Characterizing the foraging ecology of leatherback turtles (*Dermochelys coriacea*) using stable carbon and nitrogen isotopes of eggshells

Sara Paddock
Undergraduate Thesis
Cornell University

Advisors:
Jed Sparks, Cornell University
Jeffrey Seminoff, National Marine Fisheries Service
Abstract

Stable isotopes are useful tools for discerning information regarding the foraging ecology of far-ranging animals like sea turtles. Isotope signatures of prey items are integrated into the turtles’ tissues in a characteristic manner, and in the case of nesting females, eggshell isotopic signatures can provide insight into the trophic status and foraging locations of the adult females. By sampling eggshells from nesting beaches, one can obtain a unique, wide-range perspective that might not be observed by sampling turtles directly within foraging areas. In the present study, leatherback turtle eggshells were collected from 21 different nests on the nesting beach in Jamursba Medi, Papua, Indonesia in the Western Pacific in July 2003. Habitat samples, including particulate organic matter, krill, gelatinous organisms, squid, and small fish were collected with dip nets and bongo tows in the Eastern Pacific, off the west coast of the United States. Eggshell δ\(^{13}\)C values were similar for all eggshell samples (-13.2 ± 1.2 ‰). However, there was a dichotomy in δ\(^{15}\)N values, with 62% of the eggshells in the 9.2‰-10.8‰ range and 38% in the 12.5‰-14.5‰ range. Comparing these values with the δ\(^{15}\)N values of habitat samples analyzed in this study and others as well as with information on Pacific-wide nitrogen fixation/denitrification patterns, suggests that these two δ\(^{15}\)N groupings represent animals foraging in the Western Pacific and Eastern Pacific, respectively. These data are consistent with satellite telemetry data and suggest that δ\(^{15}\)N isotope analysis can be an effective, non-invasive method to gain knowledge about turtle foraging locations that may prove useful in conservation efforts.
Introduction

Stable isotope analysis has been used in the field of ecology to explore animal migration and diet (Killingley and Lutcavage 1982; Gannes et al. 1997; Hobson 1998; Burton and Koch 1999; Kelly 1999; Zhao and Yan 2000; Smith et al. 2002; Biasatti 2004; Rubenstein and Hobson 2004). Consumers integrate prey isotopic composition into their own tissues in a characteristic manner and can convey information about the foraging locations and trophic status of the consumer. This technique has only recently been applied to sea turtle ecology, with some of the first studies being done in the 1990s (Godley et al. 1998). To date, most sea turtle studies that utilize stable isotopes have sampled skin, blood, or bones (Godley et al. 1998; Biasatti 2004; Seminoff et al. 2006), often times from turtles that have been found stranded dead. A few studies have utilized whole hatchlings or egg yolk content (e.g., Godley et al. 1998), but no study to date has investigated turtle eggshell. Since eggshells can be collected from nesting beaches after hatchlings have left the nest, this is a non-invasive sampling procedure that may provide significant information on turtle diet and foraging behavior without causing any stress to the organism.

The stable isotope composition of an organism in terms of carbon ($^{13}$C:$^{12}$C; $\delta^{13}$C) and nitrogen ($^{15}$N:$^{14}$N; $\delta^{15}$N) is an expression of the source material consumed and some degree of fractionation, or enrichment in $\delta^{13}$C and $\delta^{15}$N, due to excretion. In the case of carbon, fractionation is minimal (<1‰) and consumers reflect an integration of their prey item (DeNiro and Epstein1978, 1981; Tieszen et al. 1983; Minagawa and Wada 1984; Hobson 1999). Therefore, $\delta^{13}$C values do not provide trophic level information, but are often useful in determining foraging location. Previous studies have shown that there
exists a spatial gradient in $\delta^{13}C$ values in marine systems, whereby pelagic regions have more negative $\delta^{13}C$ values than do coastal areas (Hatase et al. 2002). Loggerhead turtles, *Caretta caretta*, that feed in pelagic areas have $\delta^{13}C$ values of $-19‰$ to $-18‰$, while those that feed in neritic zones off the coast of Asia have $\delta^{13}C$ values of $-17‰$ to $-16‰$ (Hatase et al. 2002). For nitrogen, excretion processes generate an enrichment of $\delta^{15}N$ of approximately $3‰$ per trophic level in most animals, including sea turtles (DeNiro and Epstein 1981; Minagawa and Wada 1984; Seminoff et al. 2006a; Reich et al. in press). The precise mechanism of this enrichment has not been completely explored, but it is likely due to the preferential excretion of the lighter $^{14}N$, and thereby gradual $^{15}N$ accumulation through trophic levels (Seminoff et al. 2006b). Using this knowledge of carbon spatial gradients and nitrogen discrimination through trophic levels, stable isotope analysis can be used to determine trophic status and foraging location of organisms and is therefore an especially useful tool in the conservation efforts of highly migratory animals.

Leatherback turtles (*Dermochelys coriacea*), believed to be the most pelagic of all sea turtles, are critically endangered (IUCN 2006). They spend the vast majority of their time at sea, with only the females returning to land to nest. Leatherbacks are specialist consumers on gelatinous organisms, and are believed to migrate long distances between foraging grounds and nesting beaches every 2-4 years (Bjorndal 1997).

Nesting females use nutrients obtained from their foraging areas to produce eggs, and therefore egg component isotope values can be used to determine where and on what trophic level female turtles are foraging (Godley et al. 1998). Leatherback eggshells contain both organic carbon and nitrogen, and inorganic carbon in the aragonite form of calcium carbonate (Simkiss 1962; Baird and Solomon 1979; Congdon and Gibbons 1985;
Bilinski 2001). These two different types of carbon can be separated chemically and then used to determine isotope values for the total mean diet of the female (δ\textsuperscript{13}C inorganic) and the proteinaceous portion of the diet (δ\textsuperscript{13}C organic) (Schaffner and Swart 1991; Johnson et al. 1998). Using eggshell samples from nesting beaches, rather than skin or blood samples from turtles in foraging areas, can give researchers a unique ocean-wide perspective. For example, it allows for the determination of where turtles are migrating once they leave the nesting beach and the exploration of whether nesting groups tend to migrate to the same foraging grounds or if a nesting group is comprised of several different foraging groups.

The goal of this study was to explore the foraging behavior of the Jaumrsba Medi nesting leatherbacks using δ\textsuperscript{13}C to determine relative foraging distance from shore and δ\textsuperscript{15}N to determine trophic level. In addition, we compared the δ\textsuperscript{15}N of eggshells to δ\textsuperscript{15}N of various organisms, including gelatinous organisms, that were collected in the Eastern Pacific to identify potential prey items used by foraging turtles. We hypothesized that δ\textsuperscript{15}N would support that Jamursba Medi leatherbacks are specialists on gelatinous prey and that the single nesting group is a single foraging group that migrates long distances to pelagic foraging areas.

Materials and Methods

Study site

Five eggshells, from which hatchlings had already emerged, were collected from each of 21 nests on a nesting beach in Jamursba Media, Papua, Indonesia on July 24, 2003 (Fig. 1). Habitat samples, including particulate organic matter, krill, gelatinous
organisms, small crustaceans, squid, and small fish were collected in the Eastern Pacific during the NOAA PICEAS 2005 and CSCAPE 2005 cruises from May 6, 2005 to July 22, 2005 (Fig. 1). These samples were collected at latitudes of 32° 13.7’ N to 48° 27.3’ N and longitudes of 119° 10.1’ W to 129° 55.93’. Samples were collected near the surface of the water using dip nets and at approximately 210m depth using bongo tows. Collected organisms were then identified to species, if possible, and placed in Whirl-Pak bags. The bags were labeled with the organisms in the sample as well as the date, time, and location of collection. They were then placed in a freezer at -80°C. At the time of this study, these samples were thawed and separated into subsamples: particulate organic matter and krill, gelatinous organisms, small crustaceans, squid, and fish before they were prepared for isotope analysis.

**Sample preparation and analysis**

Eggshell samples were cleaned with DI water and cut and homogenized using scissors and a mortar and pestle. The eggshell sample was then separated into two fractions. The first was reacted with 3.5% NaOCl to completion (approximately 24 hours) and the product rinsed with DI water and dried at 60°C for 5 hours to obtain eggshell inorganic carbon. The second portion of eggshell and all Eastern Pacific habitat samples were processed to obtain organic carbon and nitrogen. The samples were cleaned with DI water and homogenized using mortar and pestle. Lipids were removed from this fraction using a Soxhlet apparatus with a 1:1 solvent mixture of petroleum ether and ethyl ether for two 6-hour cycles. Samples then were dried at 60°C for 24 hours to remove any residual solvent and then reacted to completion with 2M HCl to remove
carbonate. They were then rinsed with DI water and dried in an oven at 60°C for 5 hours. Subsamples (~1.0mg) of all processed samples were loaded into sterilized tin capsules and analyzed by a continuous-flow isotope-ratio mass spectrometer (Deltaplus, Finnigan MAT, Bremen, Germany) in the Stable Isotope Laboratory at Scripps Institution of Oceanography, La Jolla, California, USA. The mass spectrometer was outfitted with a Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT, Bremen, Germany). Sample stable isotope ratios relative to the isotope standard are expressed in the following conventional delta (δ) notation in parts per thousand (‰):

\[
\delta = (\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1) \times 1000
\]

where \(R_{\text{sample}}\) and \(R_{\text{standard}}\) are the corresponding ratios of heavy to light isotopes (\(^{13}\text{C}/^{12}\text{C}\) and \(^{15}\text{N}/^{14}\text{N}\)) in the sample and standard, respectively. International standards were used for \(R_{\text{standard}}\), Peedee Belemnite (PDB) limestone formation for \(^{13}\text{C}\) and atmospheric \(\text{N}_2\) for \(^{15}\text{N}\). In order to calibrate the system, all runs included samples of standard materials (Baker Acetanilide \((\text{C}_8\text{H}_9\text{NO}; \delta^{13}\text{C} = -10.4)\) and IAEA NI Ammonium Sulfate Sulfate \((\text{(NH}_4)_2\text{SO}_4; \delta^{15}\text{N} = +0.4))\) inserted every 6 to 7 samples, calibrated against the international standards. Stable isotope ratios, as well as %C and %N, were measured for eggshell and habitat samples. Samples were combusted in pure oxygen in the elemental analyzer, and resultant \(\text{CO}_2\) and \(\text{N}_2\) gasses were passed through thermal conductivity detectors and element traps in order to ascertain percent carbon and nitrogen. Acetanilide standards (10.36% N, 71.09% C) were used for calibration. For 19 of the 21 nests, one eggshell was used for analysis. For the other two nests, five eggshells from each nest
were used to determine that there is very little intra-clutch variation in isotope values and that one eggshell is representative of the isotope values for an entire clutch.

**Statistical analysis**

Means and standard deviations were calculated for $\delta^{13}C$ (organic and inorganic) for eggshell samples and for $\delta^{15}N$ for eggshell as well as habitat samples. Differences in the $\delta^{15}N$ values of eggshells were evaluated with student’s t-tests with a significance value of $\alpha \leq .05$.

**Results**

Eggshell $\delta^{15}N$ expressed a total range of 9.2‰-14.5‰. A student’s t-test ($\alpha = .05$) indicated that this is comprised of two significantly different $\delta^{15}N$ groups: 9.2‰-10.8‰ (mean: 9.9 ± 0.53‰) and 12.5‰-14.5‰ (mean: 13.6 ± 0.73‰) (Table 1; Fig. 2).

Unlike the eggshell $\delta^{15}N$ values, there were no statistically different groups within either $\delta^{13}C$ (inorganic) or $\delta^{13}C$ (organic). The $\delta^{13}C$ values had smaller ranges than that of $\delta^{15}N$, and $\delta^{13}C$ values were distributed relatively evenly throughout the range (Fig. 2). $\delta^{13}C$ values $\delta^{13}C$ (inorganic) had a range of -14.8‰ to -11.6‰ and mean of -13.2 ± 1.2‰. $\delta^{13}C$ (organic) had a range of -17.7‰ to -15.8‰ and mean of -16.8 ± 0.56‰ (Table 1).

Eastern Pacific habitat samples showed increasing $\delta^{15}N$ values with increasing trophic levels (particulate organic matter and krill = 10.2 ± 1.5‰; gelatinous organisms (salps and jellyfish) = 10.4 ± 1.3‰; small crustaceans = 11.3 ± 1.6‰; squid = 11.5 ± 0.7‰; fish = 13.0 ± 2.1‰) (Fig. 3). Of particular interest are gelatinous organisms
(\(\delta^{15}N = 10.4 \pm 1.3\%\)), as these are conventionally considered to be the exclusive diet of leatherbacks.

**Discussion**

Within the Jamursba Medi eggshell samples, there were two significantly different groups described by \(\delta^{15}N\). One of these groups, which comprised 38% of the turtle eggshells sampled, had a mean \(\delta^{15}N\) value of 13.6 \(\pm 0.73\%\), while the rest of the samples had a lower mean \(\delta^{15}N\) value of 9.9 \(\pm 0.53\%\). The wide range of \(\delta^{15}N\) values (9.2\%\%-14.5\%) was broader than that expected for a specialist consumer. Since \(\delta^{15}N\) values increase by approximately 3\% per trophic level, monotrophic consumers feeding on the same prey items should exhibit a \(\delta^{15}N\) range of approximately 3\% (Seminoff et al. 2006b). Therefore, it is possible that the Jamursba Medi nesting group is comprised of multiple foraging groups feeding in different areas.

Habitat samples from the Eastern Pacific showed the expected increases in \(\delta^{15}N\) values through higher trophic levels (Fig. 3). Gelatinous organisms from the Eastern Pacific had a mean \(\delta^{15}N\) value of 10.4\%, and therefore, leatherbacks feeding in the Eastern Pacific would be expected to have \(\delta^{15}N\) values of approximately 13.4\%. Therefore, it is likely that the group of Jamursba Medi turtles with a mean \(\delta^{15}N\) value of 13.6\% are foraging in the Eastern Pacific.

Eggshell \(\delta^{15}N\) values were compared to \(\delta^{15}N\) values from skin samples taken from turtles in the Monterey Bay foraging ground in the Eastern Pacific (J. Seminoff, unpublished data; Fig. 4). The \(\delta^{15}N\) values for these skin samples had a mean value of 13.0 \(\pm 0.7\%\), which is consistent with Eastern Pacific foraging, and further supports the conclusion that the group of Jamursba Medi turtles with a mean \(\delta^{15}N\) value of 13.6\% are
likely foraging in the Eastern Pacific. The Monterey Bay skin samples had a $\delta^{15}$N range of 2.6‰ (Fig. 4), indicating that these leatherbacks are feeding on a single trophic level, which is consistent with the knowledge that they are specialists on gelatinous organisms. The eggshells examined in this study had a much larger range of $\delta^{15}$N (5.3‰), and superficially, this suggests these animals are polytrophic consumers. However, since there is an increase of 3‰ in $\delta^{15}$N per trophic level and one of the eggshell $\delta^{15}$N groups had a mean value that was approximately the same as the $\delta^{15}$N value for particulate organic matter and krill in the Eastern Pacific, it can be concluded that this group of Jamursba Medi turtles is not foraging in the Eastern Pacific. Therefore, it is likely that rather than being comprised of a single polytrophic foraging group, the Jamursba Medi nesting group is comprised of two separate monotrophic foraging groups (Fig. 3), one in the Eastern Pacific and one elsewhere.

While the overall range of eggshell $\delta^{15}$N was 5.3‰, the ranges for the two separate $\delta^{15}$N groups were much smaller: 1.6‰ and 2.1‰ (Fig. 4). These smaller ranges are indicative of monotrophic foraging, supporting the conclusion that the Jamursba Medi nesting group contains two foraging groups. This conclusion shows the importance of sampling at nesting beaches, as it gives an ocean-wide perspective and multiple foraging areas can be determined by sampling at just one site, a perspective that could not be obtained by sampling in foraging areas.

The two different $\delta^{15}$N groups observed within the Jamurba Medi turtles likely represent groups foraging in different parts of the Pacific. Differing nitrogen cycling regimes result in different baseline $\delta^{15}$N values in different parts of the Pacific. Denitrification is highly prevalent in oxygen-depleted areas, including the Eastern
Pacific, resulting in higher baseline $\delta^{15}N$ values (Fig. 5; Saino and Hattori 1987; Wallace et al. 2006). In contrast, in areas where nitrogen fixation dominates, such as in the Western Pacific, there are lower baseline $\delta^{15}N$ values (Fig. 5; Saino and Hattori 1987; Gruber and Sarmiento 1997; Berman-Frank et al. 2001; Wallace et al. 2006). This difference in $\delta^{15}N$ values is propagated through trophic webs, resulting in consumers that forage in the Western Pacific having lower $\delta^{15}N$ values than consumers that forage in the Eastern Pacific, even though they are foraging at the same trophic level. In the Western Pacific, salps, gelatinous tunicates, have a $\delta^{15}N$ value of 5.3‰, and jellyfish have a $\delta^{15}N$ value of 10.4‰ (Hatase et al. 2002). Therefore, if leatherbacks had a diet of 50% salps and 50% jellyfish in the Western Pacific, they would have a $\delta^{15}N$ value of 10.8‰, the upper range of one of the Jamursba Medi $\delta^{15}N$ groups. If turtles consume slightly more salps than they do jellyfish, then they would have a slightly lower $\delta^{15}N$ value. Based on the isotopic evidence, we suggest the Jamursba Medi nesting group is comprised of two monotrophic foraging groups, and that 62% of the turtles sampled are foraging in the Western Pacific and 38% are foraging in the Eastern Pacific.

This intra-Pacific baseline $\delta^{15}N$ difference has been used to explain $\delta^{15}N$ isotope values in sooty shearwaters, *Puffinus griseus* (Minami and Ogi 1997). However, until now, the only differing baseline $\delta^{15}N$ values used to explain foraging location in sea turtles has been intra-basin differences between the Pacific and Atlantic (Wallace et al. 2006). This study shows that baseline $\delta^{15}N$ values can also be useful in determining sea turtle foraging location within a single ocean basin. This can be useful in determining the relative amounts of migration and foraging by leatherbacks in various parts of the Pacific,
knowledge that can be useful in conserving this population that is declining in abundance (Spotila et al. 2000).

In previous studies, $\delta^{13}C$ values have been used to determine foraging distance from shore (Hatase et al. 2002). In these studies, animals foraging farther from shore have more negative $\delta^{13}C$ (organic) values ($-19\%$ to $-18\%$) than do animals foraging closer to shore ($-17\%$ to $-16\%$). In this study, the eggshells had a $\delta^{13}C$ (inorganic) range of $-14.8\%$ to $-11.6\%$ and a $\delta^{13}C$ (organic) range of $-17.7\%$ to $-15.8\%$. Since these ranges are relatively small and there is fairly uniform distribution of values within these ranges, it can be concluded that either all the Jamurba Medi leatherbacks are foraging relatively the same distance from shore or that $\delta^{13}C$ may not be an adequate indicator of foraging distance from shore for leatherbacks. The $\delta^{13}C$ marine spatial gradient has not been substantiated for sea turtles, and a recent study (Hess et al. 2007) showed that there was no correlation between $\delta^{13}C$ values of olive ridley sea turtle ($Lepidochelys olivacea$) skin and foraging distance from shore. Therefore, additional study is needed in order to determine whether $\delta^{13}C$ spatial gradient corresponds with foraging distance from shore for sea turtles and researchers should be cautious of their interpretations of $\delta^{13}C$ data for sea turtles.

This study is unique in that it was able to use $\delta^{15}N$ values to determine both trophic status and foraging location. Using knowledge of baseline $\delta^{15}N$ values as well as the food source $\delta^{15}N$ values, we concluded that 62% of the leatherbacks studied may have foraged in the Western Pacific and 38% foraged in the Eastern Pacific. This result is significant in that not only does it show that single nesting groups are comprised of multiple foraging groups, but it also shows the relative abundance of turtles in different
parts of the Pacific, which is knowledge that may prove critical to conservation efforts. While 38% of the leatherbacks studied did partake in a trans-Pacific migration, the majority of turtles foraged much closer to the nesting site, presumably in the Western Pacific. A recent study (Benson et al. in press) that used satellite telemetry to track females leaving the Jamursba Medi nesting beach found similar migration patterns, supporting the results obtained from this stable isotope study. Stable isotopes can therefore give us much of the same information that is obtained through satellite telemetry but in a much less expensive and invasive manner.

Acknowledgments

I gratefully acknowledge Jeff Seminoff and Jed Sparks for their valuable advising during this project. I thank Lindy Barrow, Scott Benson, Kangana Beri, F. Chavez, Bruce Deck, Peter Dutton, Candice Hall, Lauren Hess, Melinda Kelley, Erin LaCasella, Bryan Wallace, and Elizabeth Zele for their contributions. I also thank the NOAA Hollings program for providing the funding which allowed me to do this project.

Literature Cited


Benson, S.R., Dutton, P.H., Hitipeuw, C., Samber, B., Bakarbessi J. and D. Parker. in press. Post-nesting migrations of leatherback turtles (Dermochelys coriacea) from
Jamursba Medi, Birds Head Peninsula, Indonesia. Chelonian Conservation and Biology 6(1).


ecology: assumptions, caveats, and a call for more laboratory experiments.


Leatherback turtles as oceanographic indicators: stable isotope analyses reveal a
trophic dichotomy between ocean basins. Marine Biology.

Zhao Z., and Z. Yan. 2000. Stable isotopic studies of dinosaur eggshells from the
Figure 1. Map showing the Jamursba Medi nesting beach, Monterey Bay foraging area, locations of habitat sample collections by the CSCAPE 2005 and PICEAS 2005 cruises, and the location where habitat δ¹⁵N data already exists in the literature.

Table 1. δ¹³C and δ¹⁵N values for eggshell samples.

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ¹³C (inorganic)</td>
<td>-14.8‰ to -11.6‰</td>
<td>-13.2 ± 1.2‰</td>
</tr>
<tr>
<td>δ¹³C (organic)</td>
<td>-17.7‰ to -15.8‰</td>
<td>-16.8 ± 0.56‰</td>
</tr>
<tr>
<td>δ¹⁵N Group 1</td>
<td>9.2‰ - 10.8‰</td>
<td>9.9 ± 0.53‰</td>
</tr>
<tr>
<td>Group 2</td>
<td>12.5‰ - 14.5‰</td>
<td>13.6 ± 0.73‰</td>
</tr>
</tbody>
</table>
Figure 2. $\delta^{15}$N and $\delta^{13}$C (organic) for Jamursba Medi eggshell samples, showing the two significantly different $\delta^{15}$N groups.

Figure 3. $\delta^{15}$N for Eastern Pacific habitat samples.
Figure 4. $\delta^{15}$N for eggshells from the Jamursba Medi nesting beach and for skin samples from the Monterey Bay foraging area.

Figure 5. World-wide nitrogen fixation patterns (Berman-Frank et al. 2001).

A: N2 fixation in the Western Pacific results in lower $\delta^{15}$N;
B: Denitrification in the Eastern Pacific results in higher $\delta^{15}$N.