uncertainties, expressed as  $\pm 1$  standard deviation of  $n = 25$  measurements of a laboratory reference material, were  $\pm 0.23\text{‰}$  for  $\delta^{13}\text{C}$  and  $\pm 0.10\text{‰}$  for

$\delta^{15}\text{N}$ . For  $\delta^{34}\text{S}$  analyses approximately 3 mg of skin was placed into a tin capsule and sent to Washington State University Stable Isotope Core Laboratory. These samples were analyzed with a ThermoFinnigan Delta PlusXP continuous flow isotope ratio mass spectrometer (Brenna et al. 1997) with a measurement uncertainty, expressed as  $\pm 1$  standard deviation of  $n = 8$  measurements of a laboratory reference material, of  $\pm 0.09\text{‰}$  for  $\delta^{34}\text{S}$ . Sulfur isotopic ratios are reported in per mill relative to VCDT by assigning a value of  $-0.3$  per mill to IAEA S-1 silver sulfide (Coplen and Krouse 1998).

### ***Satellite Transmitter Attachment***

Transmitters (Wildlife Computers SPOT-352B) for the three nesting females tracked during the 2015 nesting season were attached using methods commensurate with Mansfield et al. (2009), using AnchorFix™ two part adhesive as a base layer covered by SonicWeld™ putty epoxy. Position information was provided by Service ARGOS. The other 12 satellite-tracked turtles sampled for this study are part of a different research project, with terminal position data (Figure 2) provided by Bagley et al. (in prep).

### ***Cluster Analysis and Isotope Patterns***

Due to the greater amount of tissue required for  $\delta^{34}\text{S}$  analyses, we collected enough epidermis to acquire isotope ratios for only 115 of the 119 turtles sampled for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  (Table 1). From a theoretical perspective, individuals that forage closer to one another

should incorporate isotopic ratios that are more similar than individuals that are more distant, provided they have a similar diet/feed at the same trophic level. Foraging adult green turtle populations in the northwest Atlantic occupy very similar trophic levels, feeding on seagrasses (primarily *Thalassia testudinum*) and red and green algae (Bjorndal 1997). To evaluate numerical isotope data for patterns of clustering we used the package “mclust” in the R (CRAN) statistical framework (R Core Team 2014; Fraley et al. 2012; Fraley and Raftery 2002). Functions within the package generate a series of normal mixture models fitted using an EM algorithm with varying covariance parameterizations and number of clusters. The model with the highest BIC score was selected and used to classify individual turtles into putative foraging clusters and provide information on model classification uncertainty. We chose to use this model-based clustering assignment method over others (e.g. discriminant function analysis, Ceriani et al. 2012), to allow direct statistical interpretation of trends in isotopic space, rather than potentially biasing assignment by defining distinct foraging areas within the small geographic area identified by Schroeder et al. (2008) and Bagley et al. (in prep). For each isotope, model selection using AICc was performed on a suite of additive and multiplicative models to test for the effects of size (SCL) and year on isotopic patterns in R (CRAN) using the package “bbmle” (Bolker and R Core Team 2014; R Core Team 2014). Models with  $\Delta AICc$  less than 2.0 were considered indistinguishable. These models only included females from 2013 and 2014, as sample sizes for males in all years and females in 2015 were too low to incorporate and would likely bias model performance.



## Results

### ***Telemetry***

For nesting females satellite-tracked in 2015 ( $n = 3$ ), tags ceased transmitting prior to turtles' arrival in their post-nesting habitats. Ten of the turtles tracked by Bagley et al. (in prep) transmitted long enough to establish foraging areas (Table 2). Nine of these individuals (4 females, 5 males) migrated to the region around the Florida Keys and Florida Bay, while one male migrated to the coastal waters off of southeastern peninsular Florida (Figure 2). The sampled female previously tracked by Schroeder et al. (2008) established a foraging area near the Marquesas Keys, Florida (position not included in Figure 2).

### ***Cluster Analysis***

Cluster analysis using stable isotope values of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  identified a model containing three clusters with variable volume, equal shape, and orientation along the coordinate axes as the best parameterization of the data using BIC (model VEI, 3 components, Figure 3). Of these 115 turtles, 88 were classified into Cluster 1 (black circles, Figure 4A), 5 into Cluster 2 (grey triangles, Figure 4A), and 22 into Cluster 3 (open squares, Figure 4A). Figure 4B illustrates an elevated degree of classification uncertainty for individuals in the isotopic border region between Cluster 1 and 3. Of the 11 satellite-tracked turtles whose foraging areas were known, seven had foraging locations on the Gulf of Mexico side of the Florida Keys or around the Marquesas Keys), while one individual occupied an area southeast of Key Largo. These

turtles were assigned to Cluster 1 (Figure 2). Of the three remaining turtles, one foraging off the southeastern coast of peninsular Florida was assigned to Cluster 2, while 2 foraging south of Big Pine Key, Florida were assigned to Cluster 3 (Figure 2). Based on a chi-squared test of count data, there is no indication of significant differences in cluster contribution to the nesting population between 2013 and 2014.

### ***Isotope Patterning Model Selection***

Model selection using AICc was performed on a suite of 6 models (including the null model) with  $n = 109$  turtles for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  models and  $n = 106$  turtles for  $\delta^{34}\text{S}$  models. For  $\delta^{15}\text{N}$  models, the null model was most informative, with no other models having a  $\Delta\text{AICc}$  value less than 2.0. Although two other models for  $\delta^{34}\text{S}$  had  $\Delta\text{AICc}$  value less than 2.0, they remain indistinguishable from the null based on the above criteria, and are considered uninformative. Isotopic patterns influenced by size and year did appear in the results for  $\delta^{13}\text{C}$  model selection, however. The top two models combined carried 99% of the model weight: SCL plus year, and SCL plus year and the interaction of SCL and year, respectively. Evaluating the terms within the top model revealed that larger turtles had significantly ( $p < 0.001$ ) depleted (more negative)  $\delta^{13}\text{C}$  values, and turtles in 2014 had significantly ( $p < 0.001$ ) more enriched (less negative)  $\delta^{13}\text{C}$  values than 2013 turtles.

## Discussion

This study is the first to examine the migratory connectivity of Florida green turtles using stable isotope analysis in combination with satellite telemetry, and is one of the first marine turtle studies to incorporate  $\delta^{34}\text{S}$  values (Table 3). The reduced cost of SIA compared to traditional tracking technologies permitted us to investigate a wider breadth of individuals using the ACNWR green turtle rookery, while the inclusion of a subset of transmitter foraging locations allowed us to examine the most informative output of clustering scenarios for accuracy. Combined approaches like these augment efforts to better assess migratory patterns of highly migratory organisms on the population and individual scales.

Based solely on the cluster analysis using stable isotope values, model results indicate a disproportionate contribution of the putative Cluster 1 foraging area to the ACNWR nesting population (77%), followed by Cluster 3 (19%), and Cluster 2 (4%). As evident in Figure 4A, model classification differences appear to be largely driven by  $\delta^{15}\text{N}$  values. Although isotope values for Cluster 1 and Cluster 3 appear to be tightly grouped, the large spread in values for Cluster 2, particularly in the  $\delta^{13}\text{C}/\delta^{15}\text{N}$  biplot (Figure 4A) draws into question the validity of those individuals being placed into the same cluster, if the assumption that individuals foraging closer to one another should exhibit more similar isotope ratios holds.

Model selection results indicating larger turtles exhibit more depleted  $\delta^{13}\text{C}$  values may suggest that these larger turtles feed at higher latitudes, deeper waters, more pelagically, or any combination of these (Reich et al. 2009). However, as almost all satellite turtles foraged within the Florida Keys/Florida Bay region (an area with a small latitudinal gradient), and as

foraging adult populations feed primarily on *Thalassia testudinum* and (to a lesser extent) red and green algae (Bjorndal 1997), foraging higher in the water column is unlikely; therefore, larger turtles feeding in deeper waters is the most plausible of these potential drivers of  $\delta^{13}\text{C}$  variation. Although  $\delta^{13}\text{C}$  values varied between 2013 and 2014, the relative contributions of foraging clusters did not. Differences in environmental cycling could contribute to this  $\delta^{13}\text{C}$  variation. However, additional samples from subsequent “high” and “low” years would be needed to begin addressing whether this pattern is maintained throughout multiple cycles of this biennial pattern.

Satellite telemetry data validated important aspects of the clustering output (Figure 2). The male foraging off the southeastern coast of peninsular Florida, assigned to Cluster 2, had the most depleted  $\delta^{13}\text{C}$  value ( $-18.61\text{‰}$ ) and the most enriched  $\delta^{34}\text{S}$  value ( $16.92\text{‰}$ ) of all 115 turtles included in this study. As this individual was the only satellite-tracked turtle to not transition to the Florida Keys/Florida Bay region, its segregation from those individuals in geographic space is reflected in isotopic space. Although four other turtles were assigned to Cluster 2, their spread within isotopic space calls into question the accuracy of assigning them to the same foraging cluster. The lack of surrounding data within isotopic space could have influenced their inclusion within the same cluster. It is possible that some of these individuals should be assigned to Cluster 1 or 3, or perhaps originated from unknown foraging areas that contribute disproportionately low amounts of turtles to the nesting population.

When considered separately from cluster analysis results, the relatively tight distributions of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Figure 5) and satellite telemetry data would suggest a large

majority contribution from one foraging area, the Florida Keys/Florida Bay complex. Although the range in  $\delta^{34}\text{S}$  is broad, the mean and standard deviation of  $\delta^{34}\text{S}$  values in this study ( $9.04^{0}/_{00} \pm 2.48$ , Table 1) is very similar to the values Tucker et al. (2013) found for loggerheads foraging in the Florida Keys ( $8.81^{0}/_{00} \pm 4.08$ ). Research investigating intra- and interpopulation differences in  $\delta^{34}\text{S}$  values of the saltmarsh plant *Spartina alterniflora* found elevated levels of intrapopulation variability, but also rapid shifts in values at small geographic distances between populations (Connolly et al. 2004). Connolly et al. (2004) also recommended the incorporation of  $\delta^{34}\text{S}$  values into SIA, as sulfur isotope ratios outperformed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in separating producers isotopically.

Satellite telemetry data indicate Florida Keys foraging areas close enough together to be well within the 100-km limit of isotopic resolution in the region due to the Loop Current suggested by Tucker et al. (2014). Yet, cluster analysis results still identified two clusters (Cluster 1 and 3) into which turtles foraging in the Florida Keys/Florida Bay complex were assigned. The only two satellite-tracked individuals assigned to Cluster 3 both foraged south of Big Pine Key, on the Atlantic side of the Florida Keys (Figure 2). An analysis of isotope patterns for *Thalassia testudinum* within the Florida Keys (Fourqurean et al. 2005) shows little structure for  $\delta^{13}\text{C}$  values, but identifies larger, more structured regions of enriched  $\delta^{15}\text{N}$  values that overlap with the foraging area for satellite-tracked turtles assigned to Cluster 3. This lends support to the cluster analysis results, in which  $\delta^{15}\text{N}$  values were the primary driver of differences in assignment for individuals into Cluster 1 or 3 (Figure 4A). The  $\delta^{15}\text{N}$  values of the ACNWR breeding population in this study ( $7.20^{0}/_{00} \pm 1.15$ ), however, are closer to the Tucker et

al. (2014) foraging loggerhead values for the Northern Caribbean ( $7.26^{0}/_{00} \pm 1.21$ ) including the Bahamas and Cuba, rather than the Florida Keys ( $8.43^{0}/_{00} \pm 3.28$ ). This may indicate a reduced  $\delta^{15}\text{N}$  structure in the larger region, although differences in trophic position between green turtles and carnivorous loggerheads hinder direct interpretation of this comparison. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of satellite-tracked green turtles in this study fall within the predicted isoscape interpolations generated from loggerhead data by Ceriani et al. (2014b). The large border region of classification uncertainty between Cluster 1 and 3 (Figure 4B) implies that assignment using isotopes alone may be impractical for green turtles foraging within this relatively small geographic region. It appears that the Florida Keys/Florida Bay complex functionally operates as a single foraging region, although more fine-scale structuring may be assessed with increased sampling from individuals at their foraging location.

Conceptually (Figure 1), we would expect nesting beaches with single or heavily disproportionate foraging contributions to exhibit narrower, highly unimodal distributions of  $\delta^{13}\text{C}$  values; this assumption appears verified by the relatively small variation ( $-8.82^{0}/_{00} \pm 1.67$ , Table 1) in  $\delta^{13}\text{C}$  in Figure 5, and validated by transmitter data. In contrast, loggerheads nesting in the ACNWR dispersed to three (Table 3) post-nesting foraging regions, and displayed a wider breadth of  $\delta^{13}\text{C}$  values ( $-14.61^{0}/_{00} \pm 2.48$ , Ceriani et al. 2012). These lines of evidence suggest a highly disproportionate contribution of the Florida Keys/Florida Bay complex foraging area to the green turtle ACNWR rookery in central Florida.

The recovery of threatened and endangered species is often hampered by the transboundary nature of animal movements and the need for multiple nations to work together

to form conservation partnerships. The fact that both endpoints of the migration routes for the majority of green turtles nesting in the ACNWR appear to fall within the state and federal waters of the United States allows for more concerted management of this rookery and its in-water habitat, some of which is already protected (e.g. Florida Keys National Marine Sanctuary and Key West National Wildlife Refuge). In theory, strongly disproportionate migratory connectivity (Figure 1) has benefits and risks for wildlife populations and managers. Movements of the majority of the breeding aggregation to one geographic foraging area make them easier to locate and protect. However, these aggregations are more vulnerable to fine spatial scale perturbations that can have direct and long-lasting ramifications for the survival and reproduction of the population.

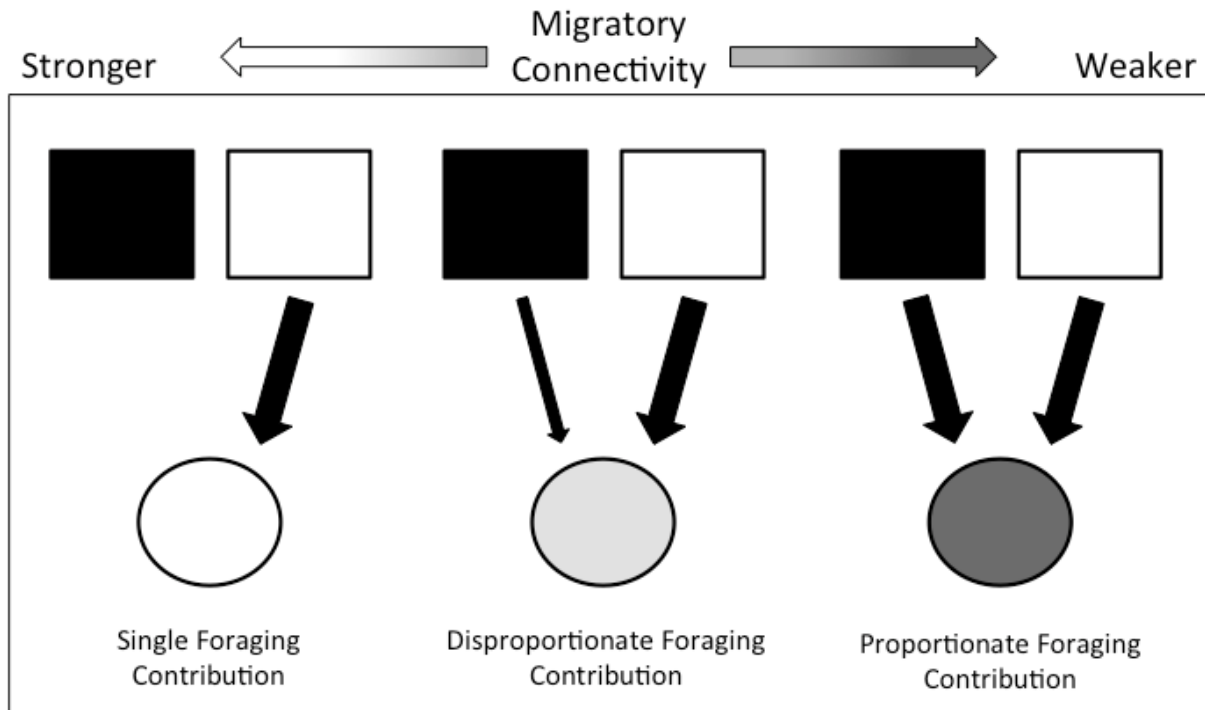


Figure 1: Conceptual model modifying the definitions of “strong” and “weak” migratory connectivity endpoints described in Webster et al. (2002). Here, systems on the “strong” endpoint are described as having single foraging contributions, in which a breeding population (circle) is made up solely of individuals from one foraging population (square). On the opposing end of the spectrum, “weak” connectivity breeding populations are described as having approximately proportionate contributions of individuals from any number of foraging areas. Between these two endpoints lie breeding populations that have some level of disproportionate contributions of individuals from different foraging populations.



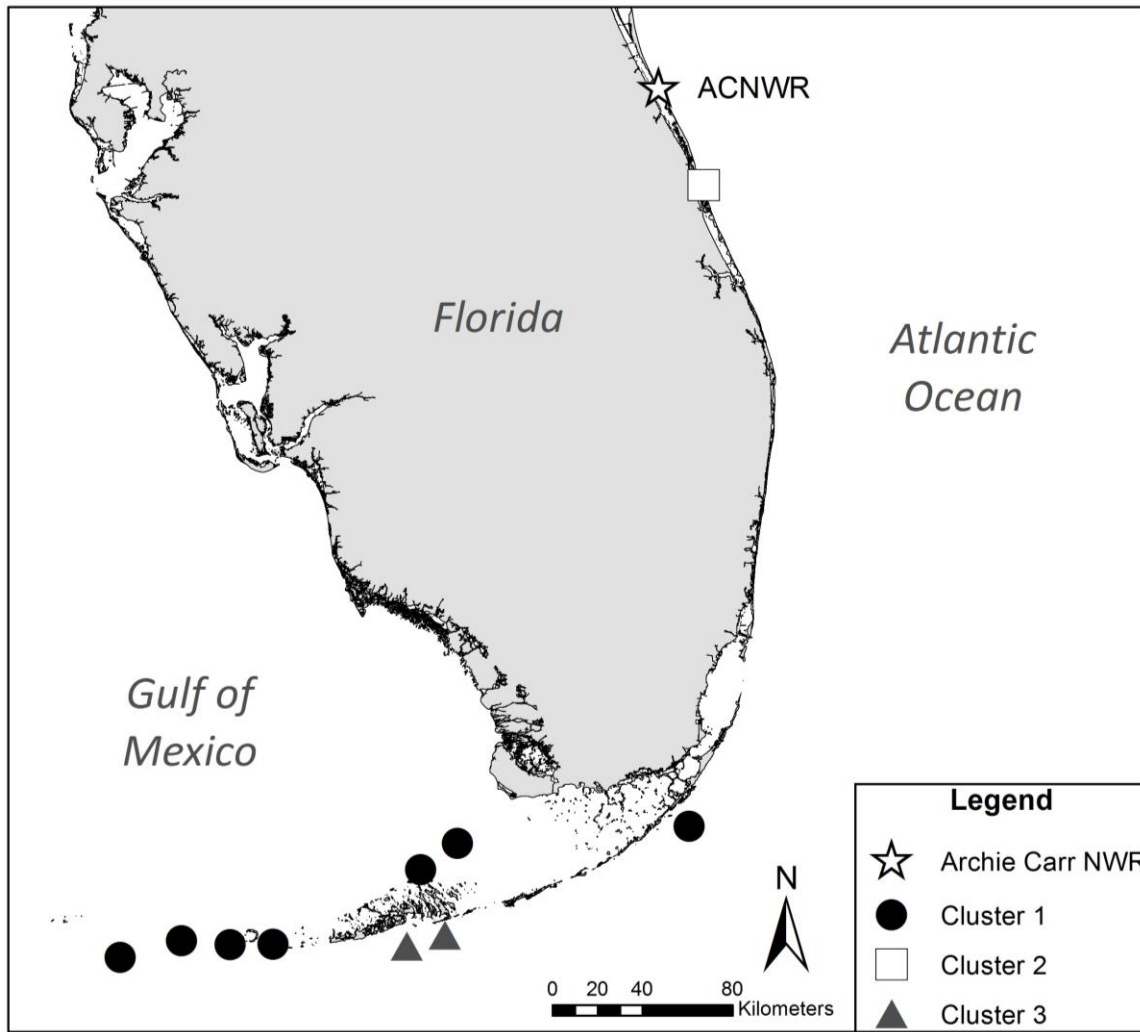


Figure 2: Map detailing the location of the ACNWR green turtle rookery, as well as terminal positions for satellite-tracked individuals from Bagley et al. (in prep). Shapes and colors identifying cluster assignment correspond to those used in Figure 4A.

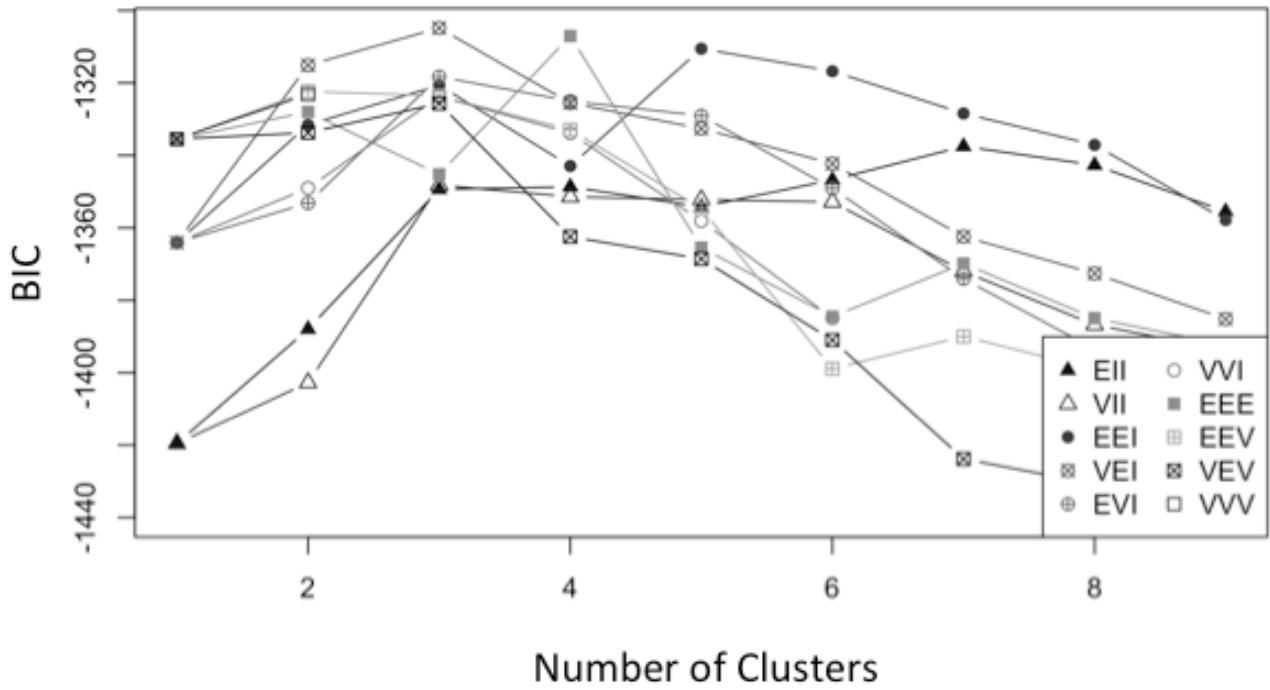


Figure 3: Plot visualizing the combination of models and number of clusters used in a model selection framework to identify the best combination. The classification scheme described by model VEI with three clusters was selected, as it had the highest BIC score.

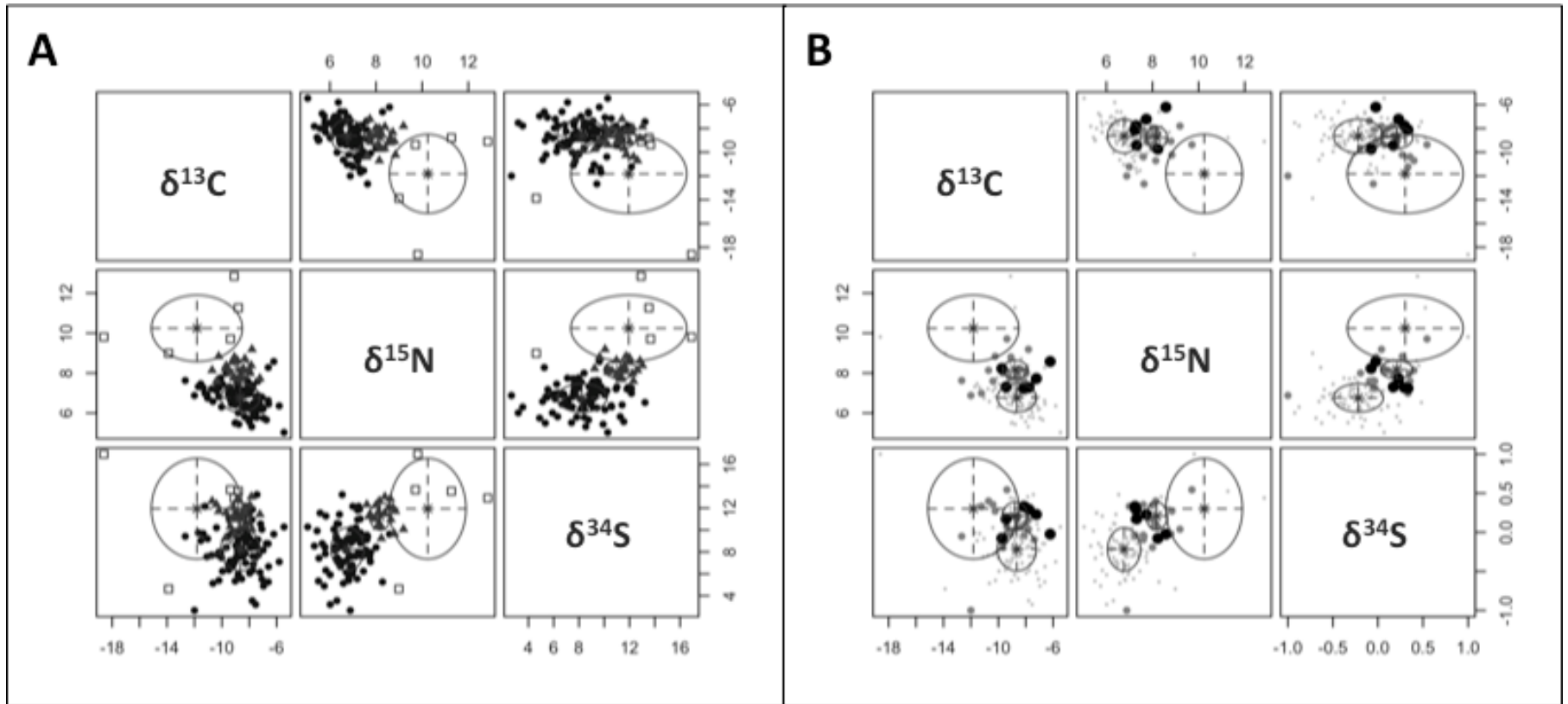


Figure 4: Panel A shows classification results based on stable isotope values of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  for the 115 turtles included in this study. Individuals were divided into multiple clusters: Cluster 1 (black circles,  $n = 88$  turtles), Cluster 2 (hollow squares,  $n = 5$  turtles), and Cluster 3 (grey triangles,  $n = 22$  turtles). Panel B demonstrates the classification uncertainty of each individual; larger and darker circles indicate higher uncertainty in that individual's cluster assignment.

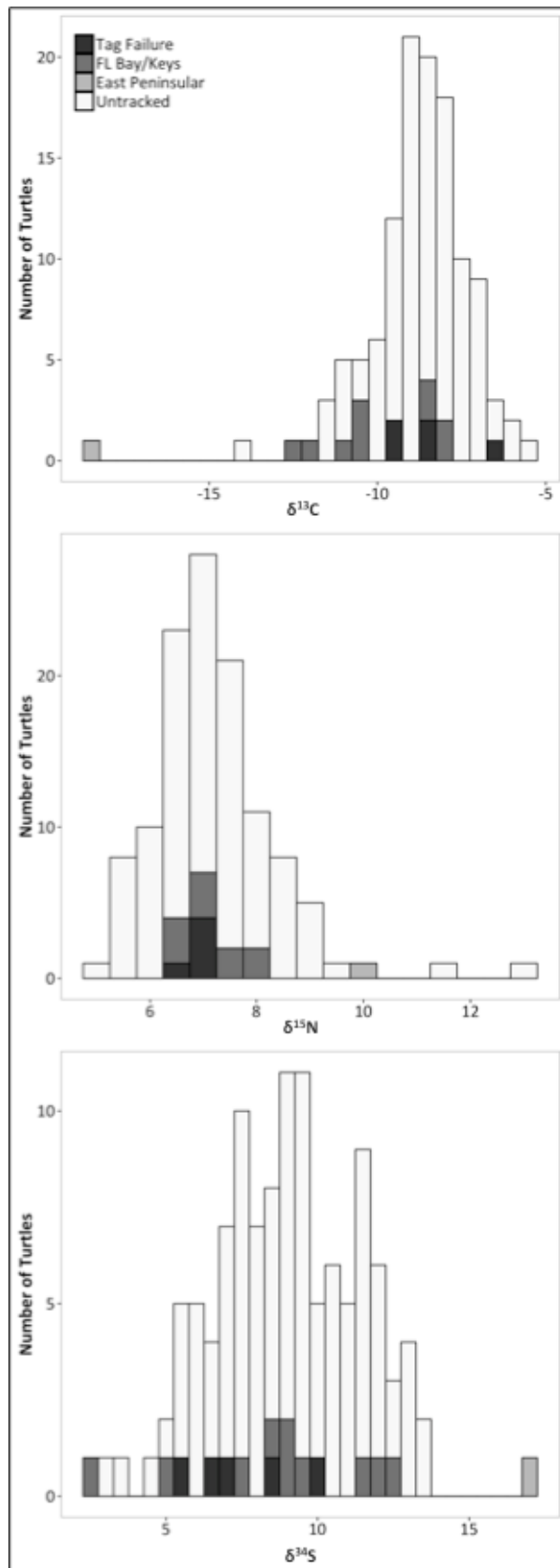


Figure 5: Histograms representing the distribution of isotope values for all turtles included in this study. Bar color indicates the animals foraging destination, or whether the satellite transmitter failed before or during migration to a foraging area.

Table 1: Summary of stable-carbon ( $\delta^{13}\text{C}$ ), -nitrogen ( $\delta^{15}\text{N}$ ), and -sulfur isotope ( $\delta^{34}\text{S}$ ) values for green turtle (*Chelonia mydas*) skin tissue collected from adult green turtles at the ACNWR, Florida, USA.

Year	n ( $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ )	n ( $\delta^{34}\text{S}$ )	Mean SCL (cm) $\pm$ SD (Min SCL, Max SCL)	Mean $\delta^{13}\text{C}$ (‰) $\pm$ SD (Min $\delta^{13}\text{C}$ , Max $\delta^{13}\text{C}$ )	Mean $\delta^{15}\text{N}$ (‰) $\pm$ SD (Min $\delta^{15}\text{N}$ , Max $\delta^{15}\text{N}$ )	Mean $\delta^{34}\text{S}$ (‰) $\pm$ SD (Min $\delta^{34}\text{S}$ , Max $\delta^{34}\text{S}$ )
<b>2013</b>	58	57	97.6 $\pm$ 5.5 (81.5, 110.4)	-9.20 $\pm$ 1.86 (-18.61, -7.01)	7.17 $\pm$ 0.88 (5.40, 9.81)	8.88 $\pm$ 2.27 (3.55, 16.92)
<b>2014</b>	53	51	100.5 $\pm$ 5.4 (87.0, 115.7)	-8.28 $\pm$ 1.17 (-11.09, -5.46)	7.24 $\pm$ 1.46 (5.03, 12.85)	9.31 $\pm$ 2.58 (3.20, 13.67)
<b>2015</b>	7	7	96.5 $\pm$ 2.9 (92.6, 101.1)	-9.45 $\pm$ 2.16 (-12.67, -6.62)	7.18 $\pm$ 0.41 (6.63, 7.78)	8.51 $\pm$ 3.30 (2.66, 12.16)
<b>Total</b>	119	115	98.9 $\pm$ 5.5 (81.5, 115.7)	-8.82 $\pm$ 1.67 (-18.61, -5.46)	7.20 $\pm$ 1.15 (5.03, 12.85)	9.04 $\pm$ 2.48 (2.66, 16.92)

Table 2: Summary of satellite transmitter deployments on adult green turtles at the ACNWR, Florida, USA for this study and Bagley et al. (in prep).

<b>Year</b>	<b>No. of Satellite- Tracked Turtles</b>	<b>Female:Male Ratio</b>	<b>Reached Foraging Location</b>
<b>2013</b>	5	2:3	4/5 (80%)
<b>2014</b>	3	3:0	2/3 (67%)
<b>2015</b>	7	3:4	4/7 (57%)
<b>Total</b>	15	8:7	10/16 (63%)

Table 3: Foraging area origin for nesting sea turtles based on stable isotopes and satellite telemetry. The current project is one of the first sea turtle studies to incorporate  $\delta^{34}\text{S}$  values. †Although the model identified 3 putative foraging areas, validation procedures using satellite telemetry provide evidence for the strongly disproportionate contribution of one functional foraging area (the Florida Keys/Florida Bay Complex) to the ACNWR green turtle rookery.

Species	Breeding Area	No. Foraging Areas	Research Tools				Reference
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$	Satellite telem.	
<i>C. mydas</i>	Archie Carr National Wildlife Refuge, FL, USA	3†	●	●	●	●	This study
<i>C. mydas</i>	Tortuguero, Costa Rica	5	●	●		●	Vander Zanden et al. 2013
<i>C. mydas</i>	Ogasawara Islands, Japan	2	●	●		●	Hatase et al. 2006
<i>C. caretta</i>	Archie Carr National Wildlife Refuge, FL, USA	3	●	●		●	Ceriani et al. 2012
<i>C. caretta</i>	Casey Key, FL, USA	5	●	●	●	●	Tucker et al. 2014
<i>C. caretta</i>	Zakynthos, Greece	2	●	●		●	Zbinden et al. 2011
<i>C. caretta</i>	North Carolina and Georgia, USA	3	●	●		●	Pajuelo et al. 2012
<i>C. caretta</i>	Minabe and Yakushima, Japan	2	●	●		●	Hatase et al. 2002
<i>D. coriacea</i>	Jamursba Medi, Papua Barat, Indonesia	2	●	●		●	Seminoff et al. 2012
<i>D. coriacea</i>	Yalimapo beach, French Guiana	2	●	●			Caut et al. 2008

## CHAPTER 2: OPTIMIZING TISSUE TYPE ANALYSES TO ASSESS THE RELATIONSHIP BETWEEN FORAGING AREA AND FITNESS METRICS

### Introduction

Sexually reproducing organisms employ a myriad of strategies in order to maximize the production of offspring: from sessile, broadcast-spawning corals (Richmond and Hunter 1990) to highly migratory whales with long gestation and parental care intervals (Witteveen et al. 2009). In placental mammals, environmental characteristics surrounding the female have the capacity to continuously influence the health and survival of the embryo(s) throughout development. In contrast, egg-laying organisms encapsulate embryos in an external shell, where nourishment is provided by a yolk. While the egg yolk, composed of lipids, proteins, and carbohydrates, is still derived solely from direct female inputs, environmental effects on the developing embryo in oviparous animals are a composite of two states: first, the indirect effects on female health and energetic investment pre-oviposition, and second, the direct effects of the surrounding environment on the egg post-oviposition without possible buffering effects of the mother (Flatt et al. 2001). These direct effects are likely magnified in life history strategies in which parental care during incubation (e.g., brooding) is minimal or absent (Shine et al. 1997). Because of this spectrum of inputs, care must be taken when choosing fitness metrics to evaluate reproduction in wildlife populations with these characteristics.

Green turtles (*Chelonia mydas*) are highly migratory oviparous organisms that as adults, like other marine turtles species, regularly utilize the same nesting and foraging sites over many years (Limpus et al. 1992; Broderick et al. 2007). Reproductively active females return to



nesting beaches every 2-10 years (depending upon the availability and quality of resources) from foraging areas that may lie thousands of kilometers away, laying multiple clutches of eggs per breeding season (Bjorndal 1982; Mortimer and Carr 1987). As capital breeders (*sensu* Bonnet et al. 1998), green turtles store energy in the form of fat, often over one or more years before acquiring sufficient reserves to trigger migration to the nesting beach (Bjorndal 1982). Green turtles forgo or negligibly feed during intervals between nesting events, collectively entering a fasting period that can last 2-4 months (Hays et al. 2002). The energetic costs of migration and habitat quality of foraging areas may affect a female's condition and reproductive potential. These "carry-over effects" (Harrison et al. 2011) have been documented in the loggerhead (*Caretta caretta*) turtle (Zbinden et al. 2011; Vander Zanden et al. 2014a; Ceriani et al. 2015), but not in green turtles.

After a nesting female deposits her eggs, she will spend an extended period of time (30 minutes to one and a half hours; personal observation) camouflaging the nesting site; beyond this, she will not provide any additional maintenance or protection to the nest, or to her offspring. In marine turtle studies, a number of fecundity metrics have been used to evaluate questions regarding reproductive output, including clutch frequency (the number of nests a female lays in a season), clutch size, egg size, hatching success (the proportion of eggs in a nest that hatch), and emerging success (the proportion of hatchlings that successfully extricate themselves from the nest out of the total number of eggs). Although clutch frequency can be useful to track reproductive output over the course of the nesting season (Broderick et al. 2003) and estimate the number of females using a nesting beach (Broderick et al. 2002), it is very

difficult to find females each time they nest even at low-density rookeries, and infeasible at high-density rookeries. Among green turtles, female size is correlated with clutch size and egg size, with larger females typically laying larger clutches (Bjorndal and Carr 1989; Van Buskirk and Crowder 1994; Hirth 1997), and larger eggs (Bjorndal and Carr 1989). These two metrics are determined solely by factors affecting the nesting female before oviposition, and do not incorporate environmental effects during incubation. Hatching success and emerging success are tightly coupled and are often commensurate, although emerging success does incorporate the factors of hatchling vigor and ability to escape the nest environment. These metrics can be highly influenced by both coarse and localized environmental conditions inherent at the nest site, including nest temperature, sand grain type, distance to the sea, and sand grain size (Bustard and Greenham 1968; Maloney et al. 1990; Mortimer 1990; Hays & Speakman 1993; Ackerman et al. 1997). In this respect, hatching success and emerging success are determined by this composite of pre- and post-oviposition environmental conditions. This variation in parameters that affect different reproductive metrics, and the degree to which each affects them, must be addressed so that the most informative variables are selected for evaluating carry-over effects.

Understanding patterns linked to specific geographic regions are essential to the development of proper management strategies for migratory species of conservation concern, like the green turtle. Beside their role as indicators of carry-over effects from foraging areas on fitness metrics, sea turtle nests (more specifically, sea turtle eggs) can act as tracers to identify these foraging areas. Unlike some other tissue types (e.g., skin), which require researchers to

have access to nesting females themselves, unhatched, “addled” eggs (eggs that never began the process of embryonic development) are an easily acquired and potentially useful tissue type, are found in almost every green turtle nest excavation, and are much less invasive than other collection techniques. Stable isotope analysis (SIA) of sampled tissue has proven to be a valuable tool used to understand migratory connectivity between foraging areas and nesting beaches in a host of sea turtle studies (Hatase et al. 2002; Caut et al. 2008; Ceriani et al. 2012; Seminoff et al. 2012; Vander Zanden et al. 2013). As different tissues types are added to the growing repertoire available to these studies, the relationship between these tissues needs to be evaluated. Understanding the isotopic relationship between tissue types would allow for the generation of conversion equations that would act as a “common currency” across stable isotope studies, providing researchers with a means of comparing results. Most recently, Vander Zanden et al. (2014b) developed these equations for conversion between loggerhead (*Caretta caretta*) skin and scute values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and Ceriani et al. (2014) evaluated  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of unhatched eggs compared to red blood cells, skin, serum, and fresh egg yolk. These relationships have not yet been identified for any green turtle tissues, and represent a significant knowledge gap in the sea turtle isotope literature.

Foraging area assignments nesting female green turtles (n = 100; Chapter 1) were used in a model selection framework to evaluate the relative influence of foraging area on the reproductive metrics of clutch size, egg mass, hatching success, and emerging success in relation to other relevant variables that may influence these metrics. We also developed the first conversion equations for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  between green turtle skin and addled eggs.

## **Methods**

### ***Study Site***

Our study was conducted on the 21-km Brevard county portion of the Archie Carr National Wildlife Refuge (ACNWR), located in Melbourne Beach, Florida, USA (Figure 2). Green turtle nesting numbers from this rookery follow a relatively consistent pattern, with a “high” year (in terms of nest numbers) being followed by a “low” year (UCFMTRG unpublished data). This study took advantage of this biennial pattern, with sampling of females occurring during the “high” 2013 nesting season and the “low” 2014 season.

### ***Turtle Sampling and Measurement***

One hundred total nesting female green turtles were sampled in 2013 and 2014. Females were sampled from June through September of each year following a spatial distribution to approach equal coverage across the study area. Weekly sampling effort followed predicted trends through the nesting season based on the previous 5 years of weekly nest numbers (2013 range: 1-5 females, 2014 range: 1-4 females). Straight carapace length (SCL) was recorded for each sampled individual. Two Inconel flipper tags were applied per turtle (one to each front flipper) and passive integrated transponder (PIT) tags were inserted subcutaneously in the right front flipper to prevent resampling. Two epidermis biopsies were obtained from sampled individuals using a sterile 4 mm biopsy punch. In 2013, one shoulder biopsy was obtained from the right shoulder midway between the neck and flipper, and another epidermis

sample was acquired by splitting a rear flipper biopsy. In 2014, two shoulder biopsies were obtained from sampled turtles. Similar anatomical sampling locations have been used in loggerhead (*Caretta caretta*) turtles with no significant differences in isotopic signatures between sites (Ceriani unpublished data).

### ***Nest Marking, Excavation, and Assessment***

Following oviposition, sampled females' nests were marked using GPS locations and by measuring distances seaward from stakes placed in the dune. Date, beach section (one of seven 3-km sections along the 21-km nesting beach), distance to the dune line and the mean high water line were also recorded for each nest. Additionally, all nests in 2014 were excavated immediately after the female returned to the water to determine exact clutch size and evaluate *in situ* individual mass of twenty-five randomly selected eggs using a portable scale, following protocols described by Miller (1999) and used by Tiwari and Bjorndal (2000) and Long (2013). Eggs were returned to nests within three hours of deposition. All nests were monitored for hatchling emergence and excavated at least three days after emergence, or at least seventy days after deposition if no emergence was observed. Data collected during nest excavations include clutch size (if not previously known), hatching and emerging success, numbers and stages of eggs arrested in their development, and number of eggs affected by a variety of stochastic events (e.g. depredated by raccoons, inundated by tides, invaded by plant roots, etc.). If available, up to five unhatched, "addled" eggs were collected for stable isotope analysis. These were stored in a -20°C non-frost-free freezer until processing. Eggs were collected only if

they appeared to have a minimal level of decomposition, were not punctured or compromised, and appeared to not contain a large embryo.

### ***Added Egg Preparation and Stable Isotope Analysis***

Frozen added eggs were thawed, and egg contents were placed into individual bags; up to three added eggs per nest that appeared the least decomposed upon inspection of the egg contents were freeze dried for 48h. Dried contents were then homogenized with a mortar and pestle. From there, a subsample of each egg was lipid extracted using petroleum ether as solvent in a soxhlet device for 24h. Approximately 0.5 to 1.0 mg of each added egg subsample was placed in a small tin capsule and sent for analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Nitrogen and carbon isotope and bulk composition were measured by CF-EA-irms (Continuous Flow Elemental Analyzer Isotope Ratio Mass Spectrometry) at the University of South Florida College of Marine Science Stable Isotope Biogeochemistry Laboratory using commonly accepted procedures (Werner et al 1999). Isotope compositions were measured on a ThermoFinnigan Delta+XL IRMS, are reported in per mil (‰) notation and are scaled to VPDB ( $\delta^{13}\text{C}$ ) and AT-Air ( $\delta^{15}\text{N}$ ). Secondary reference materials were used to normalize raw measurements to the VPDB ( $\delta^{13}\text{C}$ ) and AT-Air ( $\delta^{15}\text{N}$ ) scales (Werner et al 2002, Qi et al 2002, Coplen et al 2006). Measurement uncertainties, expressed as  $\pm 1$  standard deviation of  $n = 32$  measurements of a laboratory reference material were  $\pm 0.14\text{‰}$  for  $\delta^{13}\text{C}$ ,  $\pm 0.09\text{‰}$  for  $\delta^{15}\text{N}$ . For  $\delta^{34}\text{S}$  analyses approximately 3 mg of added egg was placed into a tin capsule and sent to Washington State University Stable Isotope Core Laboratory. These samples were analyzed with a Thermofinnigan Delta PlusXP continuous flow

isotope ratio mass spectrometer (Brenna et al. 1997) with a measurement uncertainty, expressed as  $\pm 1$  standard deviation of  $n = 9$  measurements of a laboratory reference material, of  $\pm 0.29\text{‰}$  for  $\delta^{34}\text{S}$ . Sulfur isotopic ratios are reported in per mill relative to VCDT by assigning a value of  $-0.3$  per mill to IAEA S-1 silver sulfide (Coplen and Krouse 1998).

### ***Independent Variables and Model Selection***

The different model selection scenarios used to evaluate each fecundity metric contained suites of explanatory variables associated with female-centric and nest-centric environmental factors. Within each model selection scenario, AICc was used to compare models; models with a  $\Delta\text{AICc}$  score of  $< 2.0$  were considered indistinguishable. Clusters derived from analyses in Chapter 1 were used as assignments to particular foraging areas. Cluster 2 contained only four nests with reproductive information, therefore these nests were excluded from all analyses. Year (not included in egg mass analyses) was chosen because interannual variation in environmental conditions at foraging and nesting areas could affect reproduction, and SCL because size is a known correlate to clutch size and egg size in the green turtle (Bjorndal and Carr 1989; Hirth 1997; Van Buskirk and Crowder, 1994). As females lay successive clutches throughout the nesting season, shifts in investment or output may occur between nests, like the increasing clutch sizes observed by Broderick et al. (2003). To account for this, we included ordinal lay day (OLD) into the model selection framework for all metrics. Ordinal lay date is the  $n^{\text{th}}$  day during a particular year; as an example, the OLD correlate of February 5<sup>th</sup> is 36. To account for the influence of nest environment, we incorporated beach section and cross-

shore percentage of beach in which a nest was laid into models for hatching and emerging success. Percentage of beach, expressed as a proportion, is the distance (in meters) from the nest to the mean high water line, divided by the sum of this distance and the distance from the nest to the mean dune line. Unlike a “traditional” percentage, which is bounded between zero and one, percentage of beach can be greater than one, if a nest is deposited landward of the mean dune line. Incorporating dune and mean high water distances into one metric helps to account for the variability in width of the beach in different beach sections. Finally, clutch size was included in model suites for hatching and emerging success, to investigate the potential for tradeoffs between increasing clutch size and the “quality” of the overall clutch. Because of the correlation between turtle size and clutch size observed in other marine turtle studies, SCL and clutch size were never included in the same model.

Cluster, year, SCL, and OLD were included in a suite of 37 simple, additive, and interactive linear models to elucidate the most informative model explaining clutch total. Cluster, OLD, and SCL were used in a collection of 22 simple, additive, and interactive linear models to assess egg mass. Hatching and emerging success data are proportions; historically, these data were typically arcsine transformed, and then evaluated using simple linear models, although this method is now discouraged because of more robust generalized linear models (GLMs) (Warton and Hui 2011). For hatching and emerging success, the proportion data were used as the response variable in GLMs using a quasibinomial distribution (to account for overdispersion) and weighting each nest by the clutch total, as recommended by Zuur et al. (2013). Both hatching and emerging success were evaluated using the same suite of 51 simple,



additive, and interactive models including OLD, year, beach section, SCL, percentage of beach, cluster, and clutch total. An  $\alpha$  value of 0.05 was used to test for significance in all cases.

### ***Developing Conversion Equations***

For all nests that contained at least 2-3 eggs that were sampled for SIA, we calculated a standard deviation (SD) for each nest per isotope to evaluate intraclutch variation. We used a modified Thompson Tau test on the distribution of SD to determine a threshold for each isotope, above which would identify a nest as an outlier. From there, the aberrant egg within the nest was eliminated from the data set. If, after eliminating that egg, the SD for that nest was still above the threshold, or there were no longer at least two eggs, that nest was removed completely. We then constructed simple linear models evaluating the relationship between skin values (from Chapter 1) and addled egg values for each isotope. The residuals and leverage plots for each of these models were assessed, and nests that were having a disproportionate influence on model output or performance were eliminated. Finalized conversion equations were then generated using the reduced dataset. Confidence intervals for coefficients within each isotope model were used to determine whether the intercept differed significantly from zero, and if the slope differed significantly from zero and a 1:1 ratio between the two tissue types, following methodologies described in Vander Zanden et al. (2014b). The SD of isotopic values was evaluated across nests to better understand the intraclutch isotopic variability of green turtle addled eggs.

## Results

### ***Model Selection – Clutch Size***

Before analysis, nests without clutch size data, nests without a cluster assignment, or those nests assigned to Cluster 2 were removed from the dataset. Clutch totals of 94 nests (mean  $\pm$  SD: 125 eggs  $\pm$  25; range: 64 to 183 eggs) were included in a model selection framework that identified 4 of the 37 models tested as having  $\Delta$ AICc scores  $<$  2.0 (Table 4). Within these four models, only SCL was a significant factor, with the simple linear model of SCL (Figure 6) carrying the highest weight. This model demonstrates a significant positive correlation between SCL and clutch size. Although not significant terms in any of the top models, cluster is found in two of the other three models, and year in one of the other three. Ordinal lay date was not included in any of the top models based on AICc scores. All four of the top models each explained approximately 25% of the variation within the clutch size data based on  $R^2$  values.

### ***Model Selection – Egg Mass***

Prior to analyses, nests without clutch size data, those assigned to Cluster 2 or without a Cluster assignment, and those without egg mass data were removed from the dataset. Egg mass data from 51 nests (mean  $\pm$  SD: 49.88 g  $\pm$  4.3; range: 40.05 to 59.21 g) were used in a model selection framework that identified 5 of the 14 models tested as having  $\Delta$ AICc scores  $<$  2.0 (Table 5). Within the 4 models with the greatest weight, only OLD was a significant term; in

the 5<sup>th</sup> highest weighted model, there were no significant terms. Evaluating the top model revealed a significant relationship between egg mass and OLD; as the nesting season progresses, egg mass on the population level increases. Because SCL is not significant within the top model, for ease of graphical interpretation, only the relationship between OLD and egg mass is shown (Figure 7). Although not significant terms in any of the top models, cluster is found in two of the five top models, and year in three of the five. The five top models each explained between 10-13% of the variation within egg mass data, with the highest weighted model explaining 12.35% based on R<sup>2</sup> values.

### ***Model Selection – Hatching Success***

Nests without reproduction information, with a Cluster assignment of 2, and those that had been depredated by raccoons were removed from the dataset. Hatching success data from 78 nests (mean  $\pm$  SD: 70%  $\pm$  27; range: 0.1 to 100%) were used in a model selection framework that identified only one of the 51 models tested as having an  $\Delta$ AICc score  $<$  2.0 (Table 6). Only beach section is a significant term within this model, although year and the interaction of year and beach section are included as well. Figure 8A demonstrates a large spread in hatching success for Beach Sections 1 and 5, and 2 and 3, to a lesser extent. Figure 8B illustrates how differences between years drive some of that variation. Because analyses for these data were performed with GLMs (which do not produce R<sup>2</sup> values), explained deviance was used as the most commensurate calculation. The top model explained 32.53% of the variation within hatching success data.

### ***Model Selection – Emerging Success***

The same nests removed from the dataset for hatching success were also removed for emerging success. Emerging success data from 78 nests (mean  $\pm$  SD: 68%  $\pm$  28; range: 0 to 100%) were used in a model selection framework that identified only one of the 51 models tested as having an  $\Delta$ AICc score  $<$  2.0 (Table 7). Beach section, and the interaction of clutch size and beach section (particularly as it relates to Section 3 and 4) are significant terms within the model, while clutch total is not. Figure 9 illustrates the generally negative relationship between clutch total and emerging success in Beach Sections 1, 2, and 7, a flat relationship for Beach Sections 5 and 6, and a positive relationship for Beach Sections 3 and 4. The top model explained 31.6% of the variation within emerging success data.

### ***Isotope Conversion Equations***

Out of the 100 nests marked for this project, 72 contained at least 2 addled eggs that were collected for stable isotope analysis. The modified Thompson Tau test identified an intraclutch  $\delta^{13}\text{C}$  SD threshold of 0.385‰, a  $\delta^{15}\text{N}$  SD threshold of 0.48‰, and a  $\delta^{34}\text{S}$  SD threshold of 0.86‰. This resulted in nine original outlier nests for  $\delta^{13}\text{C}$ , twelve for  $\delta^{15}\text{N}$ , and five for  $\delta^{34}\text{S}$ . From there, the aberrant egg was removed from each of these nests; a total of 7 nests were fully removed from the dataset after this step, because their intraclutch SD was still above the threshold for at least one isotope, or there were not at least two eggs left. Four additional nests were removed after evaluating residual and leverage plots of tissue isotope conversion models

and determining that these nests were having a disproportionate amount of influence on model parameters. We used the remaining 62 nests to construct finalized skin to added egg conversion models with equations (Figure 10A, 10B, 10C) for each isotope, and to evaluate the intraclutch variability per isotope at the nest and population level (Figure 10D, 10E, 10F). The intercept of the  $\delta^{13}\text{C}$  model was significantly different from zero ( $p < 0.05$ ), while the intercepts of the  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  models were not. The slopes of all the models were significantly different from zero. The slope of the  $\delta^{13}\text{C}$  model is significantly lower than 1 (the confidence intervals around the model coefficient do not contain 1). However, the slopes of the  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  models are not significantly different from 1. Overall, intraclutch isotopic variability (measured as the SD in isotopic values of the eggs sampled from a nest) is relatively low, with a population-level average intraclutch isotopic SD of 0.107‰ for the  $\delta^{13}\text{C}$  model, 0.124‰ for the  $\delta^{15}\text{N}$  model, and 0.246‰ for the  $\delta^{34}\text{S}$  model (Figure 10D, 10E, 10F).

### **Discussion**

The four metrics of fecundity evaluated in this study were best explained by different top models and variables, suggesting different drivers of variation. The fact that SCL alone (and as the only significant term in the other three top models) best explains the variation in clutch total (Table 4) is supported by a positive relationship between female size and clutch size in other marine turtle studies (Bjorndal and Carr 1989; Van Buskirk and Crowder 1994; Hirth 1997). The amount of variation in clutch size explained by female size in our study (~25%) is extremely similar to the value observed by Bjorndal and Carr (1989) in Tortuguero, Costa Rica

(26%). Although non-significant terms within any model, year and foraging area assignment (cluster) were included in the top models, suggesting their inclusion may carry some explanatory power. Broderick et al. (2001) suggested that the green turtle's principally herbivorous diet might increase its vulnerability to the effects of environmental stochasticity on primary productivity, which may result in inter-annual differences in reproductive output. Broderick et al. (2003) found that green turtles nesting in years with lower nest numbers did have reduced reproductive output in the form of reduced number of clutches produced, not clutch size, suggesting a possible tradeoff to maximize propagule quality. Vander Zanden et al. (2014a) and Ceriani et al. (2015) identified differences in the clutch sizes produced by loggerhead turtles from distinct foraging areas, even after accounting for the effect of differences in body size. This factor (cluster) was not significant in our analyses of green turtle clutch size. Results from Chapter 1 suggest that foraging areas used by this nesting population of green turtles are extremely close geographically, which may dampen the effect of these habitat differences. This methodology should be tested for another nesting population of green turtles that utilize more geographically distinct foraging habitats, to determine whether these particular carry-over effects exist within this species. Although Broderick et al. (2003) observed increasing clutch sizes with each successive clutch laid within a nesting season (a rough correlate to OLD), this variable was not included in any of our top models evaluating clutch size. The fact that our data were assessed on the population level (each nest laid by a different female) rather than the individual level, however, may be the reason we did not detect this pattern.

Ordinal lay date was the only significant term in four of the five top models for egg mass (the other had none). This is in contrast to the results from Bjorndal and Carr (1989) who found no seasonal trend at the population (comparable to our study) or the individual level. Possible explanations for this include an increase in reproductive investment or efficiency throughout the nesting season, or females arriving from different foraging areas at different times to the nesting beach producing eggs of different sizes. The latter is not supported by our data, or the fact that cluster does not appear as a significant term in any of the top models; the former would be difficult, if not impossible, to disentangle at the population level, and would require an individual level approach. Tiwari and Bjorndal (2000) found evidence to support optimal egg size theory (tradeoffs between the number of eggs produced and their size to maximize fitness) in three distinct loggerhead nesting populations. They observed that the relative variation in egg size is smaller (i.e., more constrained) than the relative variation in clutch size, based on the coefficient of variation  $[(SD/mean)*100]$  of each. Our own coefficients of variation for egg mass (8.6) and clutch size (20.2) support these conclusions for green turtles, as well. It is worth noting that, although SCL was not a significant factor in any of the top models for egg mass, the three models for which it is included (Table 5) have an explanatory power ( $R^2$ ) between 12-13%, very close to the 13% explained by female size in the Tortuguero green turtle population (Bjorndal and Carr 1989).

Unlike model selection frameworks for clutch size and egg size, frameworks used to evaluate hatching success and emerging success identified only one top model each, with a very large  $\Delta AICc$  between the first and second models. The most informative model evaluating

hatching success included beach section, year, and the interaction of beach section and year (Table 6). This model likely outperforms all others by such a significant margin because these two variables (and their interaction) capture the effects of a number of latent variables that we did not directly measure. These variables (e.g. temperature, sand grain size, etc.), which have an effect on clutch survival and hatching success (Bustard and Greeham 1968; Maloney et al. 1990; Mortimer 1990; Hays & Speakman 1993; Ackerman et al. 1997), may vary considerably between different nesting beaches, individual nests, and years. The most informative model evaluating emerging success was clutch size, beach section, and the interaction of clutch size and beach section (Table 7). Although hatching and emerging success, by their nature, are often tightly coupled, the fact that the top models for these reproductive metrics are slightly different highlights subtle differences in potential environmental drivers. Ackerman (1980) described the importance of gas exchange to the incubating clutch, and suggested that limitations on that exchange (increased clutch size, changes in sand density or water content) could impact embryonic growth and hatching success. Although he does not directly address effects on emergence success, it is possible that increased clutch size (if it is accompanied by a similar drop in gas exchange) could reduce hatchling vigor as they attempt to extricate themselves from the nest. The overall differences in relationships between clutch size and emerging success based on beach section described by the top model, though, are difficult to assess. As latent variables were not collected, these patterns could be indicative of variation in habitat characteristics between these zones, or be an artifact of relatively low sample sizes for a variable (emerging success) that fluctuates drastically between nests.



Detection of foraging habitat characteristics on green turtle reproduction using hatching and emerging success may be dampened or completely eroded by strong, localized environmental factors at the nesting beach or even nest level. Although not a significant term in any of the most informative models evaluating clutch size or egg mass, foraging area assignment (cluster) was present in 2 of 4 of the clutch size models and 2 of 5 of the egg mass models. The close geographic proximity of the foraging areas 1 and 3 described in Chapter 1 (Figure 2) may have reduced our ability to perceive carry-over effects in these variables. It is our recommendation that future marine turtle studies attempting to evaluate these carry-over effects use clutch size, egg size, and other variables affected only by female-centric characteristics for their investigations, and to avoid hatching and emerging success as metrics. It may be possible to use these two metrics to evaluate carry-over effects (or even genotype influences on reproductive output) if nests are incubated in a homogenized environment, such as a hatchery. Hatching success and emerging success are still useful in tracking long-term trends in reproduction at nesting beaches. In these cases, it would be useful for researchers and managers attempting to evaluate these trends at large spatial scales to incorporate nesting beach and beach section as random effects into models, in order to reduce the influence of localized environmental conditions on the interpretation of model results.

These are the first stable isotope conversion equations produced for green turtle tissues (and the first for any marine turtle species, in the case of  $\delta^{34}\text{S}$ ). Although the intercepts are similar between the  $\delta^{13}\text{C}$  model in this study (Figure 10A) and Ceriani et al. (2014) for loggerheads (-3.292 and -3.415, respectively), the slope for this study's equation is lower

compared to the loggerhead study (0.772 to 0.936). This may suggest interspecific differences in tissue isotopic discrimination factors that make broad applications of conversion equations generated using one marine turtle species to another inappropriate and impractical. While the slopes of this study's  $\delta^{15}\text{N}$  model (Figure 10B) and the model in Ceriani et al. (2014) (0.840 to 0.875, respectively) are similar, this study's intercept for the  $\delta^{15}\text{N}$  model (0.029) is much lower than the one in Ceriani et al. (2014) (2.162). This is not surprising, as loggerheads generally feed at much higher trophic levels compared to green turtles, and would likely have elevated  $\delta^{15}\text{N}$  values in their tissues, comparatively (Hobson 1999; Ben-David & Flaherty 2012). The  $R^2$  values of our  $\delta^{13}\text{C}$  model (0.70) and  $\delta^{15}\text{N}$  model (0.63) are lower than those for skin to addled egg isotope conversion models for loggerheads (0.83 and 0.86, respectively; Ceriani et al. 2014). There is no similar study in any marine turtle species with which to compare the goodness-of-fit of the  $\delta^{34}\text{S}$  model ( $R^2 = 0.64$ ). This loss of explanatory power is not likely due to increased intraclutch isotopic variation, as mean intraclutch isotopic SD for all three isotopes evaluated (Figure 10D, 10E, 10F) is fairly similar to laboratory measurement uncertainties. It is possible that the relationship between isotope discrimination factors for skin and eggs is more decoupled in green turtles than in loggerheads. Replicating this study for other green turtle nesting populations dispersed globally would elucidate whether these particular patterns are population-, or species-specific. Although intraclutch variation is generally low, and our method for eliminating aberrant eggs is relatively conservative, we still recommend that at least 2-3 addled eggs be sampled and their isotopic values be averaged per nest, since approximately 14% of the originally sampled nests were removed using our protocol.

In summary, these conversion equations will allow researchers to compare datasets using different tissues, facilitating understanding of green turtle movement at larger spatial scales. As an example, only a few research groups in the United States actively engage in nighttime patrols to encounter nesting green turtles, limiting sampling of skin to a few nesting beaches. However, most areas of coastline hosting sea turtle nesting are monitored by surveyors who mark nests, perform nest excavations, and can collect addled eggs. Combining these two levels of isotopic data and reproductive metrics across a wide breadth of important nesting habitat would allow for stronger inference when attempting to understand the relationship between migratory ecology and reproduction in the green turtle.

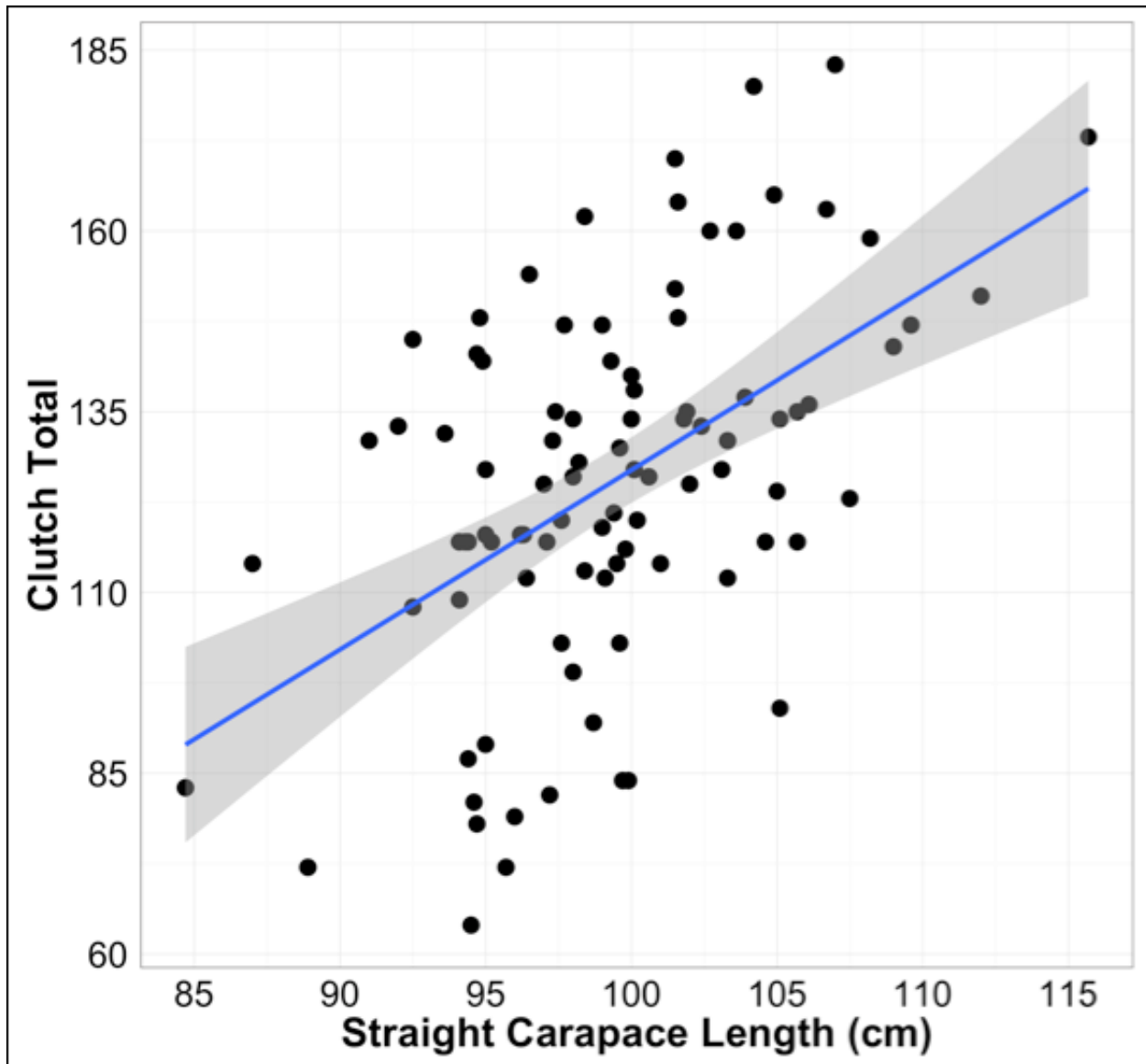


Figure 6: Graphical representation of the significant, positive relationship between nesting green turtle straight carapace length and clutch size. The blue line represents the linear regression, while the shaded area is the 95% confidence interval.

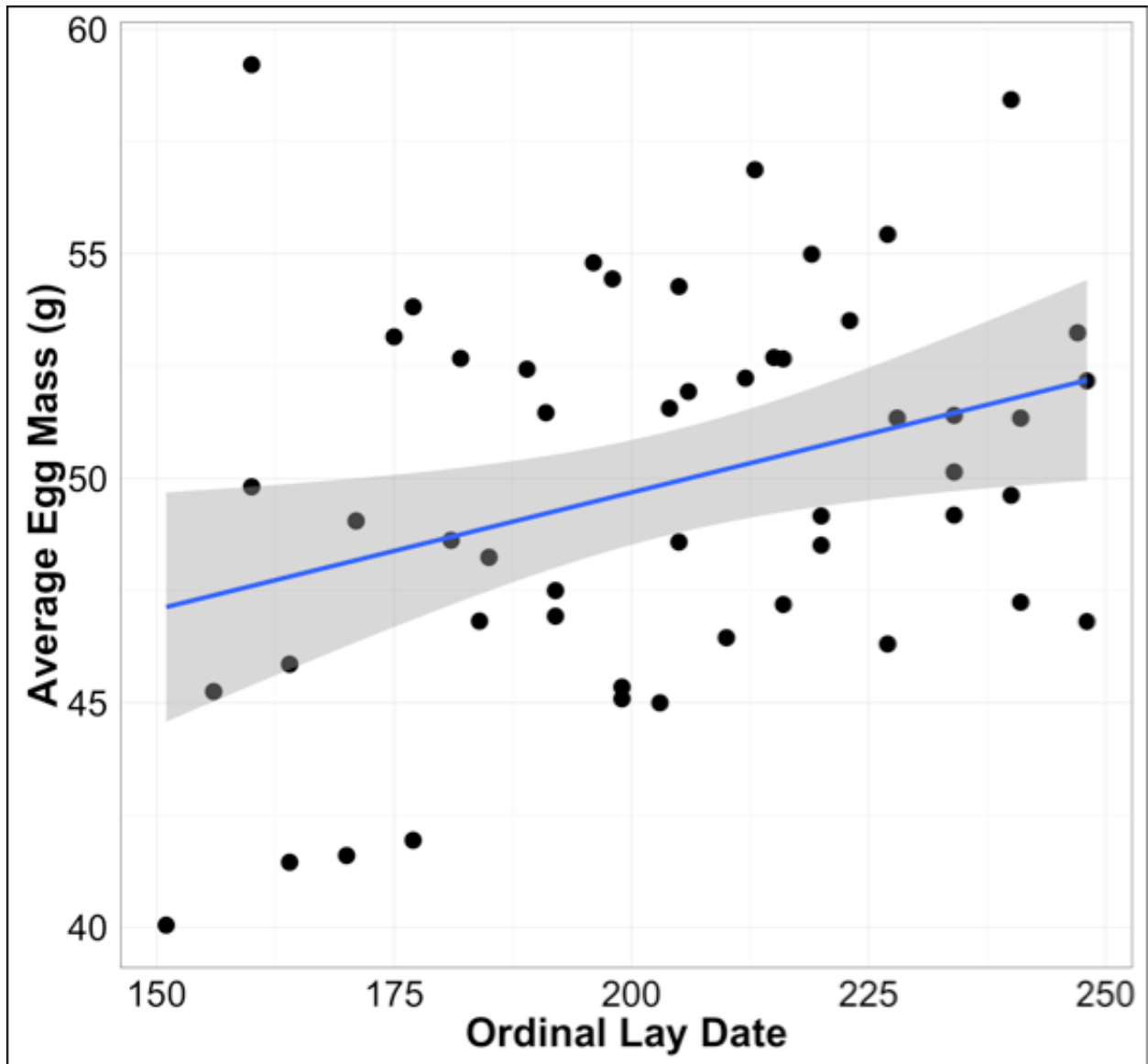


Figure 7: Graphical representation of the significant, positive relationship between lay date and nesting green turtle egg mass. The blue line represents the linear regression, while the shaded area is the 95% confidence interval.

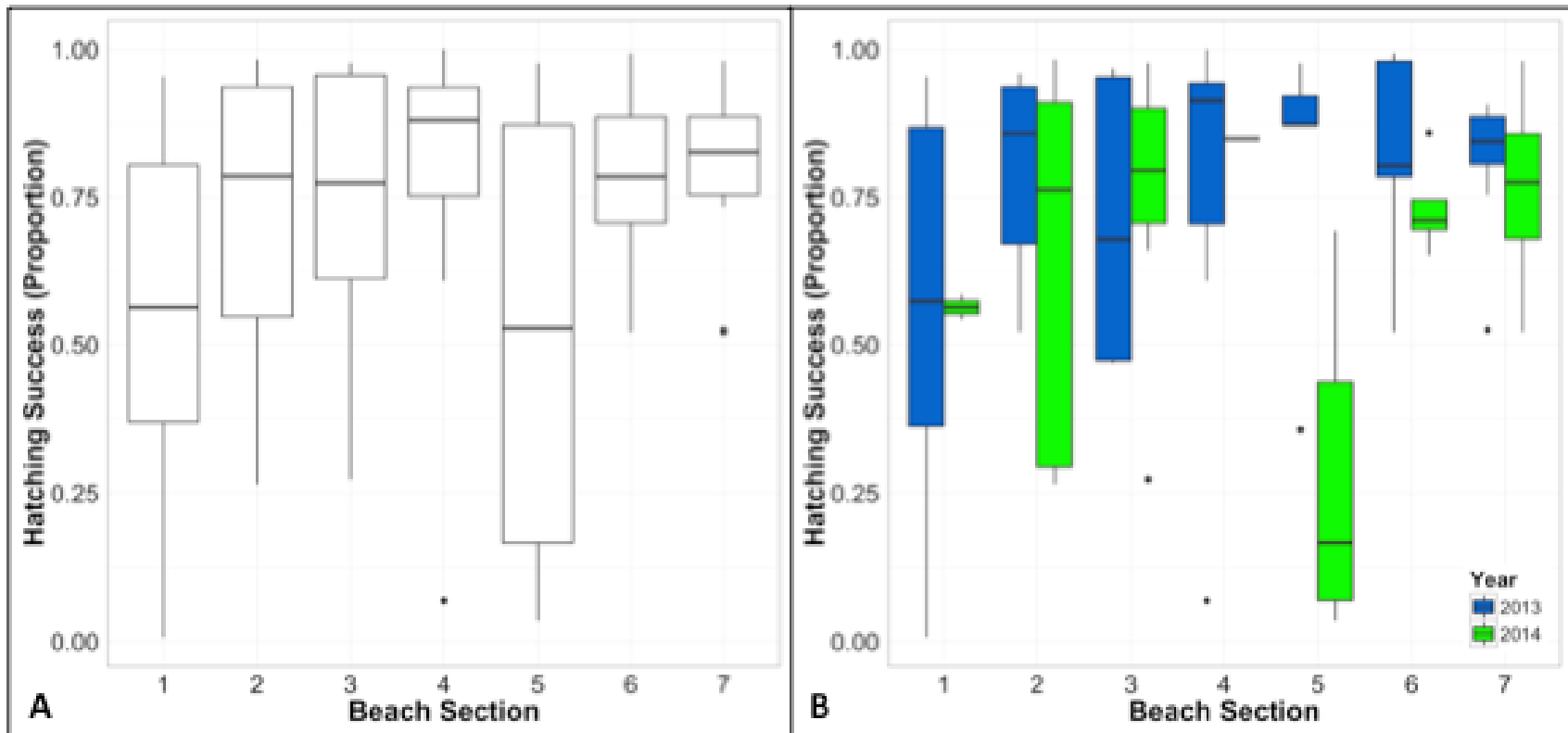


Figure 8: Boxplots showing the comparisons of hatching success among the different beach sections used in this study (A), and the same comparisons incorporating year to illustrate interannual variation (B). The only significant variable within the most informative model explaining variation in hatching success was beach section. However, large differences in hatching success within certain sections between years seemed to drive a large portion of this variation.

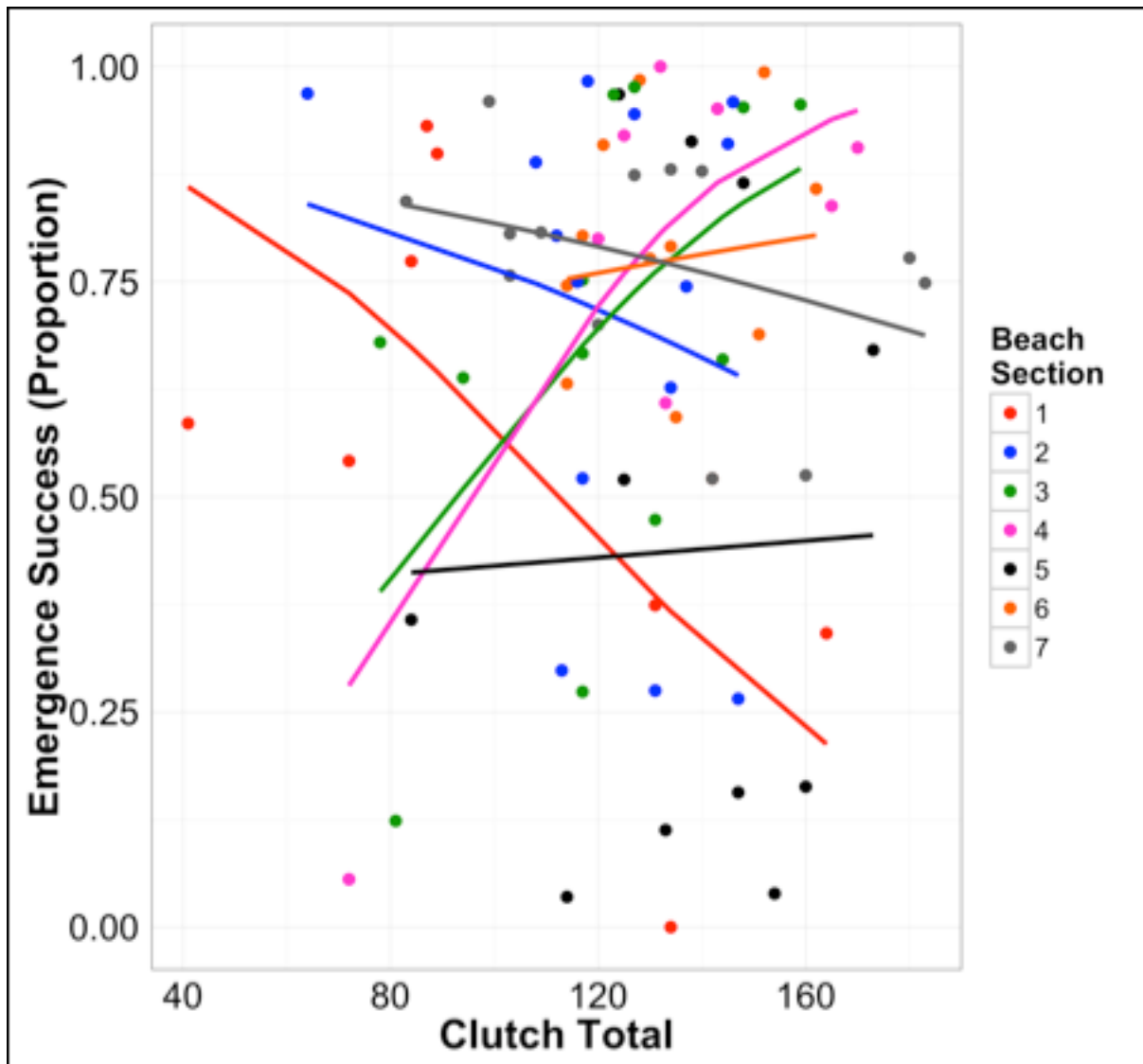


Figure 9: The relationship between clutch size and emergence success is highly influenced by which beach section a nest is laid in, based on the top model evaluating emergence success. While this could be an effect of localized nest conditions, it may also be an artifact of small sample size. Points are raw data, while the lines are predicted emergence success curves by beach section.

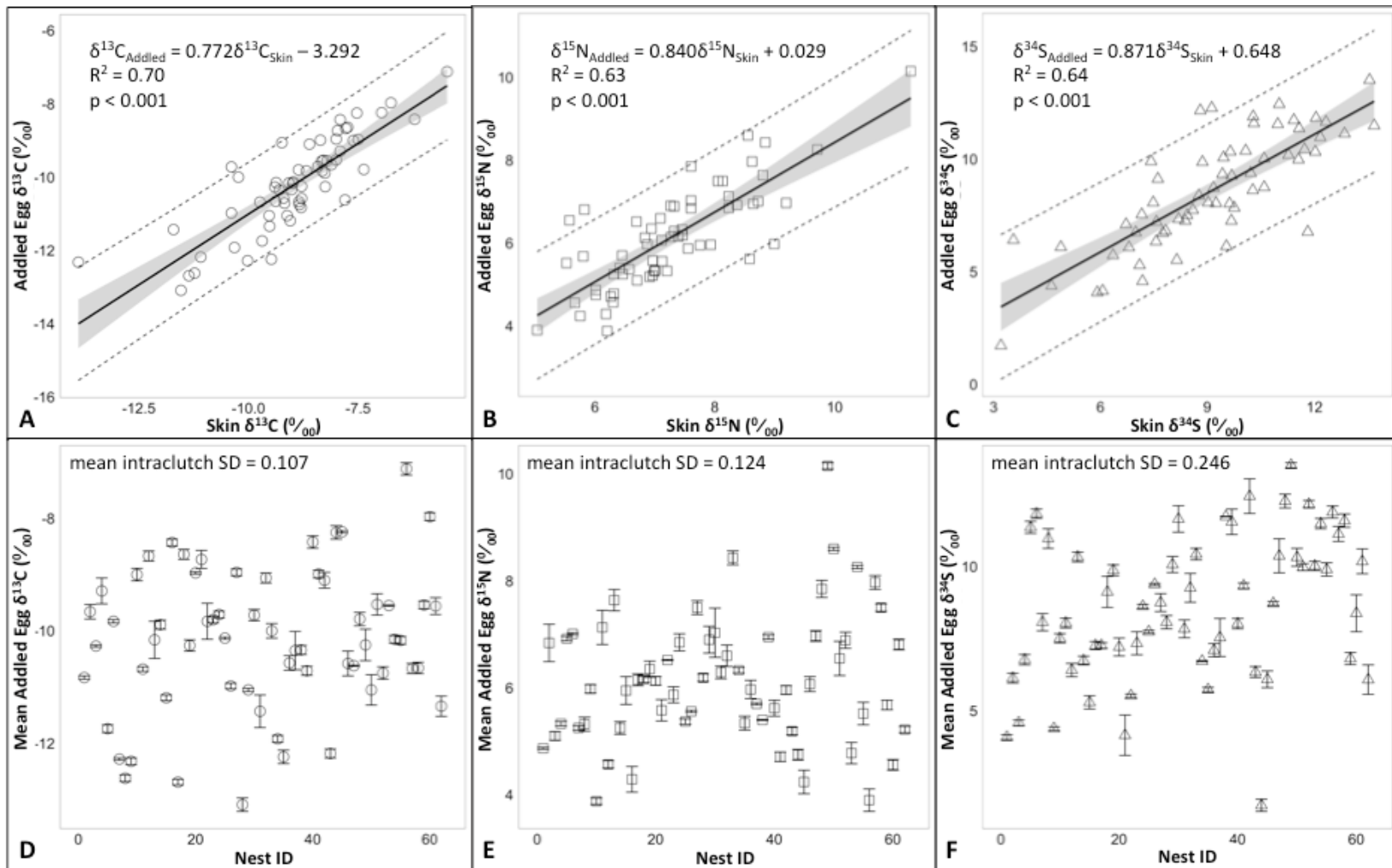


Figure 10: Panels A, B, and C depict the relationship between isotopic values in green turtle skin and added eggs for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$ , respectively. Panels D, E, and F show that there is minimal isotopic variation within the 3 added eggs sampled per nest, with correspondingly low levels at the population level, as well.



Table 4: Summary of the four top models evaluating clutch size whose  $\Delta AICc$  values were less than 2.0. Significant terms in models are bolded. Not only is the simple model of SCL the highest weighted model, but SCL is the only significant term in any of the models.

<b>Model</b>	<b>df</b>	<b>AICc</b>	<b><math>\Delta AICc</math></b>	<b>Weight</b>	<b>R<sup>2</sup></b>
<b>SCL</b>	3	851.6	0.0	0.1937	0.2501
<b>SCL</b> + Cluster	4	852.2	0.5	0.1489	0.2551
<b>SCL</b> + Year	5	853.0	1.3	0.0987	0.2581
<b>SCL</b> + Cluster + SCL:Cluster	5	853.1	1.5	0.0936	0.2572

Table 5: Summary of the five top models evaluating egg mass whose  $\Delta AICc$  values were less than 2.0. Significant terms in models are bolded. Although the top weighted model also included SCL, OLD was the only significant term in that model, or three of the other four models. The top model with the lowest weight had no significant terms.

<b>Model</b>	<b>df</b>	<b>AICc</b>	<b><math>\Delta AICc</math></b>	<b>Weight</b>	<b>R<sup>2</sup></b>
<b>OLD</b> + SCL	4	292.5	0.0	0.2168	0.1235
<b>OLD</b>	3	293.2	0.7	0.1544	0.0887
<b>OLD</b> + SCL + Cluster	5	293.6	1.1	0.1227	0.1278
<b>OLD</b> + Cluster	4	293.7	1.2	0.1204	0.1031
OLD + SCL + Lay Date:SCL	5	294.1	1.6	0.0994	0.1206

Table 6: For the suite of models evaluating hatching success, there was only one model which had a  $\Delta AICc$  of less than 2.0. The model of year, beach section, and their interaction took almost the entirety of AICc weight, suggesting the significant factor within the model (beach section) has a strong impact on hatching success.

Model	df	AICc	$\Delta AICc$	Weight	Explained Deviance
Year + <b>Beach Section</b> + Year:Beach Section	14	2810.9	0.0	1.0	0.3253
Clutch Total + Beach Section + Clutch Total:Beach Section	14	3008.5	197.6	<0.001	0.2704
Year + Beach Section	8	3126.5	315.6	<0.001	0.2334

Table 7: For the suite of models evaluating emerging success, there was only one model which had a  $\Delta AICc$  of less than 2.0. The significant terms of beach section and their interaction of clutch size and beach section took almost the entirety of AICc weight, suggesting that the influence of clutch size on hatching emergence is significantly impacted by localized factors.

Model	df	AICc	$\Delta AICc$	Weight	Explained Deviance
Clutch Total + <b>Beach Section</b> + <b>Clutch Total:Beach Section</b>	14	2954.6	0.0	1.0	0.3160
Year + Beach Section + Year:Beach Section	14	3082.7	128.1	<0.001	0.2819
OLD + Beach Section + OLD:Beach Section	14	3158.3	203.7	<0.001	0.2617

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